Altered Brain Activation During a Verbal Working Memory Task in subjects with Amnestic Mild Cognitive Impairment

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Abstract

In subjects with Mild Cognitive Impairment (MCI) memory disorders indicate a high risk for conversion to Alzheimer's disease (AD). The objective of this study was to delineate the differences in brain activation between amnestic MCI and age-matched healthy controls (HC) during a verbal working memory task. The verbal working memory task was a delay match to sample design. Brain activation was measured using functional magnetic resonance imaging. There were 8 subjects in each group and were matched for performance. The task was analyzed as an event-related design. Group differences were calculated using Analysis of Covariance (ANCOVA) with statistical significance at $p < 0.05$ corrected. Both groups activated a wide network in the posterior and frontal areas of the brain. There was higher activation in the parietal and frontal lobes in the MCI compared to the HC during the maintenance phase. There were no areas in the HC that activated higher than the MCI subjects. Response time in the task in the HC group was correlated to the left hippocampus during encoding phase and to the parietal and frontal areas during the recall phase. In the MCI group there was strong correlation to the inferior and middle temporal gyrii during encoding, the middle frontal gyrus during the maintenance phase, and hippocampus during recall phase. The activation differences between groups may be compensatory mechanisms within the MCI group for the effects of the putative AD neuropathology. This has been the first study that has examined verbal working memory in MCI.

1. Introduction

Alzheimer's disease (AD) is one of the most common psychiatric disorders in older subjects and the first clinical symptoms are in the memory domain. One of the high risk groups for Alzheimer's disease are subjects with Mild Cognitive Impairment (MCI) [1], a group that has cognitive impairments in memory or in other domains but the severity of impairment does not meet the criteria for AD. Within the MCI group there are various subgroups, with the amnestic Mild Cognitive Impairment (aMCI) group characterized by a single memory disorder [2]. This group is at higher risk for converting to AD compared to healthy subjects and other MCI subtypes such as non-amnestic MCI [2-7].

Working memory (WM), the ability to hold information in memory while performing another mental operation [8], is a key cognitive domain important for higher cognition and is impaired in MCI subjects [9] and AD patients [10]. WM tasks recruit a network of regions that include bilateral frontal and parietal regions, as well as the cingulate cortex, and cerebellum in young healthy subjects [11-13] and older healthy subjects [14-15]. WM has also been investigated in MCI and AD patients with both groups recruiting alternate networks compared to the HC. For example using the n-back WM task in MCI subjects Goekoop and colleagues [16] found a network of activation that included the bilateral parietal lobes and prefrontal cortex with the extent of activation increasing in the 2-back compared to 1-back WM task. Another study that examined a verbal 0-, 1-, and 2-back WM tasks found that the MCI had reduced activation compared to HC bilaterally in the parietal and frontal regions [17]. A recent study found that aMCI and HC in a 2-

back WM task using visually presented emotional stimuli activated a wide network including bilaterally the parietal and frontal lobes with a group by emotion interaction effect in the left cuneus, where the HC group had lower activation for the positive valence stimuli compared to the neutral valence stimuli [18]. The aMCI group revealed a signal increase in the right precuneus for negative compared to neutral valence stimuli.

The alterations in brain activation described previously in the MCI and AD groups reflect compensatory mechanisms in the brain. The level of compensation may be an index of disease progression, for example, Celone and colleagues found that a mild MCI group compensated with higher activation in the hippocampus but in the more advanced MCI group the activation in the hippocampus decreased compared to the mild MCI [19]. Disease severity can also be quantified by measurement of the hippocampal volume, for example there was increased recruitment of the posterior hippocampus and fusiform gyrus in the MCI compared to the HC during an associate encoding task, with the increased recruitment linearly correlated to the atrophy of the hippocampus in the MCI group [20]. In an encoding task with visual stimuli, the aMCI subjects had higher activation in right parahippocampal gyrus compared to HC and those that showed the greatest activation in this region within the aMCI group, had the greater cognitive decline within the following 2.5 years [21]. Compensatory mechanisms have been found not only in memory but also in a visual perception task in MCI subjects [22]. In addition in a study of AD patients there was increased recruitment of frontal lobe regions for performance of short term tasks and the recruitment of frontal regions was associated with performance of the task [23-24].

To further investigate the brain compensatory mechanisms in MCI subjects, we used a delay-match-to-sample (DMTS) working memory task, which has not been investigated in MCI subjects. We hypothesized that the aMCI group would have higher activation, indicative of a compensatory mechanism compared to the HC group. The stimuli were letters and brain activation was measured using functional magnetic resonance imaging (fMRI). The design of the DMTS task allowed for quantification of the different phases of the working memory task.

2. Methods

2.1 Subjects

There were 5 males and 3 females in the HC group and 6 males and 2 females in the MCI group, with an average age ± standard deviation of 66.6 ± 3.9 (age range 60 to 71) and 70.8 ± 5.3 (age range 63 to 76) in the HC and MCI subjects, respectively (neuropsychological profiles in Table 1). All subjects were right handed. The MCI subjects were recruited from a specialized memory problems unit at a university hospital. The clinical assessment included detailed medical history, neurological and neuropsychological examinations, and laboratory tests (routine hematology and biochemistry screen, thyroid function tests). Major systemic, psychiatric, or neurological illnesses were carefully investigated and excluded in all subjects by clinical and neurological examinations, blood testing (complete blood count, sedimentation rate, electrolytes, glucose, blood urea nitrogen, creatinine, liver-associated enzymes, cholesterol, high-density lipoprotein, triglycerides, antinuclear antibodies, rheumatoid

factor, HIV, serum B12, folate, thyroid function tests, and urine analysis), and psychiatric examination. Subjects were excluded if they had cortical infarction, subcortical vascular disease, space-occupying lesions, depression, and any other psychiatric or neurological disease. FLAIR and T2 weighted scans were utilized to rule out vascular pathology (clinical judgment by radiologist).

The diagnostic criteria [25-26] were (a) memory impairment for the age and education of the subject, (b) memory impairment was corroborated by a close family member, (c) relatively preserved cognition outside of memory domain for age (fulfilled by the various subtests in the Consortium to establish a Registry for Alzheimer's Disease battery (CERAD) [27], (d) no impairment in activities of daily living, (e) no dementia. Clinical judgment was utilized to determine whether there was impairment in activities of daily living. The patients were systematically evaluated for the presence of affective symptoms, particularly depression and if any symptoms appeared that might have indicated the possibility of depression the subject was evaluated with a Hamilton Test (21 item version) and any subject with a score higher than 7 was excluded; none of the MCI subjects had depression. The threshold for determining a cognitive impairment was 1.5 standard deviation below the age norms [28-29] in the CERAD neuropsychological test battery. The diagnosis of the MCI subjects was established through consensus among the responsible psychiatric consultants (SJT, MO and HH). In particular, none of the MCI subjects could be classified as AD using standard clinical criteria [30].

The HC were recruited from the community, did not have an active neurological or psychiatric illness, or an illness that could affect cognitive function, and were independently functioning members of the community. The HC did not complain about cognitive problems, and there was no evidence of cognitive deficits as measured by neuropsychological testing using the CERAD [27]. If there was possible presence of depression, the Hamilton Test (21 item version) was performed and any subject with a score higher than 7 was excluded.

Subjects were excluded on MRI criteria such as pacemaker implant, recent metallic implants and claustrophobia. All subjects had normal vision or corrected by use of MR-compatible eyeglasses. All subjects gave written informed consent to participate in the study after the study was explained to them. The study was performed in accordance with the Declaration of Helsinki and the Ethics Committee of the Faculty of Medicine at Ludwig-Maximilian University approved the study.

