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# Functional Alterations in Brain Activation and Deactivation in Mild Cognitive Impairment in Response to a Graded Working Memory Challenge

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**Key Words**
Mild cognitive impairment · Alzheimer's disease · Functional magnetic resonance imaging · Working memory · Memory · Default mode network

**Abstract**
***Aim:*** To investigate dynamic changes in functional brain activity in mild cognitive impairment (MCI) in response to a graded working memory (WM) challenge with increasing memory load. ***Methods:*** In an event-related functional magnetic resonance imaging (fMRI) study, 35 MCI and 22 cognitively normal subjects performed a visuospatial associative WM task with 3 load levels. Potential performance differences were controlled for by individually calibrating the number of items presented at each load. ***Results:*** An interaction between group and WM load was observed during stimulus encoding. At lower loads, greater activity in the right anterior cingulate and right precuneus was observed in MCI subjects. As the load increased to higher levels, reduced activation in these regions and greater deactivation in the posterior cingulate-medial precuneus were observed in MCI compared to control subjects. Stronger expression of load-related patterns of activation and deactivation in MCI subjects was associated with greater clinical severity and a more abnormal pattern of performance variability. ***Conclusion:*** Patterns of overactivation, underactivation and deactivation during successful encoding in MCI subjects were dependent on WM load. This type of graded cognitive challenge may operate like a 'memory stress test' in MCI and may be a useful biomarker of disease at the predementia stage.



## Introduction

There is an increasing focus on the preclinical stage of Alzheimer's disease (AD) and other dementias in an attempt to identify biomarkers that can be used to predict the development of dementia. Volumetric structural brain changes are evident at the predementia stage, but these are still of uncertain diagnostic value [1]. There is a great deal of interest in the possibility of functional neuroimaging markers which may allow diagnosis at an earlier stage of disease since alterations in synaptic activity precede cortical atrophy [2]. Altered patterns of brain activity under the stress of cognitive test performance have been demonstrated using functional magnetic resonance

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imaging (fMRI) in older persons with mild cognitive impairment (MCI), an intermediate syndrome between normal ageing and dementia [3]. However, MCI is known to be clinically heterogeneous, with only a proportion of individuals progressing to dementia. In conjunction with a suitable cognitive challenge, fMRI has the potential for identifying those at high risk of progression [4–8]. One such candidate involves the graded increase of working memory (WM) load, which would allow examination of dynamic changes in brain activity over large-scale brain networks.

WM is a limited capacity system responsible for temporary storage and manipulation of information and is critical for reasoning, learning and comprehension [9]. WM is one of the most severely impaired cognitive functions in AD, outside of the characteristic episodic memory deficit [10–13]. Impairment in WM is also evident in MCI [14, 15]. The few fMRI studies of WM in MCI have produced variable results, with both increased and decreased brain activity being reported [16, 17]. One reason for this variability is likely to be the level of WM load presented since brain activity is known to be modulated by WM load. Specifically, young healthy individuals characteristically display increased brain activity in response to increased WM load, predominantly in prefrontal and parietal cortices and the hippocampus [18–24]. Reductions in neural response are also observed when cognitive demands are increased above an individual's WM capacity [e.g. 25]. In comparison to young adults, brain activity in healthy older adults peaks at a lower load level in the prefrontal cortex and then diminishes more quickly over further increases in load, accompanied by a decline in performance [26–28].

To date, only 1 study [18] has investigated the effect of increasing WM load on brain activity in older adults with MCI. Alterations in deactivation were noted in MCI subjects in the early phase of the fMRI signal for the lower, but not the higher, load condition in the precuneus, which is a component of the putative default mode network (DMN) [29]. Task-induced deactivation in the DMN has consistently been observed in healthy individuals using a wide range of cognitive tasks [30–32], and is thought to reflect reallocation of neurocognitive resources away from unconstrained general monitoring processes during rest or a low-demand task toward an attention demanding goal-directed task [29]. Investigation of the DMN has been the subject of intense research interest because of reports of alterations in DMN activity both at rest [33–37] and during cognitive task performance [38–41] in MCI and AD populations, and also for its potential prognostic value in preclinical AD [7].

Most previous studies of WM have not explicitly controlled for individual or group performance differences and have applied the same levels of load to all subjects. This approach risks the possibility that some individuals will experience greater difficulty on the selected task than others. Indeed, a number of studies have demonstrated an association between task performance levels and magnitude of brain activity in young and healthy older individuals [27, 42, 43]. This suggests that when performance is not matched between groups, whether old and young or healthy subjects and clinical patients, it may not be possible to separate the variable contributions from task difficulty or performance factors and intrinsic neural-related factors such as neurodegeneration related to age or disease. This is an important consideration for MCI, which is a heterogenous syndrome with a wide spectrum of clinical impairment [44], particularly since recent studies have demonstrated divergent task-related patterns of brain activity that are dependent on clinical severity [41, 45].

The aim of this fMRI study was to investigate the effects of increasing WM load on functional brain activity in MCI, while carefully controlling for performance levels. We employed a visuospatial associative WM task that required subjects to remember pictures and their locations at 3 load levels (low, medium and high) in an event-related parametric fMRI design, which has been shown to elicit robust task- and load-related cortical changes in young healthy adults [46]. Set size for each load was individually calibrated before the scan in order to match performance level between individuals and groups. We hypothesized that differences in brain activity between an MCI group and a group of healthy control (HC) subjects would be dependent on the level of WM load presented. More specifically, the most informative changes would not be expressed at any given WM load or in any specific brain region associated with WM, but rather would reflect changes in the dynamic range from low to high load across a number of cortical regions. Importantly, by controlling performance at each load level, we can eliminate alternative explanations of our effects in terms of differential task difficulty and performance differences. We also hypothesized that load-related differences in activity would be more strongly expressed in MCI subjects who had a greater degree of clinical impairment.

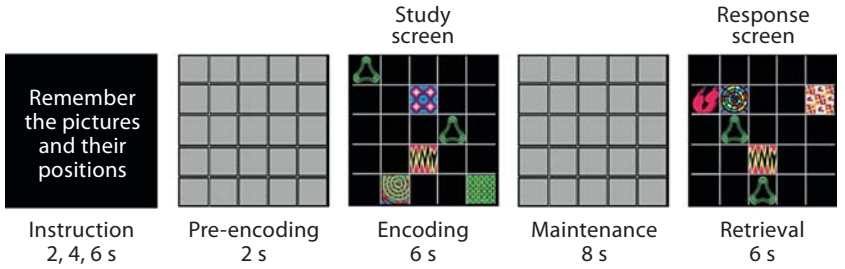


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**Fig. 1.** Paradigm sequence, stimuli and timing in a single trial. This is a schematic representation of a true-positive trial. Each box represents a trial component with duration of each (in seconds) indicated. During the study screen, targets were presented for participants to remember. During the response screen, another set of stimuli were displayed and participants indicated with a button press (yes/no) if any one of the targets were repeated from the immediately preceding study screen. Abstract designs represent target stimuli to be remembered, including their position on the grid. Curved triangular shapes represent nontarget (filler) items.

