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Alterations in working memory networks in amnestic Mild Cognitive Impairment

Shortened title: Working memory networks in aMCI

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Abstract

Patients with amnesic Mild Cognitive Impairment (aMCI) show preserved or mildly impaired working memory, despite their deficits in episodic memory. We aimed to identify performance and/or neural differences between aMCI patients and matched controls on a standard working memory fMRI task. Neuropsychological assessment demonstrated aMCI impairments in verbal and visual episodic long-term memory, with intact IQ and executive function. Participants completed a standard three-level N-back task where patients were unimpaired. Functional activations in the control group were found in expected areas, including the inferior parietal lobule and dorsolateral prefrontal cortex. Group differences were found in the insula and lingual gyrus and, in a region of interest analysis, in the hippocampus. In all cases these were caused by a absence of task-related deactivations in the aMCI group. The results are consistent with reports of failure in task-related deactivations in aMCI and could be early indications of pathology.

(145 words)

Keywords

Mild cognitive impairment, working memory, fMRI, N-back

Word Count

6845

Introduction

Amnesic Mild Cognitive Impairment (aMCI) is the memory variant of MCI, where the main presenting feature is memory disorder (Petersen, 2004). MCI patients have greater cognitive decline than would be expected from normal aging, but not sufficient to be labelled as dementia (Petersen et al., 2001). MCI is often considered as a prodromal stage of dementia (J. C. Morris et al., 2001; Petersen, 2004), with an annual conversion rate of 10% across studies (Bruscoli & Lovestone, 2004). aMCI in particular shows a higher conversion rate and is strongly linked to Alzheimer's Disease (AD; Maioli et al., 2007; Schmidtke & Hermeneit, 2008).

Much work with aMCI has focussed on episodic memory, particularly delayed recall (Petersen et al., 1999). By definition, aMCI patients have impairments in episodic memory and performance on these tasks best predicts further cognitive decline (Conde-Sala et al., 2012; DeCarli et al., 2004; Tabert et al., 2006). However, many studies have shown that this is not a purely amnesic syndrome, with impaired performance evident on other cognitive tasks, including measures of executive function and processing speed (Brandt et al., 2009; Economou, Papageorgiou, Karageorgiou, & Vassilopoulos, 2007; Lopez et al., 2006; Tabert et al., 2006). Furthermore, in amnesic syndromes, there is evidence for working memory impairment, which has been associated with hippocampal dysfunction (e.g., Olson, Page, Moore, Chatterjee, & Verfaellie, 2006).

In AD, episodic long-term memory usually shows the most prominent impairment and is one of the earliest changes observed. However, working memory is also frequently impaired at early stages (Baddeley, 1992; Delbeuck, Van Der Linden, & Collette, 2003; Huntley & Howard, 2010; R. G. Morris & Kopelman, 1986). Working memory describes a cognitive process of limited capacity, used for the temporary storage and

manipulation of information for a more complex task (Baddeley, 2000, 2010). If aMCI is considered to be, at least in part, a prodromal stage of AD, it is important to understand the links between behavioural differences on all types of affected memory and brain function.

Investigating working memory in aMCI may help to understand neural changes that occur before there is a profound deficit in behaviour. Evidence for working memory deficits in aMCI remains mixed (Economou et al., 2007; Guarch, Marcos, Salamero, Gastó, & Blesa, 2008; Kessels, Meulenbroek, Fernandez, & Olde Rikkert, 2010; Kramer et al., 2006) with differing findings possibly due to variation in patients' cognitive abilities on the spectrum of progression towards dementia. Another large factor will be the particular tasks employed and how sensitive they are to MCI. Studies investigating working memory using multiple tasks with the same group of patients show impairments on only some tasks (e.g., Belleville, Chertkow, & Gauthier, 2007; Rose, Olsen, Craik, & Rosenbaum, 2012). Linking behaviour to changes in the recruitment of neural systems may help us understand early pathophysiological changes that precede the development of behavioural deficits.