2.2 Stimuli and tasks

The WM task was composed of an encoding phase, a delay phase, and a recall phase (see Figure 1). The encoding phase was 4 seconds long and 5 capital letters were presented. The maintenance phase was 6 seconds long and a fixation cross was presented in the center of the screen. After 6 seconds one single lower case letter appeared, for which the subjects were to decide if it was among the 5 letters they had seen in the encoding phase. The subject indicated a positive answer by pressing a button in the right hand with the right index finger and a negative answer by pressing a button in the left hand with the left

index finger. No vowels were included among the letters utilized so as to minimize the probability that the subjects would encode the letters as a word or pseudoword.

Each subject performed three runs of the working memory task. Each run contained 3 blocks of working memory (50 seconds per block) and each block had 3 trials. Each subject performed 27 trials. The working memory trials alternated with four resting state blocks (20 seconds per block). Before each block, the subjects saw a short instruction for 10 seconds.

The task was explained to the subjects using examples on paper and once understood they were shown a short demonstration version of the task on a computer. The stimuli were presented using VAPP [31].

2.3 Scanning

The imaging sequence was an interleaved T2* weighted echoplanar (EPI) sequence with 28 axial slices (4 mm slice thickness and slice gap = 1 mm, repetition time (TR) = 3.60 s, echo time (TE) = 60 ms, flip angle = 90º, field of view = 240 mm. Matrix = 64 x 64) and 87 volumes acquired per run (each volume was measured in 2.8 sec with 0.8 sec gap between volumes) on a 1.5 Tesla Siemens Magneton Vision scanner (Erlangen, Germany). For anatomical reference in each subject a high resolution T1-weighted 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) structural image was acquired (TR = 11.4 ms, TE = 4.4 ms, flip angle = 8º, FOV = 270 mm, matrix = 224 x 256, Rect. FOV = 7/8, Effective Thickness = 1.25 mm).

2.4 Data Analysis

The data was analyzed on an Intel Pentium III computer (San Jose, California, USA) running Linux (Red Hat version 7.0, Red Hat Inc, Raleigh, North Carolina, USA) using AFNI [32] and FSL [33].

The initial step was to delete the first 4 volumes of each scan to remove the initial T1 magnetic transients. The remaining data were corrected for the timing differences between each slice using Fourier interpolation and then corrected for motion effects (6-parameter rigid body) (programs utilized were within the AFNI software package unless specifically noted). The structural images were utilized to convert the EPI data from native space to Montreal Neurological Institute/International Consortium for Brain Mapping 152 standard (MNI/ICBM) space. To accomplish this, the structural images were first edited of the non-brain tissue using BET from the FSL software package. The EPI images were co-registered to the MPRAGE image (6 parameter rigid body), and the MPRAGE image was registered to the MNI/ICBM template (12 parameter affine) using FLIRT from the FSL package. The transformation matrices were utilized to convert the EPI data from native to MNI/ICBM stereotaxic space. The data were smoothed (Gaussian filter at full width at half maximum = 6 x 6 x 6 mm) and high pass filtered with a cutoff at (1/100) Hz.

The event related design of the task allowed us to match the performance between the two groups. The activation maps and comparison were based on the trials that were

successfully encoded and the subjects were matched for performance. In addition, not only were the activation maps and comparisons based only on the successfully encoded trials, but also the same number of trials was included in the regressor for calculation of the activation maps. For example, if an MCI subject responded correctly to 7 trials in a run and a HC to 8 trials in the run, the regressor for the "correct trials" included 7 trials in the MCI and 7 trials in the HC group. The additional correct trial in the HC group was modeled by another regressor. Thus in this way, the activation maps in both groups included the same number of correct trials in both groups and the correct response rate was matched.

Each run for each subject was analyzed using a fixed effects general linear model using AFNI. Each model was composed of the regressor modeling the encoding phase, the delay phase, and the matching phase. These three regressors were for the correctly performed trials and not-correctly performed trials for a total of 6 regressors. If needed there were additional regressors (one for each phase) for the correctly performed working memory trials that were not required for performance matching with the subject in the other group (see previous paragraph). There were additional regressors for the instruction before each block, and the fixation period between blocks. The regressors were square wave-forms (on-off) which were convolved with a standard double gamma hemodynamic response function.

The group activation statistical analyses maps were based on a mixed effects model with a voxel wise threshold of $p < 0.01$ and was corrected for multiple comparisons at the $p <$

0.05 level using Monte Carlo simulations. The simulation included the process of image generation, spatial correlation of voxels, voxel intensity thresholding, masking and cluster identification. Based on the combination of individual voxel probability thresholding and minimum cluster size thresholding, the probability of a false positive detection per image is determined from the frequency count of cluster sizes. The models for obtaining the activation maps of the functional tasks compared to the control task was the one sample t-test. To compare the activation between groups an analysis of covariance (ANCOVA) was utilized with group membership as the random variable and age as covariate. The ANCOVA analysis consisted of three parts: an analysis of linear correlation of the activation with age was performed, which shows where significant activity was correlated with subjects' age; the actual analysis of differences in activation of HC and MCI patients; the analysis of interaction between the age effect and the difference between the two groups.

A conjunction overlay was performed to delineate area of common activation in the HC and MCI groups. The individual activation analysis maps (for each phase of the WM task) of each group were thresholded at a voxel level of $p < 0.01$ and corrected to $p < 0.05$.

In addition, it was examined if performance (response time) in each group was linearly associated with brain activation in the three phases of the working memory task. Thus a linear regression model was utilized with performance as independent variable and brain

activation as dependent variable. Statistical significance was tested using a t-test, with voxel level significance at $p < 0.01$ and cluster level at $p < 0.05$ level.

Structural data was analysed with FSL-VBM, a voxel-based morphometry style analysis [34-35] carried out with FSL tools [33]. First, structural images were brain-extracted using BET [36]. Next, tissue-type segmentation was carried out using FAST4 [37]. The resulting grey-matter partial volume images were then aligned to MNI152 standard space using the affine registration tool FLIRT [38-39], followed optionally by nonlinear registration using FNIRT, which uses a b-spline representation of the registration warp field [40]. The resulting images were averaged to create a study-specific template, to which the native grey matter images were then non-linearly re-registered. The registered partial volume images were then modulated (to correct for local expansion or contraction) by dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 6 mm. Finally, voxelwise GLM was applied using parametric testing, correcting for multiple comparisons across space.

The Talairach and Tournoux template [41] was used as reference for locating the activation in the brain. The MNI/ICBM coordinates were converted to the Talairach and Tournoux coordinates using a non-linear transformation developed by M. Brett for transforming coordinate location between both stereotaxic spaces (http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html).

3. Results

3.1 Neuropsychological and Behavioral Performance

There were a statistically significant differences in the mean scores between both groups in the MMSE, word list memory, word list recall, word list recognition subtests of the CERAD battery (see Table 1, t –test, df = 14, $p < 0.05$, uncorrected for multiple comparisons). In the verbal fluency, naming and constructional praxis of the CERAD there were no statistically significant differences.

The average response time (RT) of the included correct trials was 1.61 ± 0.39 sec and 1.83 ± 0.54 sec in the HC and MCI, respectively (see Table 2). There was not a statistically significant difference in the RT between groups. In addition, the overall correct response of every subject in both groups and the overall response time for the correct trials are included in Table 2. In the MCI subjects the response time of the correct trials included in the activation analysis were not linearly correlated (Pearson's correlation coefficient) to word list memory performance of the CERAD. The overall performance (correct response rate) in the WM task in the MCI group showed a trend to significance ($p = 0.06$) to the word list memory score from the CERAD. In the HC group similar tests were performed and no statistically significant correlations were found.