## Methods and Materials

*Participants*

Individuals with MCI (n = 35) and matched cognitively normal (n = 22) subjects were recruited from the Sydney Memory and Ageing Study, a longitudinal study of nondemented community-living older individuals. A detailed methodology of this large epidemiological study has been previously reported [47]. All participants gave written informed consent and the study was approved by the University of New South Wales Human Research Ethics Committee.

Inclusion and Exclusion Criteria

All participants were aged between 70 and 85 years, right-handed (mean Edinburgh handedness laterality index = 93; SD = 11.54) [48] and of an English-speaking background. Exclusion criteria included diagnosis of dementia (DSM-IV) [49] or a Mini-Mental State Examination (MMSE) [50] score <23, adjusted for age and education [51], psychiatric disorder, central nervous system disorder and acetylcholinesterase inhibitor treatment.

Criteria for MCI and Cognitively Normal Classifications

Participants underwent a clinical interview and comprehensive neuropsychological test battery measuring memory, language, attention/processing speed, visuospatial ability and executive function [for listing of tests see 52]. Classification of MCI was based on current international consensus criteria [53]. Participants were diagnosed with MCI if all of the following criteria were met: (a) complaint of decline in memory or other cognitive function, self- or informant-reported; (b) cognitive impairment on neuropsychological testing (≥1.5 SD below published normative values on 1 or more test measures); (c) not demented, and (d) essentially normal function in instrumental activities of daily living (IADL) on the informant-based Bayer ADL Scale (score <4.0) [54, 55]. Consensus diagnoses were made by a panel of experts including neuropsychiatrists, psychogeriatricians and neuropsychologists, based on all available clinical and neuropsychological data. The MCI group represented a mixed group of subtypes defined according to cognitive impairment profile [3] (14 amnestic single domain, 11 amnestic multiple domain, 7 nonamnestic single domain and 3 nonamnestic multiple domain). Participants were classified as cognitively normal HC if performance was ≥16th percentile (≥ –1.0 SD) on all neuropsychological measures compared to published normative values and if IADL were normal (score ≤2.0 on the Bayer ADL score). Conservative criteria for classification of cognitively normal such as this definition has high specificity over 4-year follow-up [56]. Cognitive complaint was allowable since this was very common in the Sydney Memory and Ageing Study cohort [57]. HC subjects were selected to match MCI subjects for age, sex, years of education and premorbid IQ – factors that should be controlled when investigating disease-related changes in brain activity [58].

*Task and fMRI Procedures*

Participants performed a delayed recognition visuospatial associative WM paradigm with parametric increases in load (i.e. increasing numbers of stimuli). Figure 1 depicts the events and timing of a single fMRI trial. Participants were asked to view a study screen consisting of a 5 × 5 grid on which pictures and filler items were presented. Picture stimuli consisted of abstract, multicolored designs obtained from an online database (Barbeau, E.J.: http://cerco.ups-tlse.fr/~barbeau/, accessed November 2005). Participants were instructed to remember the pictures and the positions they appeared in (targets). A delay period followed, during which a fixation mask was presented. Finally, a response screen, consisting of the grid and another set of stimuli (pictures and fillers), was presented. Participants were instructed to respond via button press (yes/no) according to whether any one of the targets were repeated from the immediately preceding study screen. For true-positive trials, both picture and position were repeated (65% of all trials). For true-negative trials, only 1 feature was repeated (correct picture in incorrect position or correct position with incorrect picture) or neither feature was repeated. True-negative trials were constructed to ensure that correct perfor-

mance was based on the association of the picture(s) and its position(s), thereby precluding use of a single-feature-based strategy. This task was part of a larger factorial paradigm with 2 other task variations: a picture task and a position task in which participants were asked to remember single stimulus features. The full factorial paradigm has previously been reported in a young healthy cohort [46].

WM Load Calibration

WM load was manipulated by altering the number of targets presented for encoding. Filler items were included in the study and response screens so that the total number of stimuli presented was always 6 items thereby holding overall visual input constant over load conditions. Individual customization of set size was conducted in a prescan calibration session in the week before the scan. Participants were administered the task, starting at 2 targets and incrementally increasing the number of targets until performance accuracy approached chance levels. Using this calibration method, we determined for each participant the number of targets to be administered during the scan in order to achieve approximately 75–85% accuracy for the medium-load condition and 60–70% accuracy for the high-load condition. This procedure was designed to ensure that all participants were equally challenged at each level of the task by controlling for individual differences in ability and minimizing potential floor effects at high load. In the low-load condition, 1 target was fixed for all participants.

Fourteen trials were run per load condition. Each load condition was performed once in a block. Load order (ascending or descending) and button press (left/right for yes) were counterbalanced across subjects. The presentation time for the instruction screen was jittered pseudorandomly to temporally decorrelate the evoked hemodynamic responses between trials. Participants received additional task practice on the day of the scan, including while lying in the scanner to ensure comprehension of task instructions.

*Imaging Protocol*

Functional T2*-weighted echoplanar images were acquired on a Philips (Achieva X) 3.0 Tesla scanner with an 8-channel SENSE head coil using an ascending slice sequence (29 axial slices, repetition time = 2,000 ms, echo time = 30 ms, 90° flip angle, matrix size = 112 × 128, field of view = 112 × 112 × 240 mm, voxel size = 2.14 × 2.73, slice thickness = 4.5 mm, 0-mm gap). A 3D T1-weighted structural MRI was also acquired coronally (repetition time = 6.39 ms, echo time = 2.9 ms, flip angle = 8°, matrix size = 256 × 256, field of view = 256 × 256 × 180 mm, slice thickness = 1 mm, 0-mm gap, 1 × 1 × 1 mm isotropic voxels).