3.2 Common Activation in the Healthy Control and Mild Cognitive Impairment Groups

The activation for the three different phases activated a wide network of regions primarily in the parietal and frontal lobes. In Figures 2 and 3 the areas of common activation

within each group are detailed. In the HC the regions activated in all three phases of the task were bilaterally in the ventrolateral frontal cortex (VLPFC), left dorsolateral prefrontal cortex (DLPFC), and bilateral inferior parietal cortex. The activation across all phases of the WM task varies and present a dynamic picture of regions activated only during one or two phases of the task. As with the HC, the MCI group had a dynamic recruitment of various regions of the brain during the task, with the pattern different from the HC. In the MCI group, there was no region that was recruited during all three phases of the task.

In addition a conjunction overlay was performed to delineate common activation areas in the HC and MCI groups, which was detailed in Table 3. This table shows areas of common activation across the two groups.

3.3 Differences in Activation among Differences Phases of Working Memory Task

In each group the differences in activation between each of the phases of the WM task was computed (Tables 4 & 5). In the HC group we found that there was higher activation in the maintenance phase compared to encoding and recall, as well as differences (higher and lower) in activation between encoding and recall. The areas of greater activation during maintenance compared to encoding or recall are located in visual areas (occipital and temporal lobes) and in the frontal lobe areas. The differences in encoding and recall were located in the visual areas (occipital lobes) and frontal lobe regions.

In the MCI group we found that there were regions with statistically significant higher activation during the maintenance phase compared to recall phase (as with the HC group), brain regions with greater activation in encoding compared to recall (as with HC group), and regions with higher activation during encoding compared to maintenance (different from HC group).

3.4 Differences in Activation between Groups

During the encoding phase there were statistically significant differences in activation between the HC and the MCI groups in temporal and frontal lobes and cerebellum (Table 6).

During the maintenance phase, there was a wide network of regions with statistically significant higher activation in MCI compared to HC (Table 7, Figure 4). There was no higher activation in the HC compared to the MCI group. The peaks of activation in the anterior cingulate and inferior frontal gyrus (Figures 5 & 6) in both groups show that the the estimated hemodynamic response function with confidence intervals are different between groups.

During the recall phase of the task, there was significantly higher activation in the HC compared to the MCI (Table 8).

3.5 Linear Association of Task Performance with Brain Activation

In the HC group, there was statistically significant linear association between brain activation during encoding phase and response time in the left hippocampus (t-score = 4.74, $p < 0.001$) at (28 mm left, -12 mm posterior, 18 mm inferior), left thalamus (t-score = 4.46, $p < 0.001$) at (16 mm left, -16 mm posterior, 12 mm superior), and right thalamus, (t-score = 4.49, $p < 0.001$), at (20 mm right, -16 mm posterior, 10 mm superior). During the maintenance phase we found no statistically significant linear association between brain activation and performance. During the recall phase the response time of the included trials was statistically significantly linearly associated to the left inferior parietal lobulus (54 mm left, -38 mm posterior, 36 mm superior) with a t-score = 5.601, $p < 0.001$; left middle frontal gyrus (44 mm left, 28 mm anterior, 22 mm superior) with a t-score = 5.78, $p < 0.001$; left thalamus (20 mm left, -16 mm posterior, 10 mm superior) with a t-score = 7.23, $p < 0.001$.

In the MCI group, performance (using the response time of the included trials in the analysis) has a statistically significant positive linear association to activation during the encoding phase in the right inferior occipital gyrus (48 mm left, -80 mm posterior, 10 mm superior) in BA 18 (t-score = 8.36, $p < 0.001$), and right middle temporal gyrus (34 mm right, -66 mm posterior, 2 mm superior) in BA 19 with a t-score = 10.58, $p < 0.001$. During the maintenance phase we found statistically significant positive linear association to the left middle frontal gyrus (32 mm left, 40 mm anterior, 0 mm) (BA 11) with a t-score = 4.74, $p < 0.001$; and left precentral gyrus (44 mm left, -6 mm posterior, 34 mm superior) (BA 6) with t-score = 5.49, $p < 0.001$. During the recall phase there was a statistically significant positive linear association between performance and the left

hippocampus (26 mm left, -8 mm posterior, -28 mm inferior) with a t-score = 4.58, $p < 0.001$.

3.6 Structural Differences Between Groups

The was statistically significantly decreased grey matter density in the MCI compared to the HC ($p < 0.05$ corrected) bilaterally in medial and lateral temporal lobes, hippocampus, fusiform gyrus, parahippocampal gyrus, cuneus, and medial frontal gyrus (see Figure 7). Examination of the areas of differences in grey matter density between the HC and MCI group did not overlap with the activation differences between groups in the three phases of the WM task.

4. Discussion

In this study we have identified the regions that are activated in DMTS design of the WM task in older HC and MCI subjects. The results have shown that there is not a single network activated through the three different phases of the WM. The HC group had a "core" set of regions that were active through the entire task whereas the MCI group did not recruit a set of regions activated through all three phases of the WM task. There were differences in activation between both groups in all three phases, with the largest differences during the delay phase. The response time was found to be linearly associated to different areas of the brain in both groups, with the hippocampus associated with performance level in both groups but in different phases of the task.

The two groups of subjects were matched based on performance so that the alterations were not due to differences in performance between groups. The present results suggest that even when behavioral performance between groups does not differ, the neural systems that support performance may not be the same. The differences in activation pattern that we found may occur when the optimal or ideal network (as defined by the HC) is compromised by disease. In the case of the MCI group, the putative lesion or lesions that define a subject as having MCI, may be "partial" in the sense that the ideal network for the cognitive task is only partially damaged. Thus this could lead to mild changes in performance that may go undetected unless brain imaging techniques are utilized to measure altered brain activation during performance of a cognitive task.

The first novel contribution from this study was that the networks supporting WM in MCI subjects were altered in all phases of the task compared to the HC group. In the initial analysis step, the conjunction analysis showed the areas of common activation in both groups within the encoding and maintenance phase of the task which included the ventral and dorsolateral prefrontal cortices (VLPFC and DLPFC), inferior parietal regions, and visual processing areas in occipital and temporal lobes. These areas have been shown to be activated in WM tasks in young subjects [11, 42-49] and remain as part of the network supporting memory function in older HC and MCI subjects. The alterations in activation between the two groups (shown in tables 6 – 8) were primarily located in temporal and frontal lobe areas. The largest difference between groups were during the maintenance phase with the MCI group requiring greater neural resources to maintain the information during the delay compared to the HC group. The greater

demand of resources in the MCI group were located bilaterally in frontal areas and left hemispheric temporal lobe regions. The regions of the temporal lobe are associated with the visual processing of the stimuli (fusiform gyrus and lingual gyrus) and the superior temporal gyrus are areas associated with language processing [50-53] whereas the frontal lobe regions have been shown to be involved in both language and working memory tasks [11, 42-49, 54-56]. The increased activation in the MCI group compared to the HC group in the precuneus may indicate recruitment of an additional region as a compensatory mechanism for maintenance of stimuli in working memory.