*Image Processing and Data Analysis*

fMRI (BOLD) images were preprocessed and statistically analyzed using statistical parametric mapping SPM5 software (http://www.fil.ion.ucl.ac.uk). Preprocessing included: (i) realignment of the time series to the first image using a 6-parameter rigid-body transformation; (ii) coregistration of each individual's structural T1 image to the mean BOLD image; (iii) segmentation of the T1 into white and grey matter images and creation of a bias-corrected structural image; (iv) spatial normalization of the bias-corrected structural image and coregistered BOLD images into standard [Montreal Neurological Institute (MNI)] space (MNI/CBM avg 152 T2* template) using a 12-parameter affine transformation and resampling of the BOLD image into 3 × 3 × 3 mm isotropic voxels, and (v) spatial smoothing of the normalized images using an 8-mm full-width-half-maximum Gaussian kernel. The experiment used a mixed event-related/blocked fMRI design allowing relative temporal disambiguation of BOLD activity associated with task load and memory process (encoding, maintenance and retrieval). Statistical analysis of the time series of images was conducted using the General Linear Model [59]. Only correct trials were modeled. The model estimated 4 components of each trial: pre-encoding, encoding, maintenance and retrieval. Realignment parameters were included to account for movement-related variability. For each participant, t-contrasts on BOLD signal changes were defined for all individual events of interest by combining a single memory process and load level (e.g. encoding/low load). Group-level random-effects analyses were performed on individual subject contrast images by computing a 2-way flexible factorial analysis of variance (ANOVA) with group (MCI or HC) as a between-subjects factor and load (low, medium and high) as a within-subjects factor. Separate ANOVAs were conducted for encoding, maintenance and retrieval. F-statistics testing for the load main effect and interaction of group and load were thresholded voxel-wise using family-wise-error (FWE) correction ($p < 0.05$) to control for multiple comparisons across the whole brain [60]. Planned t tests were employed to examine directional group × load differences for significant interactions and to examine between-group differences averaged over load conditions. All T-maps were threshold at $p < 0.001$ (uncorrected) and only clusters significant at $p < 0.05$ (FWE-corrected) are reported. This threshold is comparable or more rigorous than that used in prior studies in similar populations when analyzing whole brain activity [5, 61, 62].

*Behavioral Data Analysis*

To control for a potential affirmative response bias, dPrime statistic (d′) was used to examine performance accuracy instead of total correct. d′ is estimated from measurements of the hit rate (true positives) and the false alarms providing a measure of sensitivity to the 'signal' (true-positive items). A separate group (MCI, HC) × load (low, medium and high) ANOVA was performed for each of the following behavioral measures: d′, response time (RT) and coefficient of variation (CoV), a measure of intra-individual variability of RT (the ratio of the intertrial SD to the mean).

## Results

*Participant Characteristics*

Sociodemographic, clinical and neuropsychological data are shown in table 1. The MCI and HC groups did not significantly differ on age, sex, years of education, estimated premorbid IQ or APOE genotype. As expected, MCI participants had lower scores on the MMSE ($p < 0.001$), the majority of neuropsychological tests (memory and nonmemory) and IADL ($p < 0.05$).

**Table 1.** Sociodemographic, clinical characteristics and neuropsychological test scores in MCI and HC participants

| Participant characteristics | MCI participants (n = 35) mean ± SD | Healthy control participants (n = 22) mean ± SD |
|---|---|---|
| Age, years | 77.97 ± 3.88 | 77.16 ± 3.31 |
| Sex (% male) | 14 (40.0) | 10 (45.5) |
| Education, years | 12.60 ± 3.86 | 11.44 ± 3.74 |
| NART IQ | 107.18 ± 10.30 | 109.45 ± 8.74 |
| MMSE adjusted | 27.94 ± 1.56 | 29.32 ± 0.95** |
| Bayer-ADL | 1.55 ± 0.55 | 1.25 ± 0.30* |
| APOE ε4 (% positive) | 9 (25.7) | 4 (18.2) |
| Digit-Symbol-Coding (WAIS-III) | 45.66 ± 11.00 | 57.77 ± 12.8** |
| Trail Making Test A | 45.00 ± 13.28 | 36.81 ± 13.35* |
| Trail Making Test B | 125.53 ± 69.15 | 90.45 ± 34.18* |
| Logical Memory Story A delayed recall | 7.09 ± 3.43 | 12.32 ± 3.91** |
| Rey Auditory Verbal Learning delayed recall | 5.49 ± 3.57 | 9.59 ± 2.54** |
| Benton Visual Retention Test recognition | 11.71 ± 1.71 | 12.55 ± 1.54 |
| Block Design (WAIS-R) | 20.74 ± 7.39 | 22.86 ± 7.40 |
| Boston Naming Test (30-item) | 23.17 ± 3.88 | 26.86 ± 1.81** |
| COWAT | 36.51 ± 12.71 | 42.32 ± 9.87 |
| Animal Fluency | 14.20 ± 4.14 | 18.32 ± 4.30** |

WAIS-III = Wechsler Adult Intelligence Scale, ed. 3; COWAT = Controlled Oral Word Association Test (FAS).

A $\chi^2$ test was used to compare MCI and healthy control groups for sex and APOE ε4. For the remaining variables, Student's t tests for independent samples (2-tailed) were used. * $p < 0.05$, ** $p < 0.001$.

APOE ε4 positive indicates a heterozygous or homozygous genotype. NART IQ is the estimated premorbid IQ based on the error score on the National Adult Reading Test, ed. 2. The MMSE-adjusted score includes adjustments for age and education. The Bayer-ADL score is the average score for 25 items using a 10-point scale (1 = never has difficulty and 10 = always has difficulty for the item) on the Bayer Activities of Daily Living Scale, an informant-based questionnaire developed to assess deficits in everyday activities in mild cognitive impairment or mild-to-moderate dementia.

*Behavioral Data*

Pre-Scan Calibration Performance

Group performance during the calibration session was examined for the 2- and 3-target trials since these were attempted by all participants (4 of the worst performing MCI participants did not attempt the 4-target trial) using a 2-way repeated measures ANOVA. A trend was observed for lower accuracy in the MCI group [$F(1,55) = 3.73$, $p = 0.06$]. Table 2 lists the stimulus sets (SS) administered for the scan session for each group. As expected, a larger proportion of MCI participants received a low SS (1-2-3) versus a high SS (≥1-3-4; $\chi^2 = 3.83$, $p < 0.05$).

**Table 2.** Percentage of participants receiving each stimulus set

| Stimulus numbers L-M-H | MCI participants (n = 35) | HC participants (n = 22) |
|---|---|---|
| 1-2-3 | 31.4 | 9.1 |
| 1-3-4 | 62.9 | 72.7 |
| 1-3-5 | 2.9 | – |
| 1-4-5 | 2.9 | 13.6 |
| 1-4-6 | – | 4.5 |

Stimulus numbers refer to the number of target stimuli (pictures in positions on the grid) for each stimulus set (SS). A $\chi^2$ procedure (d.f. = 4) was performed to examine group differences in SS and Fisher's exact test was used as the test statistic (since some cells had a count of <5). A trend to significance ($p = 0.051$) was observed, suggesting a relative difference between groups according to the received SS. L = Low load; M = medium load; H = high load.

Scan Performance

Mean accuracy, RT and CoV are depicted in figure 2. A strong main effect of load was found for accuracy (d′) [$F(2,110) = 73.51$, $p < 0.001$] and RT [$F(2,110) = 215.34$, $p < 0.001$]. t tests revealed significant differences be-

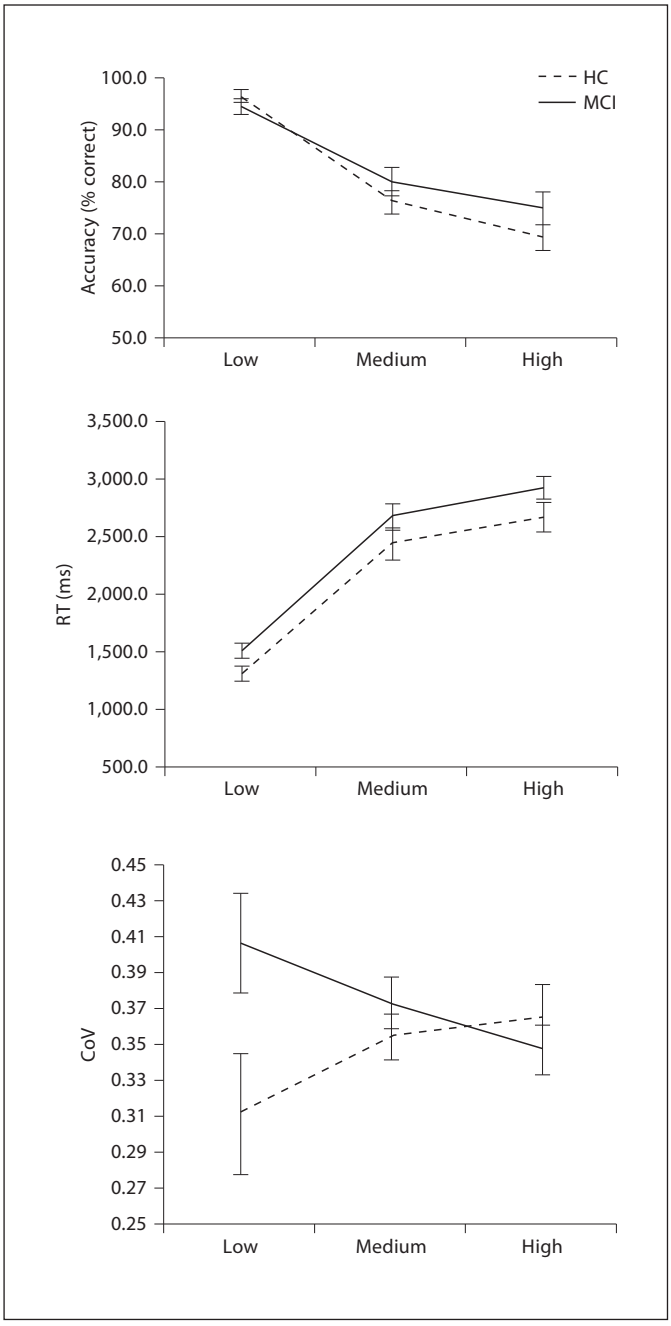


**Fig. 2.** Behavioral data for the MCI and HC groups at each load (low, medium and high): mean accuracy, mean RT and mean CoV. Error bars represent ±1 SEM.

tween each load (p values: <0.001 to <0.05) suggesting adequate differentiation between load levels. Importantly, no significant group, or group × load interaction effects were found for accuracy. A trend for longer RTs in the MCI group was observed [$F(1,55) = 3.39$, $p = 0.07$]. A significant group × load interaction was observed for CoV [$F(2,110) = 3.80$, $p = 0.05$]. As seen in figure 2, higher variability at low load and lower variability at high load was observed in the MCI compared to the HC group.

*Functional Imaging*

Main Effect of Load, and Interactions of Load and Group

We first examined the main effects of load and its interaction with group in the framework of a flexible factorial ANOVA analysis. A significant main effect for load, independent of group, was observed at encoding and maintenance, but not at retrieval. Inspection of significant activity revealed an extensive network of regions, including bilateral superior parietal lobe (BA 7), right occipital lobe (BA 18), bilateral cerebellar regions and right middle frontal lobe (BA 6), consistent with the distribution of brain regions responsive to visual WM load reported in previous studies [19, 20, 24, 25], specifically those regions that typically show increased positive load-related responses. Regions such as the bilateral parietal cortex, medial parietal cortex, cingulate and medial frontal cortex that typically demonstrate negative load-related responses in young adults [46, 63, 64] were not observed, independent of group. A significant group × load interaction was observed in the right anterior cingulate (AC) for the encoding phase, but not for maintenance or retrieval. We subsequently conducted t tests in order to explore the direction of the effects underlying this interaction which we report below.

Group × Load Interaction at Encoding

Table 3 lists regions of significant activity for the group × load interaction. A significant interaction effect was observed for the group × (high > low) contrast in the right AC (BA 24/32) and right precuneus (BA 31/7). To further explore this interaction effect, parameter estimates (β-values) for the peak voxels in each suprathreshold cluster were extracted to allow examination of the relative effect size at each load in the 2 groups. Figure 3 displays SPM maps of significant clusters and plots of the parameter estimates (from the General Linear Model) for each group over the 3 load levels for peak voxels in the AC and right precuneus clusters. As shown, greater activity at low load and lower activity at high load was observed for the MCI group and the reverse effect was observed in the HC group, in both regions. The same AC region was observed for the group × (medium > low) contrast at the uncorrected cluster threshold ($p = 0.014$, uncorrected). A