The HC group activated a "core" set of regions, that is, the regions activated over the three phases of the WM task (indicated by yellow in Figure 2) while the MCI group did not. The activation of the core regions found in the present study was consistent with previous studies, showing activation in inferior parietal areas, DLPFC, and language areas in the temporal lobe [16-18] to be key regions for verbal WM. The DLPFC is recruited for executive components of a task [57] and this region is also thought responsible for initiating the controlled processing of verbal working memory material [58-59]. Prefrontal regions and inferior parietal regions have been previously identified as part of a network for maintaining the neural representation of the stimuli during the maintenance phase [11, 42-49]. Parietal regions, with the highest extent during the encoding phase, reflect the stores affected by the working memory updating process [60-61]. The areas that we found activated in all three phases may be due to (a) maintenance processes that are activate during encoding and recall phases or (b) these regions

mediated computational processes that are common in all three phases such as phonological processing and access to long term memory. Previous studies that examined WM obtained the average activation pattern across all phases of the WM task or the n-back design was utilized, which did not allow for a separation of the different components of the task [16-18, 62].

Task performance in the HC was linearly positively associated to activation in the left hippocampus and bilaterally the thalami during encoding and to the left inferior parietal region and middle frontal gyrus during recall. Thus for the HC group, the encoding and recall phases are critical for performance while the maintenance phase did not seem to play a role in performance. Greater activation of the hippocampus during encoding may indicate stronger representations of the memory trace and thus lead to more efficient retrieval in the later phase of the WM task. In addition, the inferior and middle frontal areas during recall are key areas for maintaining information in working memory but also support executive function during working memory. The positive association with thalamus activation in both phases may indicate greater interaction among the regions activated in the HC, that is, increased or stronger connectivity among the regions of the network may lead to greater performance. This issue would need further investigation to address.

We demonstrated in this study that the MCI use alternative regions to subserve performance of a WM task. Thus performance was positively associated with activation in regions not found in HC in all three phases. Grady and colleagues [24] were the first

to find that performance was correlated to a compensatory network in an AD patient group during a semantic and episodic task.

Within each group there were differences in activation among the different phases of the WM task. Even though there were many regions of common activation (see Figures 2 & 3) the different phases of the task require activation of different regions among the three phases of the WM task. The fewer number of differences in activation between phases in the MCI group than in the HC group (see Tables 4 & 5) suggest that the MCI group may be impaired also in executive function as there is no specific neural network for each phase of the DMTS task. Further supporting this interpretation is that the DLPFC is activated throughout the WM task in HC but not in the MCI group, suggesting impairment in executive and attentional resources in the MCI group.

In addition, we found that both groups de-activated the default network during the working memory task. The HC group deactivated regions that have been shown to be part of the default network [63-66] and the MCI group deactivated only a region in the medial frontal lobe. Given the limited deactivation of the default network in the MCI group during the maintenance phase yet large activation in this phase, it suggests that activation and deactivation of different networks impact the neuronal dynamics in the brain. Given the differences in which regions activated for the WM task, it should not be surprising that the regions deactivated are different. Recent studies have indicated that performance in a memory task in not only correlated to brain activation but also to the level of

deactivation [67-68]. The present results give further support that the network recruited for a task are not only regions of activation, but also in regional deactivation.

We examined the grey matter (GM) density differences between groups using VBM. In the MCI group we found statistically significantly decreased GM density differences compared to the HC, particularly in medial temporal areas. Our two groups showed similar structural differences as shown by previous studies [69-70]. Visual examination of the fMRI and VBM results showed that the differences between the MCI and HC groups were in different regions for the two modalities. A more detailed analysis integrating the two modalities is necessary, such as performed by Oakes and colleagues [71], to make statements about the possible interaction between brain activation and GM density.

A limitation of the current study is the small numbers of HC and MCI subjects. It would be useful to reproduce the study with a larger cohort. In addition, in the current analysis it was assumed that the hemodynamic response function (HRF) was well modeled across subjects and across groups. A study by Rombouts and colleagues showed that there can be variation in the delay or shape of the HRF [72] and found that from 7 regressors used to model the variance in delay and shape of the HRF, 2 of the regressors were statistically significant between HC and MCI in the visual cortex. One of the possible interpretations is that it indicates differences in the vascular coupling between MCI and HC groups or that it may be another measure of the effects of AD related neuropathology in the brain.

Examination of these effects could potentially be used as an additional neuroimaging-based marker for discriminating between HC and patients groups.

A limitation in the study to be aware of is that in delay match to sample designs, particularly with short delay phases as in the current study, part of the activation that is due to the encoding processes may appear as activation in the delay phase because the encoding phase is short. One approach around this limitation is to have a long delay phase (for example 30 seconds) and model the activation in the delay phase with two different regressors. One regressor would model the first part of the delay phase (i.e. first 10 seconds) and the other regressor the last part of the delay phase (i.e. the later 20 seconds). Thus the activation modeled by the second regressor would be taken as the activation during the delay phase as it is unlikely to have activation due to encoding processes. Another approach would be to orthogonalize the regressors but then the challenge is that interpretation of the results can be difficult as the shape of the orthogonal regressors may not reflect the cognitive processes one wishes to investigate.

Even though the MCI group has a high risk of converting to AD, not all subjects will convert to dementia as some may remain stable in this domain or may even revert to normal cognitive domain [3, 25, 73-76]. The MCI group is likely to have subjects that will not convert to AD and thus it would be valuable to follow-up this group of subjects and see how the composition of the group develops.

This has been the first study that has examined working memory in MCI subjects using a event related design to examine the difference phases of the WM task. In addition, we examined the default network and found de-activation consistent with previous studies in MCI subjects. A next possible step for examining working memory would be examining the effective connectivity differences between groups and doing longitudinal follow-up studies.

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Table 1. Average CERAD results for HC and MCI subjects.

(a)

Group	MMSE [0-30]	Word List Memory [0-30]	Word List Recall [0-10]	Verbal List Recognition [0-10]	Verbal Fluency [0-24]	Boston Naming [0-15]	Constructional Praxis [0-11]
HC	30±0	23±3.0	8.5±1.2	10±0	19.8±2.8	14.6±0.5	10.6±0.7
MCI	**26.6±1.3**	**15.9±3.2**	**3.8±2.6**	**8.1±2.2**	18.5±3.2	14.2±0.9	11±0.0

Median values in the MCI subjects that are bolded are statistically significant different from the HC group, using t-tests (df = 14), significance at the $p < 0.05$ level, no correction for multiple comparisons. The values in brackets indicate the range of possible scores (if applicable).

Table 2. Performance in the working memory task in the HC and MCI groups. The response time of the included correct trials included in analysis, the overall correct rate and the overall response rate (all correct trials).

Subject	Working Memory Response Time (included trials in analysis) (sec)	Overall Working Memory Correct Rate (percent)	Overall Working Memory Response Time (sec)
HC Group			
1	1.97±0.29	89	1.65±0.46
2	1.84±0.35	85	1.92±0.37
3	1.76±0.38	70	1.85±0.36
4	1.53±0.40	96	1.46±0.48
5	1.51±0.40	93	1.76±0.38
6	1.39±0.56	96	1.50±0.32
7	1.46±0.47	93	1.48±0.46
8	1.42±0.25	85	1.41±0.25
Median	1.61±0.39	88±9	1.63±0.39
MCI Group			
1	1.41±0.29	63	2.58±1.44
2	3.59±0.95	74	3.59±0.95
3	1.61±0.45	93	1.62±0.45
4	1.71±0.28	93	1.70±0.31
5	1.35±0.21	70	1.54±0.34
6	1.50±0.51	74	1.45±0.45
7	1.50±0.32	93	1.61±0.36
8	2.01±1.36	89	2.01±1.37
Median	1.83±0.54	81±12	2.01±0.71

Table 3. Regions of overlapping statistically significant activation in the MCI and HC groups in the (a) encoding, (b) maintenance phases of the WM task. There are no areas of overlapping statistically significant activation in the recall phase of the WM task so the areas of activation are detailed in (c) for HC and (d) for MCI. The volume is in micro Liters. Italicized values represent subpeaks of significant clusters. The T-score refers to the Student's t-value (df = 7); a t value of 5.41 is at the p = 0.0005 significance level.