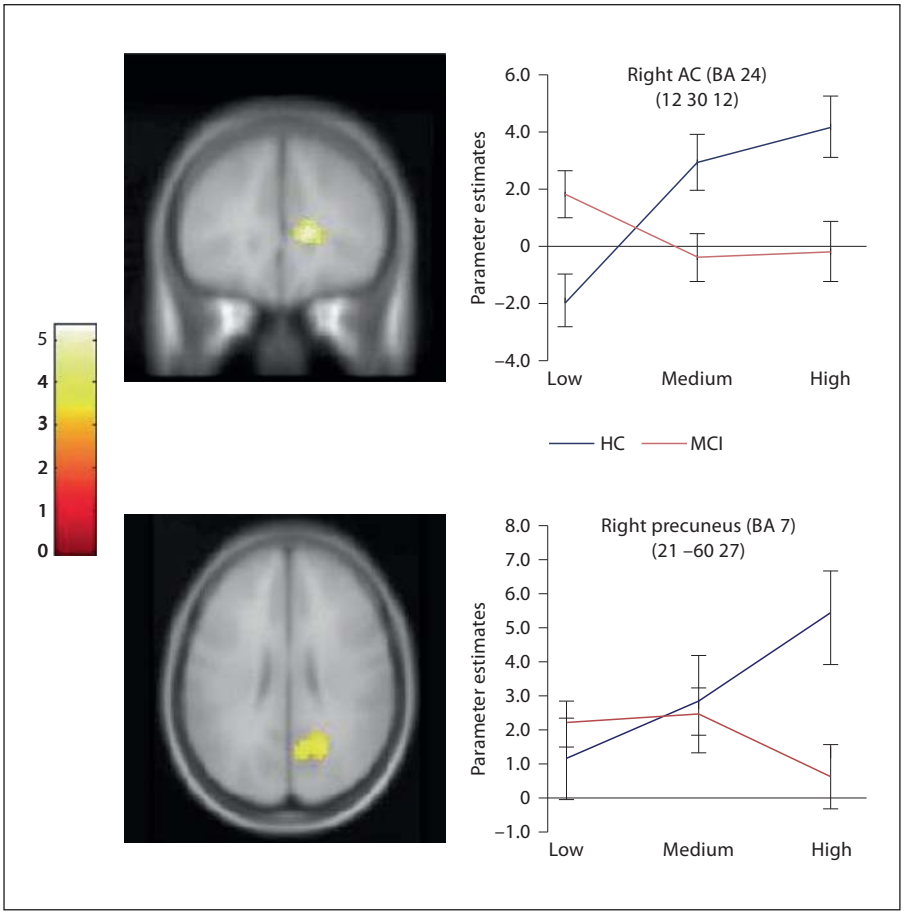


**Fig. 3.** Comparison between the HC and MCI groups as load is increased from low to high during encoding of a WM task. Compared with HC, MCI subjects showed decreased activity in the right AC and right precuneus as load was increased. Left side of panel: significant activity shown on the SPM high-resolution averaged T1-weighted image using a cluster-defining whole brain threshold of $p < 0.001$ and FWE ($p < 0.05$) cluster correction. The images are oriented in standard neurological view (right hemisphere is depicted on the right side of the image). Right side of panel: plots of the mean parameter estimates (β-coefficients) are depicted for the 2 groups (MCI, HC) at each level of WM load (low, medium and high) for peak voxels of the regions shown.

**Table 3.** Regions of significant activity for the group × load interaction at encoding

| Brain region | MNI coordinates | | | | | Cluster size | Brodmann area |
|---|---|---|---|---|---|---|---|
| | right/left/medial | x | y | z | T value | | |
| (HC > MCI) × (high > low) | | | | | | | |
| AC | R | 12 | 30 | 12 | 5.05 | 65 | 24 |
| Precuneus | R | 12 | –60 | 24 | 4.06 | 87 | 31 |
| | R | 21 | –60 | 27 | 4.02 | | 7 |
| (HC > MCI) × (high > medium) | | | | | | | |
| Posterior cingulate | M | 9 | –57 | 27 | 4.16 | 115 | 31 |
| Precuneus | M | 6 | –69 | 24 | 3.85 | | 31 |

List of significant clusters for group × load interactions at the encoding phase. The contrasts (HC > MCI) × (medium > low), (MCI > HC) × (medium > low), (MCI > HC) × (high > low) and (MCI > HC) × (high > medium) did not reach statistical significance. Standardized MNI co-ordinates represent peak voxels of significant clusters (FWE-corrected threshold). Cluster size values are listed for the primary peak only; secondary peaks from the same cluster are listed immediately underneath. Approximate Brodmann areas for peak voxels are listed.

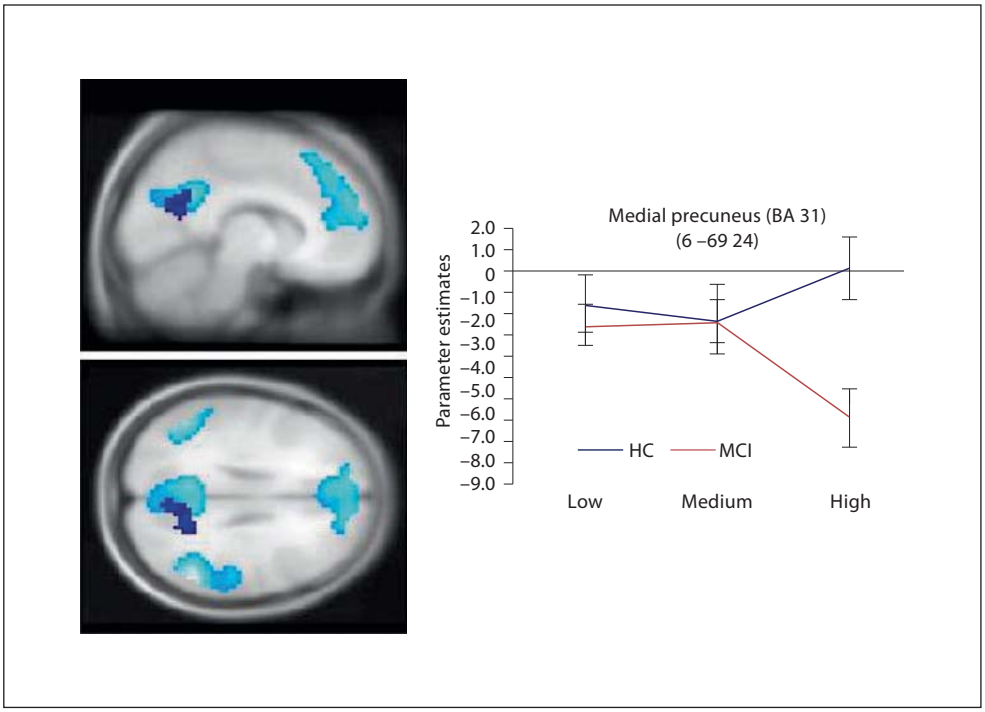


**Fig. 4.** Comparison between the HC and MCI groups as load is increased from medium to high load during encoding of the WM task. Compared with HCs, MCI subjects showed increased deactivation in a region of medial precuneus and posterior cingulate as load was increased. Left side of panel: significant activity (shown in dark blue) is superimposed on a conjunction map (light blue) of a larger set of regions that was commonly deactivated by both groups during task performance. This figure is used for illustrative purposes to show the area of overlap between the posterior region that was significant for the group × load interaction and a distributed network of regions that represents the DMN. Significant activity shown on the SPM high-resolution averaged T1-weighted image using a cluster-defining whole brain threshold of $p < 0.001$ and FWE ($p < 0.05$) cluster correction. Right side of panel: plots of the mean parameter estimates (β-coefficients) are depicted for the 2 groups (MCI, HC) at each level of WM load (low, medium and high) for a peak voxel of significant posterior cluster (shown in dark blue).

significant interaction was also observed for the group × (high > medium) contrast in a cluster comprising the posterior cingulate and medial precuneus, an area reported to be a component of the DMN [29]. Voxels exhibiting this effect are depicted in dark blue on the brain map in figure 4. The accompanying plot of parameter estimates shows that a larger deactivation (negative) response was observed for the MCI group compared to the HC group as the load increased from medium to high. To investigate whether this interaction effect was present in regions suppressed during task activity, we repeated the analysis using a task-negative mask consisting of regions deactivated during task performance at encoding in both groups. This was created by performing a conjunction analysis [65] on a contrast that defined a relative task-negative effect (compared to implicit baseline) across all load conditions for each group and is shown in light blue in figure 4. It resembles the well-established default network of regions [see examples in 66, 67]. For illustrative purposes, the regions of overlap for the group × load interaction and task-negative activity during encoding are depicted in figure 4. As can be seen, a large fraction of the original cluster lies within this task-negative mask and we also obtained a strong effect (FWE cluster corrected) when the original contrast was restricted to lie within this mask. Interestingly, the precuneus appears to have 2 subregions that are functionally distinct despite the anatomical proximity. In figure 4, the medial precuneus cluster demonstrates task-related negative responses, while the more lateralized precuneus cluster depicted in figure 3 demonstrates task-related positive responses. The former will be referred to as the medial precuneus and the latter as the right precuneus to distinguish between them.

No regions were identified that displayed increased positive load-related responses for the MCI relative to the HC group.

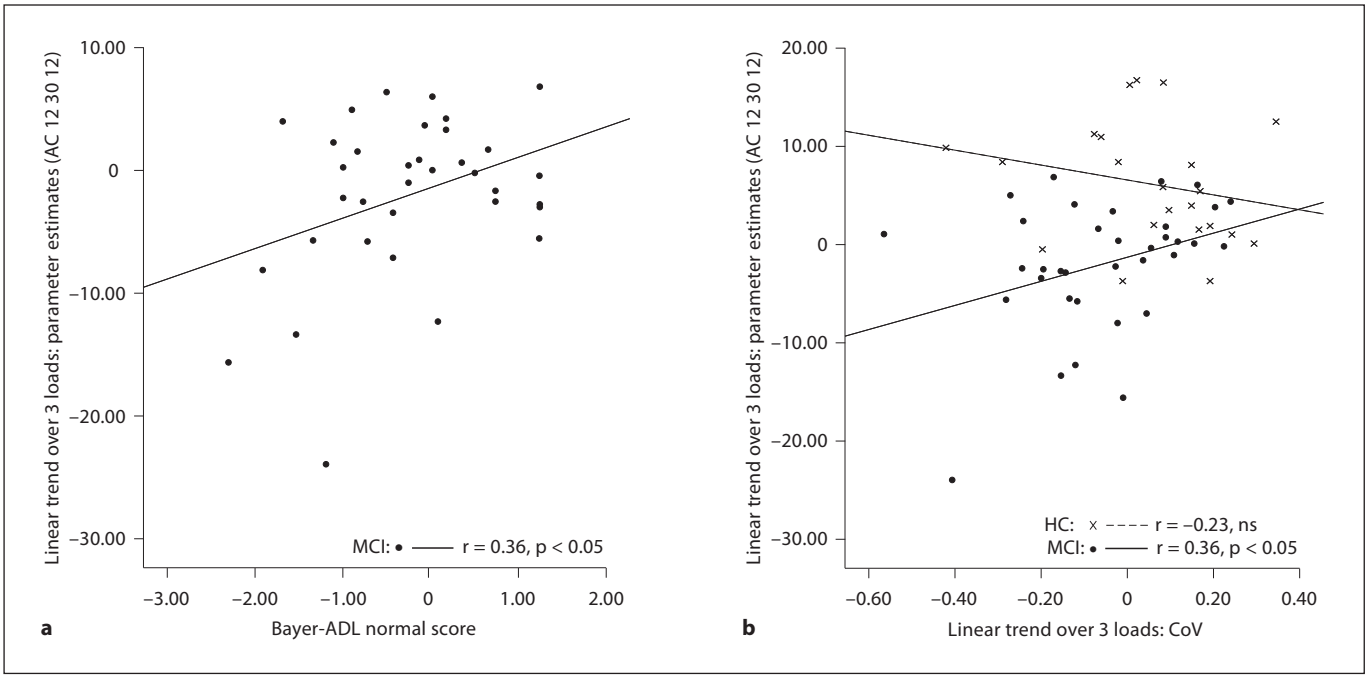


**Fig. 5.** **a** Plot depicts correlation between linear trend in parameter estimates (β-coefficients) over 3 loads in the right AC (y-axis) and the normalized Bayer-ADL score (x-axis) in the MCI group. Scores on the Bayer-ADL scale were reversed so that a higher score indicates a better performance. Normal scores represent z values of the standard normal distribution; scores below zero represent the lower half of the distribution and scores above zero represent the upper half. Values on the y-axis represent the linear change in parameter estimates from low through to high load in AC; scores below zero represent a decrease in brain response and scores above zero an increase in brain response, as load is increased. In the MCI group, individuals with relatively worse IADL function showed a greater decline in the AC response as WM load increased in comparison to those with better IADL function. **b** Plot depicts correlations between the linear trend in parameter estimates over 3 loads in the right AC (y-axis) and the linear trend in CoV values (x-axis) over 3 loads for each group. Negative values represent a decline and positive values an increase from low to high load in AC response (y-axis) and CoV (x-axis), respectively. In the MCI group, individuals who showed greater decline in performance variability also had a greater decline in response in AC as load increased. In the HC group, there was no significant association ($p > 0.1$) between change in CoV and change in AC response with increased load.

Group Differences at Retrieval

Although our main interest was in the interaction of group and load, we also examined possible group differences independent of load at all 3 phases. A significant group effect independent of load was detected at retrieval, but not for the other 2 WM phases. Reduced activity in the MCI relative to the HC group was observed in 2 regions during retrieval: the right lateral parietal lobe including the postcentral gyrus (BA 3, BA 4) and inferior parietal cortex (BA 40; peak voxel: 48 –21 39; t = 4.19; cluster size = 122; p = 0.002 FWE-corrected), and the medial parieto-occipital cortices including the medial precuneus (BA 31) and cuneus (BA 18; peak voxel: 3 –66 21; t = 4.33; cluster size = 156; $p < 0.001$ FWE-corrected). There were no areas of significantly increased activity for the MCI group relative to the HC group at retrieval.

In order to ensure that differences in brain activity observed between the groups were not due to the size of the stimulus sets received, we conducted supplementary analyses with 3 groups: (i) HC subjects who received sets equal to 1-3-4 or higher, (ii) MCI subjects who received the same sets as HC groups and (iii) MCI subjects who received a reduced set. Similar between-group effects remained present for comparisons of the HC group with both MCI groups separately, although the statistical strength of these effects was diminished, consistent with the loss of power due to the smaller numbers in the downsampled groups.