(a)

Region	Side	Volume	BA	X	Y	Z	T-score
Inferior Occipital Gyrus	R	1912	18	36	-88	-7	7.53
Lingual Gyrus			*18*	*26*	*-97*	*-4*	*6.81*
Cerebellum	R	42440		18	-63	-20	15.64
Fusiform Gyrus	*L*		*19*	*-50*	*-71*	*-12*	*13.04*
Inferior Frontal Gyrus	R	2624	47	46	15	-4	11.20
Superior Temporal Gyrus	*R*		*38*	*57*	*13*	*-6*	*6.03*
Superior Temporal Gyrus	L	1312	22	-65	-21	1	8.14
Middle Temporal Gyrus	*L*		*21*	*-61*	*-39*	*2*	*6.46*
Thalamus	R	4552		24	-23	14	13.27
	R	*4552*		*2*	*-11*	*12*	*7.48*
Inferior Parietal Lobule	L	23792	40	-40	-50	50	10.43
	R			*46*	*-44*	*54*	*9.07*
Middle Frontal Gyrus	R	9192	6	28	1	53	12.74
				51	*2*	*40*	*8.18*
Medial Frontal Gyrus	L	10592	6	-4	11	57	10.5
				-6	*-3*	*61*	*8.62*
Middle Frontal Gyrus	L	25528	9	-51	13	34	11.85
Precentral Gyrus			*4*	*-48*	*-11*	*50*	*9.94*

(b)

Region	Side	Volume	BA	X	Y	Z	T-score
Precentral Gyrus	L	4664	6	-50	4	37	14.58
Middle Frontal Gyrus			*6*	*-42*	*2*	*50*	*7.05*
Superior Frontal Gyrus	L	4368	6	-4	6	48	9.45

Medial Frontal Gyrus	*R*		*32*	*2*	*8*	*46*	*6.72*
Superior Parietal Lobule	L	1680	7	-38	-56	49	8.57
Inferior Parietal Lobule	*L*		*40*	*-36*	*-54*	*41*	*5.26*
Inferior Parietal Lobule	R	1024	40	36	-56	45	5.78
Precentral Gyrus	R	1016	4	40	-17	56	4.87

(c)

Region	Side	Volume	BA	X	Y	Z	T-score
Cerebellum	L	156760		-24	-67	-25	9.85
	R			*26*	*-62*	*-28*	*8.09*
Middle Occipital Gyrus	*R*		*37*	*56*	*-62*	*08*	*6.63*
Superior Temporal Gyrus	*R*		*22*	*60*	*-48*	*16*	*6.55*
Inferior Parietal Lobule	*R*		*40*	*52*	*-44*	*38*	*6.10*
Supramarginal Gyrus	*L*		*40*	*-60*	*-46*	*30*	*7.83*
Superior Temporal Gyrus	*L*		*22*	*-54*	*-38*	*12*	*6.66*
Superior Frontal Gyrus	L	5688	38	-48	19	-11	5.92
Hippocampus	L	2720		-2-	-2-	-7	5.45
Thalamus	R	2640		-20	16	4	5.26
Cingulate Gyrus	L	2184	23	-4	-18	30	6.93
Cingulate Gyrus	R	1072	24	2	17	27	5.17
Middle Frontal Gyrus	L	2240	9	-53	13	31	6.08
Precentral Gyrus	L	1616	6	-40	-1	28	5.23
Precentral Gyrus	L	3200	6	-38	-10	63	6.37
Middle Frontal Gyrus	R	3968	47	22	36	-9	-7.31
Putamen	R	1016		26	9	-7	-6.61

(d)

Region	Side	Volume	BA	X	Y	Z	T-score
Medial Frontal Gyrus	L	4664	6	2	23	55	5.13
Anterior Cingulate	R	6840	10	6	40	-7	-5.84
Medial Frontal Gyrus	*L*		*10*	*-8*	*49*	*5*	*-5.22*
Middle Temporal Gyrus	R	992	21	40	6	32	-4.74

Table 4. Statistical comparison of activation levels between the different phases of the WM task in the HC group (a) maintenance greater than encoding; (b) encoding greater than recall; (c) recall greater encoding; (d) maintenance greater than recall. The cluster volume is in micro Liters. Italicized values represent subpeaks of significant clusters. The T-score refers to the Student's t-value (df = 7); a t value of 5.41 is at the p = 0.0005 significance level.

(a)

Region	Side	Volume	BA	X	Y	Z	T-score
Precentral Gyrus	L	23760	6	-46	-10	32	11.01
Inferior Frontal Gyrus	*L*		*9*	*-55*	*13*	*25*	*7.19*
Anterior Cingulate	*L*		*33*	*-6*	*20*	*19*	*6.71*
Precuneus	L	247752	7	-26	-65	29	9.90
Precuneus	*R*		*7*	*32*	*-75*	*46*	*7.31*
Cuneus	*R*		*23*	*2*	*-77*	*12*	*7.82*
Inferior Occipital Gyrus	*L*		*18*	*-36*	*-88*	*-7*	*7.67*
Hippocampus	*R*			*34*	*22*	*-12*	*6.79*
Middle Temporal Gyrus	R	1336	22	44	-30	-2	6.76
Middle Frontal Gyrus	R	3664	6	30	3	59	6.30

(b)

Region	Side	Volume	BA	X	Y	Z	T-score
Transverse Temporal Gyrus	R	8632	41	48	-19	10	6.35
Inferior Parietal Lobule			*40*	*65*	*-33*	*31*	*4.85*
Transverse Temporal Gyrus	L	3392		-53	-25	10	6.48
Middle Temporal Gyrus	R	2008	38	46	14	-39	4.94
Superior Temporal Gyrus	L	3133	22	-61	-57	19	6.81

(c)

Region	Side	Volume	BA	X	Y	Z	T-score
Middle Temporal Gyrus	L	1064	21	-61	-27	-2	5.20
Parahippocampal Gyrus	L	1296	20	-36	-18	-16	4.09
Precuneus	L	15712	7	-24	-68	35	8.73
Superior Parietal Lobule			*7*	*-28*	*-56*	*42*	*7.90*
Superior Parietal Lobule	R	17560	7	30	-60	51	8.56
Inferior Parietal Lobule			*40*	*42*	*-42*	*45*	*6.49*
Putamen	R	2336		24	11	-4	10.59
Putamen	L	3872		-24	10	-4	8.22
Middle Occipital Gyrus	L	2304	19	-42	-68	-8	8.58
	R	4320	18	32	-89	1	7.06
	L	6136	18	-40	-85	4	6.74
Middle Frontal Gyrus	L	17560	6	-26	-3	50	8.56
Inferior Frontal Gyrus			*44*	*-51*	*1*	*17*	*7.21*
Middle Frontal Gyrus	L	3528	46	-42	26	19	7.44
Medial Frontal Gyrus	L	3552	6	-4	5	51	9.08
Cerebellum		2136		20	-77	-23	4.92
		2944		48	-52	-33	5.64
		1008		34	-61	-46	4.38

(d)