Correlation of Brain Activity and Clinical Severity in MCI Individuals

In order to examine the relationship between clinical severity and brain activity under conditions of WM load, correlational analyses were performed between scores on clinical measures (MMSE and Bayer-IADL) and brain responses in the regions that had maximally displayed the group by load interaction effect. For this analysis, change scores were calculated between parameter estimates (betas) at the relevant loads, namely 'high minus low load' and 'high minus medium load', and normal scores were computed for the clinical measures using Blom's procedure [68] since they were not normally distributed. The linear change in parameter estimates in the right AC over increases in load (low to high) was significantly correlated with ratings on the IADL scale ($r = 0.36$, $p < 0.05$; fig. 5). MCI individuals with relatively worse IADL function showed a greater decline in AC activity as WM load increased in comparison to those with better IADL function. Change in parameter estimates in the medial precuneus (6 –69 24) over increases in load (medium to high) was significantly associated with performance on the MMSE ($r = 0.34$, $p < 0.05$). A trend was observed in the posterior cingulate (9 –57 27; $r = 0.32$, $p = 0.06$). More impaired individuals with MCI showed greater deactivation in the medial posterior regions as the load was increased from medium to high in comparison to those who were less impaired. No significant correlations were observed between the right precuneus and either clinical measure.

*Post-Hoc Analysis*

Correlation of Brain Activity with RT Variability

RT variability was the only in-scanner performance measure that significantly varied between the groups as a function of load ($p < 0.05$). Specifically, higher variability was observed at low load and lower variability at high load for MCI subjects, a reverse pattern to that evident for HC subjects. This finding motivated post-hoc examination of the association between behavioral and brain responses under conditions of increased load. Correlations were calculated between the linear trend in CoV values and the corresponding linear trend in β-values (from the peak voxels of clusters significant for the group × load interaction at encoding) over 3 levels of load, for subjects within each group separately. In the MCI group, significant positive correlations were found for CoV and brain responses in the AC ($r = 0.34$, $p < 0.05$; fig. 5b), right precuneus (21 –60 27; $r = 0.35$, $p < 0.05$) and posterior cingulate ($r = 0.34$, $p < 0.05$). Thus, individuals with MCI who had the greatest decline in RT variability as load increased were those that had the greatest decline in brain responses. No correlations were significant in the HC group.

## Discussion

To our knowledge, the present study is the first to investigate the effects of graded increases in WM load on whole-brain patterns of activation and deactivation in MCI. Consistent with our initial hypothesis, even in the absence of differences in task performance, cortical activity during WM differed between HC and MCI subjects as a function of WM load across a number of brain regions. This effect was observed during the encoding phase of the task. At lower loads, MCI subjects more strongly engaged regions in the right AC and right precuneus, in comparison to cognitively normal elders who engaged these areas to a greater degree at higher load. Conversely, at higher load these frontal and parietal regions were underactivated and a posterior cingulate-medial precuneus region more strongly deactivated in the MCI group relative to the HC group. Furthermore, consistent with our 2nd hypothesis, changes in AC activation and posterior cingulate-medial precuneus deactivation over increasing WM load were related to clinical severity in individuals with MCI. Interestingly, brain activity was also found to be associated with performance variability in the MCI group under conditions of increased load. In comparison, group differences observed at retrieval were not modulated by load.

The observed interaction between group and WM load may help clarify previous contradictory results reported from fMRI studies using a range of tasks including WM, episodic memory and other cognitive abilities. The typical approach involves the presentation of a single task condition which is compared against rest or a baseline condition, to both the MCI and the healthy comparison group. Using this type of method, previous findings have been variable, with both increased activity [6, 62, 69, 70] and decreased activity [40, 61, 71–73] reported in MCI subjects. Our findings suggest that variability amongst these studies may be due (at least in part) to differing task demands, particularly in relation to the task difficulty experienced by subjects in each group. In the present study, when task difficulty was controlled for, by equating task performance between groups, we observed increased activity in the MCI group

in the context of the relatively easier low-load condition, but decreased activity in the context of the more challenging high-load condition. By parametrically increasing load across a range of levels for both MCI and control subjects, this study has provided the opportunity to observe a more dynamic pattern of cortical activity in MCI rather than simply a fixed response to a single task condition.

Healthy elderly subjects responded to increasing WM demands during the encoding phase by increasingly engaging relevant WM-related regions in the AC and right precuneus (fig. 3). In contrast, the individuals with MCI engaged these regions maximally at the lower loads and responses declined thereafter. The AC is known to subserve multiple executive functions including control of attention and performance monitoring [74]. Previous studies have found increased activity in this region at high WM loads [75] and with greater task difficulty [76]. The right precuneus is engaged when WM tasks make higher executive demands and also has a more general function in spatial attention [77]. Our findings suggest that in MCI, engagement of specialized cognitive processes mediated by the AC and right precuneus that are typically engaged by healthy individuals at a higher WM load or in response to higher executive demands may already be required at lower task demands, but are not sustained as task demands increase.

When the load was increased to the highest level, MCI subjects more strongly deactivated an area of the posterior cingulate and medial precuneus relative to controls (fig. 4). Further investigation found this area overlapped with the medial posterior portion of a larger distributed set of regions commonly deactivated by both groups during task performance, and was thus identified as the DMN. While strong negative responses were observed throughout this network during task performance, deactivation was not strongly dependent on load given these regions were not present in our general (group-independent) load-related contrasts during encoding or maintenance. It is therefore not entirely clear whether the greater deactivation observed in MCI subjects in this small posterior region represents an extension of the normal physiological role of the DMN.

In addition to the load-mediated effects at encoding, we observed a load-independent decrease in activity in lateral and medial parieto-occipital cortex in the MCI relative to the HC group at retrieval. These findings are consistent with a study that found reduced activity in an MCI group in medial parietal regions during recognition of previously learned items in comparison to a group of healthy elders [72]. These medial parietal regions that are actively engaged during memory retrieval [22, 78] overlap with the posterior regions of the DMN in young healthy adults [79]. Our findings of functional alterations in posteromedial regions in individuals with MCI during both encoding and retrieval stages of the task suggest there may be a primary dysfunction in this anatomical region in MCI. Indeed, there is converging evidence from multiple imaging methods, including fMRI (both resting state and with a cognitive task) [e.g. 36, 40], [$^{18}$F]fluorodeoxyglucose positron emission topography [e.g. 80], volumetric structural imaging [e.g. 81] and more recently β-amyloid imaging [e.g. 82], that abnormalities in the posteromedial cortex are present in the early stages of AD and MCI. Moreover, abnormal function in this part of the brain has been found to predict progression from MCI to AD [7]. This suggests that the patterns of abnormality observed during performance of our WM task may be of some prognostic value.