Region	Side	Volume	BA	X	Y	Z	T-score
Superior Temporal Gyrus	R	1336	38	55	15	-9	4.77
Inferior Parietal Lobule	R	2528	40	51	-52	41	6.58
Cingulate Gyrus	R	1328	23	2	-12	30	5.70
Postcentral Gyrus	R	1472	1	30	-33	70	5.34
	L	7024	5	-22	-45	70	5.08
Middle Occipital Gyrus	L	2704	19	-32	-91	10	5.78

Inferior Frontal Gyrus	R	1632	45	57	20	21	5.03
	L	2504	47	-50	21	-13	5.16
Superior Frontal Gyrus	L	1752	9	-16	50	34	4.17
Anterior Cingulate Gyrus	L	1638	24	0	33	8	6.40
Cerebellum	L	36256		-24	-57	-21	9.09
	R			*30*	*-51*	*-18*	*5.88*
Lingual Gyrus	*R*		*18*	*2*	*-84*	*-8*	*5.34*

Table 5. Statistical comparison of activation levels between the different phases of the WM task in the MCI group (a) encoding greater than maintenance; (b) encoding greater than recall; (c) maintenance greater than recall. The cluster volume is in micro Liters. Italicized values represent subpeaks of significant clusters. The T-score refers to the Student's t-value (df = 7); a t value of 5.41 is at the p = 0.0005 significance level.

(a)

Region	Side	Volume	BA	X	Y	Z	T-score
Superior Temporal Gyrus	R	1466	22	52	-39	6	5.20
Putamen	R	1360		29	-17	3	5.16
Precentral Gyrus	R	1712	6	56	0	39	6.82
	L	2800	6	-56	-8	37	7.69
	R	1592	6	54	-6	30	5.33
Postcentral Gyrus	L	1064	3	-60	-10	22	5.01
Middle Occipital Gyrus	L	3224	18	-38	-89	4	8.47
Cerebellum	L	24384		-10	-75	-28	10.14
Inferior Occipital Gyrus	*L*		*18*	*-28*	*-90*	*-7*	*7.57*
Cuneus	*R*		*18*	*26*	*-97*	*-2*	*7.39*

(b)

Region	Side	Volume	BA	X	Y	Z	T-score
Superior Temporal Gyrus	R	1168	21	66	-18	-2	5.50
Medial Frontal Gyrus	L	7326	6	0	10	47	8.06
Precentral Gyrus	R	8408	6	53	0	35	10.06
	L	15936	6	-53	-10	36	11.37
Inferior Frontal Gyrus	L	2848	45	-58	12	22	5.89
Putamen	R	2200		28	-10	4	5.91
Cerebellum	L	91144		-34	-69	-20	12.65
Fusiform Gyrus	*L*		*37*	*-36*	*-44*	*-18*	*8.22*

Cerebellum	*R*			*30*	*-69*	*-20*	*7.39*
Cuneus	*L*		*31*	*-24*	*-72*	*28*	*7.39*
Cerebellum	L	1136		-2	-64	-32	5.17

(c)

Region	Side	Volume	BA	X	Y	Z	T-score
Middle Temporal Gyrus	L	1056	22	-32	-58	18	5.41
Lingual Gyrus	L	1616	18	-6	-80	-13	5.20
Cerebellum	L	1296		-6	-82	-24	5.65

Table 6. Statistically significant higher activation peaks in the encoding phase (a) for MCI compared to HC and (b) for HC compared to MCI. The cluster volume is in micro Liters. The T-score refers to the Student's t-value (df = 14); a t value of 4.14 is at the p = 0.0005 significance level.

(a)

Region	Side	Volume	BA	X	Y	Z	T-score
Middle Temporal Gyrus	R	992	22	63	-37	6	5.22
Cerebellum	L	976		-42	-71	-18	4.61

(b)

Region	Side	Volume	BA	X	Y	Z	T-score
Middle Frontal Gyrus	R	1472	10	36	38	16	4.90
Anterior Cingulate	L	1176	32	-12	41	-4	4.98

Table 7. Statistically significant clusters of higher activation in MCI compared to HC during the maintenance phase. The cluster volume is in micro Liters. The T-score refers to the Student's t-value (df = 14); a t value of 4.14 is at the p = 0.0005 significance level.

(a)

Region	Side	Volume	BA	X	Y	Z	T-score
Lingual Gyrus	L	1232	18	-16	-70	0	5.66
Fusiform Gyrus	L	1192	19	-36	-69	-13	4.82
Superior Temporal Gyrus	L	912	39	-54	-61	20	4.28
Superior Temporal Gyrus	L	12096	22	-57	10	1	7.26
Precuneus	L	1136	7	-18	-54	47	5.01
		5680	7	-6	-62	51	5.49
Posterior Cingulate Gyrus	R	2760	29	6	-44	8	5.56
Inferior Frontal Gyrus	L	2456	45	-30	28	6	6.25
Middle Frontal Gyrus	L	3360	10	-38	38	22	5.06
Superior Frontal Gyrus	R	1016	6	6	28	54	6.39
Anterior Cingulate Gyrus	L	2472	32	-8	18	42	6.39
Medial Frontal Gyrus	L	2400	6	-8	5	55	5.72
	L	1144	6	-2	-26	69	5.51
	R	4296	9	22	32	26	5.13
Caudate	L	2040		0	2	7	4.85
Cerebellum	R	1816		28	-71	-27	5.53

Table 8. Statistically significant clusters of higher activation in HC compared to MCI during the recall phase. The cluster volume is in micro Liters. The T-score refers to the Student's t-value (df = 14); a t value of 4.14 is at the p = 0.0005 significance level.

(a)

Region	Side	Volume	BA	X	Y	Z	T-score
Cuneus	L	2408	18	-12	-74	26	4.27
Superior Temporal Gyrus	L	1320	22	-44	-21	5	4.50
Precuneus	L	920	7	-6	-63	60	3.87
Medial Frontal Gyrus	R	1192	6	2	-13	52	5.85
Cerebellum	L	6296		-30	-69	120	4.65
	R	1744		4	-49	-18	4.59

Figure 1. Schematic of the working memory task. Each run had three blocks where the working memory task was performed and four blocks where the subjects fixated on a fixation point. Within each block there were 3 trials of the working memory task.

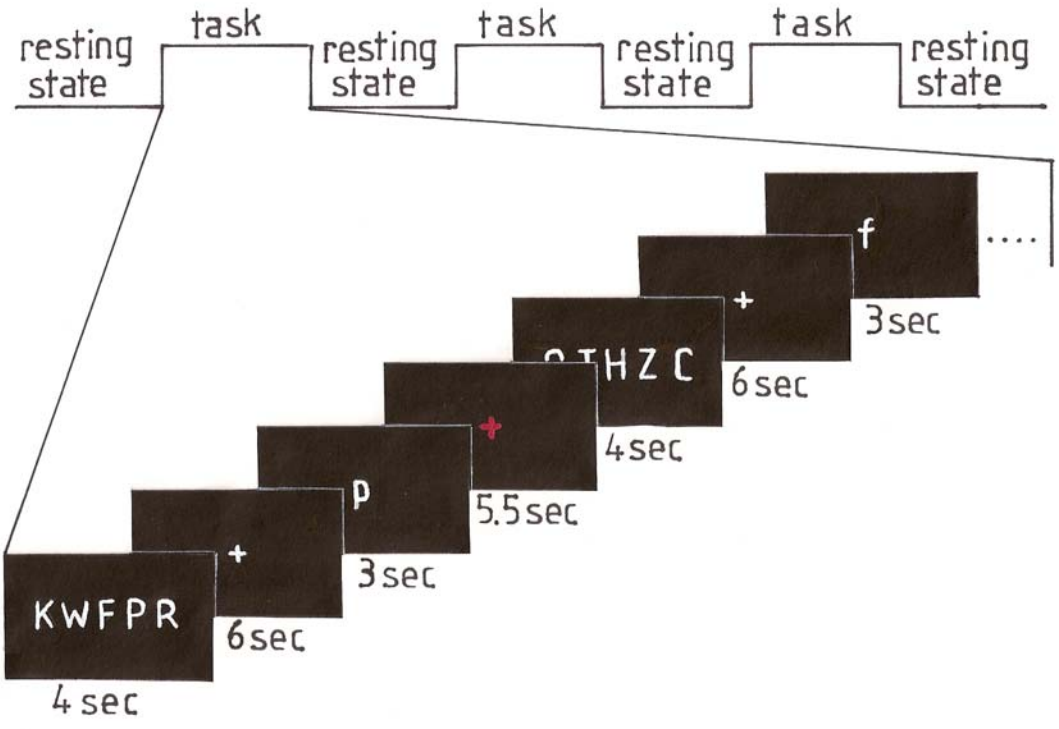

Figure 2. The activation pattern in the HC subjects during the three phases of the WM task. The different colors at each voxel indicate which WM phase or phases had significant activation in the voxel. The numbers on the images indicate the Z axis (inferior-superior) relative to the anterior commissure – posterior commissure plane in theICBM/MNI stereotaxic brain. The upper part of the image (top 3 rows) are the positiveactivation and last row are the areas deactivated. Color legend: RED, encoding; BLUE, maintenance; TAN, recall; GREEN, encoding & maintenance; CYAN, encoding & recall; VIOLET, maintenance & recall; YELLOW, all three phases.

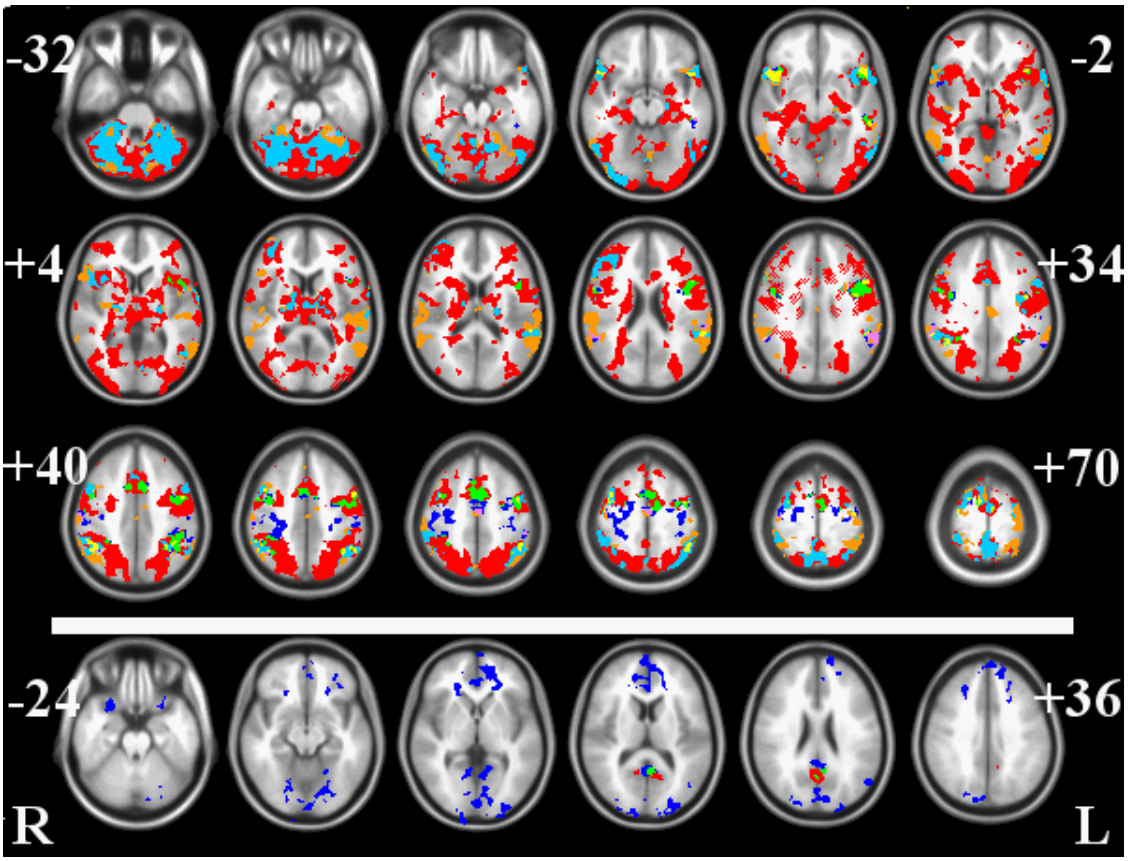

Figure 3. The activation pattern in the MCI subjects during the three phases of the working memory task. See Figure 1 for image details.

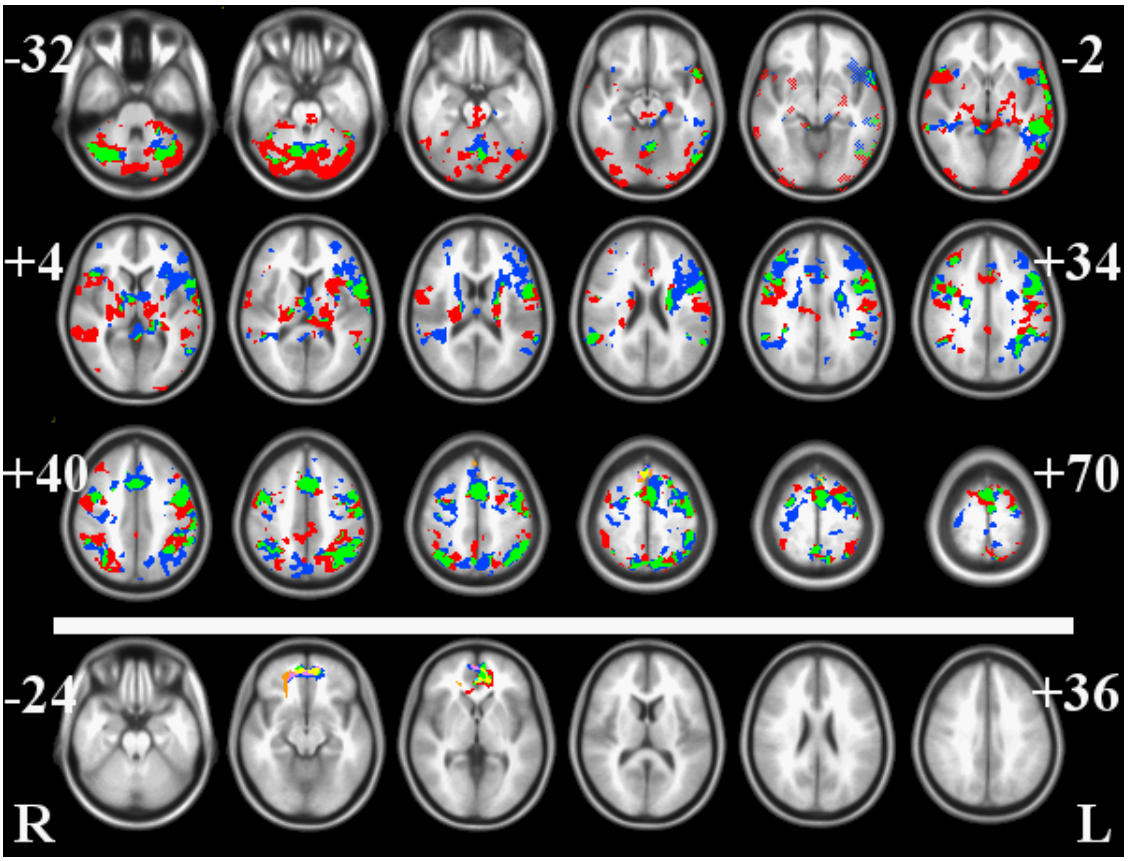

Figure 4. The pattern of greater activation in the MCI subjects compared to the HC subjects in the areas where either group had positive activation during the maintenance phase.