MCI is a heterogenous group, both in terms of profile of cognitive impairments and its prognosis; therefore, we expected a degree of variance in the expression of brain activity changes as a function of load among the individuals in the MCI group. Consistent with our prediction, patterns of brain activity were significantly associated with measures of clinical severity in the absence of task performance differences. In the MCI group, those individuals who had greatest decline in AC activity over graded increases in load were rated as having more difficulties in performing IADL. Even mild IADL restrictions appear to be predictors of cognitive decline or dementia [83–85], hence the association of brain abnormalities in AC and poorer IADL function may be indicative of more advanced disease. Additionally, individuals with MCI with the strongest deactivations in the posterior medial cortex as the load was increased to the highest WM demand performed more poorly on the MMSE, a measure of global cognitive performance used to stage cognitive impairment.

Two recent reports have demonstrated that activation follows a nonlinear trajectory across the MCI-AD continuum with increased activation in groups of less severely impaired individuals and reduced activation in groups at the more severe end of the MCI spectrum [41, 45]. One study also observed a corresponding pattern in deactivation responses [41]. In our study, however, clinical severity influenced alterations in brain activity in association with graded changes in WM load, thus our findings are not directly comparable. Furthermore, a number of methodological differences make comparisons difficult.

Nevertheless, our study highlights an important relationship between brain activity and clinical severity in MCI, independent of potential confounds of differences in task performance.

Our calibration method was successful in matching task accuracy between the MCI and the HC groups, but the groups did differ on a measure of performance variability (CoV). The greater performance variability in MCI is consistent with previous literature on MCI [86–88] and AD [87, 89]. Interestingly, variability decreased at the higher load in MCI subjects while the reverse pattern was observed for healthy elders (see fig. 2), with a similar interaction effect between group and load being observed during the encoding phase (see fig. 3). In the MCI group, stronger expression of this abnormal behavioral pattern (i.e. higher performance variability at lower loads that diminished with increased load) was related to greater decline in activation in the AC and right precuneus and greater deactivation in the posterior cingulate, as a function of load. Measures of performance variability have been shown to be sensitive to the severity of cognitive impairment across the clinical spectrum of MCI to AD [87, 88], hence we propose that the observed association with brain activity in MCI suggests that patterns of activation and deactivation elicited by graded increases in WM load may serve as potential neural markers of disease.

An interpretation of abnormal brain activity advanced in the MCI literature is the concept of development of compensatory networks. For the most part, this has been used to explain hyperactivity observed in the hippocampus [e.g. 6] or other cortical regions [70]; however, greater deactivation in parts of the DMN has also been recently reported in this light [41]. Here, we observed increased deactivation in our MCI subjects in a region known to serve as the principal node of the DMN [29]. In order to perform the more challenging load, MCI subjects may require more general cognitive resources leading to greater deactivation of default activity. This could therefore represent a compensatory mechanism to overcome deficient functioning in other task-related regions. However, compensatory brain activity is advanced as an adaptive neural response that modifies the relationship between disease and function, thereby allowing maintenance of function in the face of accumulating neuropathology. Without concurrent measures of disease burden, it is not possible to determine whether this pattern of brain activity can be genuinely considered compensatory. For example, the observed increased deactivation in the posterior cingulate-medial precuneus could equally reflect a primary pathophysiological process rather than a secondary adaptive response. This latter explanation is more consistent with our findings of diminished activity in posteromedial regions normally engaged during the retrieval phase, and also with evidence from several imaging studies that have reported primary abnormalities in this region. Moreover, the associations with greater clinical severity and performance variability support the hypothesis that this region is dysfunctional. The addition of measures of disease burden in a longitudinal design are therefore required to disambiguate these competing explanations.

The majority of fMRI studies have used samples of amnestic MCI patients from hospital memory clinics. Our sample consisted of volunteers recruited from a population-based study of ageing. The classification of MCI included all 4 subtypes since in population studies a broad definition of MCI has been found to have the highest positive predictive power for progression to dementia as compared to classifications based on MCI subtypes, which were found to be less stable [90–92]. Given that our sample of MCI subjects was recruited from the community and therefore likely to have consisted of fairly mild cases, the robust group differences in brain activity observed in this study are particularly noteworthy.

There are some limitations to this study. First, a general limitation to the calibration approach used is that scan performance did not always match pre-scan testing, and both groups equally improved their performance in the scanner, particularly for the high load, most likely due to practice effects. Nonetheless, we still observed concomitant changes in brain activity over the 3 loads. Most importantly, we achieved equal task performance in both groups by ensuring that all individuals were equally challenged at each load and intersubject variance in the MCI group was minimized. Second, it has been reported in other studies that alterations in deactivation response in MCI [38] and AD [38, 39] are present in the early phase of the fMRI signal. We used the standard hemodynamic response function which is not as sensitive to temporal change in deactivation and may have missed additional group differences in deactivation. Third, we did not observe any group differences in hippocampal activation, although previous studies have shown that the hippocampus is responsive to increased load during WM performance [21–23], and there is evidence that medial temporal lobe structures, such as the hippocampus, are the most vulnerable to early AD-related neuropathological alterations [93]. We

acknowledge that we did not optimize the fMRI acquisition sequence for detection of signals in the hippocampus as we were more interested in measuring dynamic activity changes across the whole brain. Our findings do suggest that activity differences in other cortical regions may be better able to discriminate between healthy older adults and those with MCI during performance of a WM task. Lastly, we are aware that although the intertrial period was jittered in order to decorrelate consecutive trials, we did not jitter the components of a single trial. Thus, there is a theoretical potential for a carryover effect in the BOLD signal between encoding, maintenance and retrieval. We therefore adopted a cautious approach of informally reporting differences observed within phases of the task (encoding, maintenance and retrieval), but did not perform direct contrasts between them. Importantly, linear correlations induced by the low-pass filter effect of the hemodynamic response function would tend to obscure genuine differences between cortical responses in these different phases, rather than inflate or artificially create the differences that we have reported.

In conclusion, we have demonstrated that presentation of a graded WM task may operate like a memory stress test [94, 95] by eliciting abnormal patterns of brain activation and deactivation in individuals with MCI over incremental increases in WM load. Stronger expression of abnormal load-related patterns of activity in the MCI group was associated with greater clinical severity and may be indicative of more advanced disease and possibly higher risk of progression to dementia. Determination of the prognostic value of this type of fMRI paradigm awaits longitudinal follow-up of our participants. Our findings suggest that if such memory stress tests are to be developed, it is important to examine brain activity over different levels of task difficulty and ensure that all participants are equally and sufficiently challenged, irrespective of the cognitive task used.


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