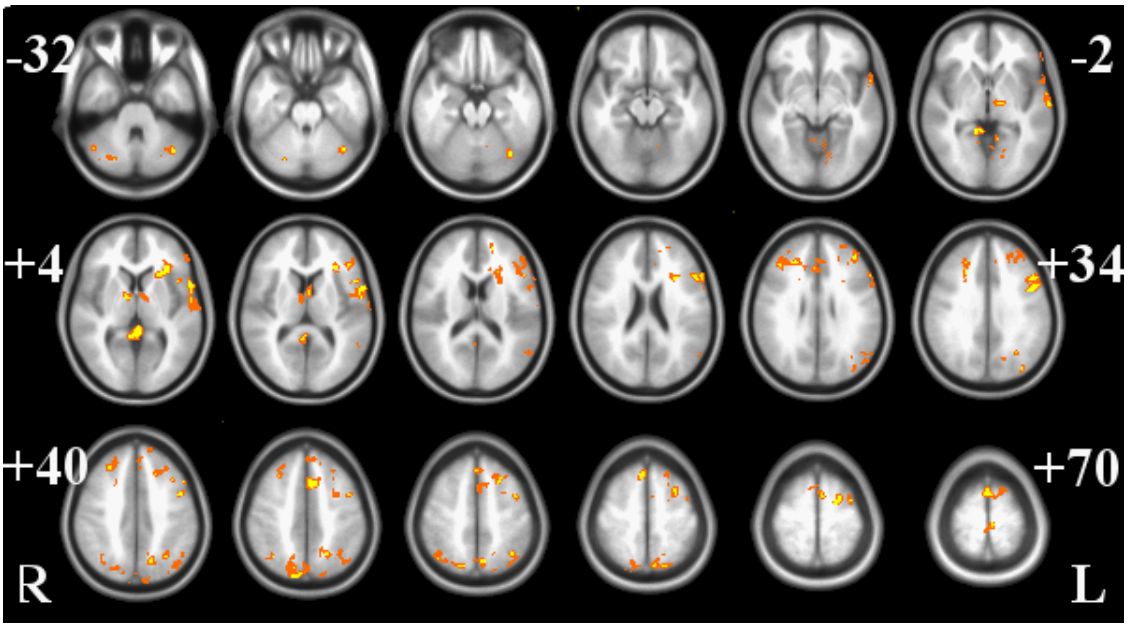

Figure 5. The percent increase activation in the MCI and HC groups at the maxima of differences between groups in the left anterior cingulate gyrus (-8, 18, 42).

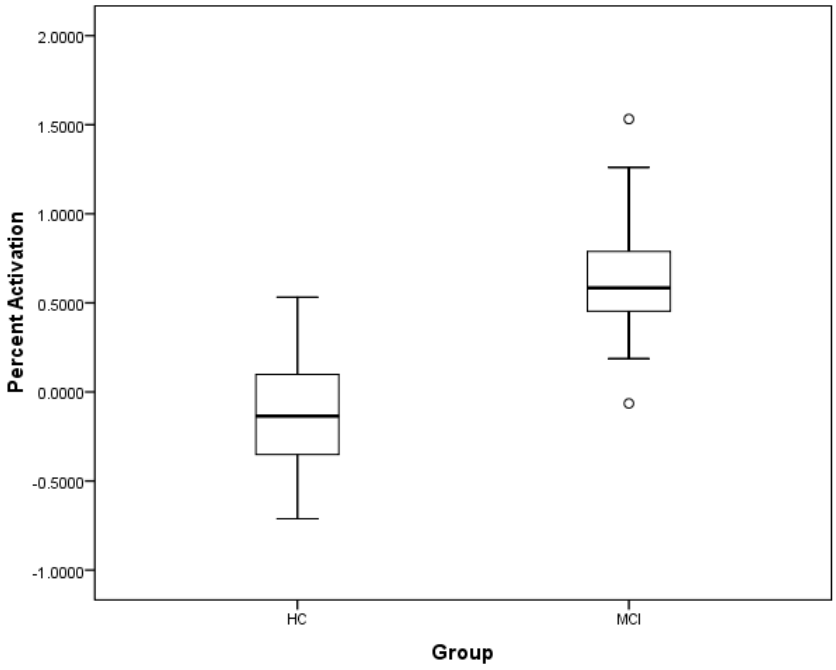

Figure 6. The percent increase activation in the MCI and HC groups at the maxima of differences between groups in the left inferior frontal gyrus (-30, 28, 6).

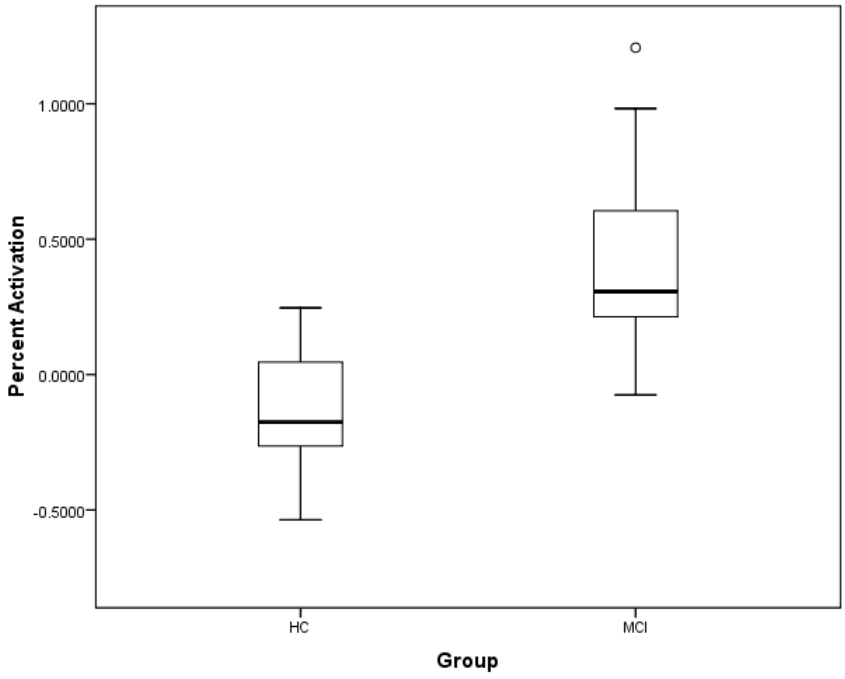

Figure 7. Statistically significant decreased grey matter density in the MCI compared to the HC group at the $p < 0.05$ corrected level. The coronal slice is at -14 mm posterior to the anterior commissure, and the axial slice is at -24 mm below the anterior commissure – posterior commissure plane. The left side of the image is right hand side of brain.