One of the most widely used working memory tests in functional neuroimaging is the N-back task, in which a stream of stimuli are presented and the participant has to respond when one is repeated. The number of trials (n -back) between the stimulus and its repeat is varied to increase working memory load, from immediate repetition to a gap of three or four items. It is therefore described as an executive working memory task requiring continuous updating, as opposed to more complex forms of manipulating information (Wager & Smith, 2003). The N-back task is characterised by a well-established network of activations in fMRI versions of the task in healthy participants

(Owen, McMillan, Laird, & Bullmore, 2005). The most consistently activated regions across studies include the posterior parietal cortex, premotor cortex, rostral prefrontal cortex, dorsolateral prefrontal cortex (DLPFC) and ventrolateral prefrontal cortex (Owen et al., 2005).

A small number of studies have investigated working memory using fMRI in broadly defined MCI (Faraco, Puente, Brown, Terry, & Stephen Miller, 2013; Kochan et al., 2010; Kochan et al., 2011; Yetkin, Rosenberg, Weiner, Purdy, & Cullum, 2006). Few studies have specifically investigated aMCI and have indicated both additional (Bokde et al., 2010) and reduced (Alichniewicz, Brunner, Klünemann, & Greenlee, 2012; Saykin et al., 2004) recruitment of frontal and parietal regions in aMCI patients. Only two studies have used visual variants of an N-back task (Döhnelt et al., 2008; Rombouts, Barkhof, Goekoop, Stam, & Scheltens, 2005). Rombouts et al. (2005) investigated BOLD activity in aMCI patients and healthy controls using a visually presented letter N-back task with three levels (0-, 1- and 2-back). Performance was matched in terms of accuracy, although the patients were slower to respond. The groups showed different whole brain patterns of task related activity when considered individually, but there were no between group differences on direct comparison.

More recently, Döhnelt et al. (2008) used an emotional pictures version of a 2-back task with aMCI patients and controls. Their performance data showed no overall effect of group in terms of hit rates or reaction times. The fMRI data showed no regions that were overall significantly different between the two groups, although the precuneus showed evidence of a significant group by emotion interaction. There is therefore, no consistency across working memory studies, in part due to methodological differences, where tasks have varied in terms of stimuli and working memory load.

There has only been one study using a standard letter N-back fMRI task with aMCI (Rombouts et al., 2005), leaving a need for replication and expansion. There have been some fMRI investigations using the N-back task in patients with AD (McGeown, Shanks, & Venneri, 2008; Yetkin et al., 2006). These studies have shown increased activation in areas such as the middle frontal gyrus, the middle temporal gyrus and caudate in the AD group compared to matched controls (McGeown et al., 2008; Yetkin et al., 2006).

As well as the limited fMRI investigations with the verbal N-back task in aMCI, a number of behavioural investigations have been carried out. These have shown that patients with aMCI perform at normal levels on the task at 1-back and 2-back levels, except where vascular damage is present (e.g., Nordahl et al., 2005). Other studies showing performance differences have used more complex tasks, such as an N-back task where colour and location needed to be monitored (Alichniewicz et al., 2012).

The use of a simple, easy to understand task such as an N-back with letters has advantages when used with an aMCI group. The task is well established with expected regions of activation (Owen et al., 2005). It is a block design task which gives enough power to detect a robust activation while importantly minimising task length for the patients. In addition, given its design we did not expect to find performance differences between our groups. This is advantageous as interpreting differences in BOLD activity can be problematic when performance differences are present between groups (Nagel et al., 2009; Price & Friston, 1999), as is often the case with episodic and spatial memory tasks in aMCI (see Kochan et al., 2010 for a similar rationale for a working memory fMRI task in MCI).

Although the N-back task is a working memory task, there is some evidence that it can be associated with BOLD activity changes outside of established working memory

networks and may be useful to see activation changes within the medial temporal lobes. The most robustly reported differences in BOLD activity in aMCI are found within the hippocampal area (e.g., see Pihlajamäki, Jauhiainen, & Soininen, 2009). Multiple studies have reported hyperactivations in patients with MCI in episodic and semantic memory tasks (e.g., Celone et al., 2006; Clement & Belleville, 2010; Woodard et al., 2009) and this hyperactivation is usually interpreted as an early indicator of neuronal damage, seen in relatively mildly impaired MCI patients (Dickerson & Sperling, 2008). In healthy participants, the hippocampus is sensitive to changes in working memory load (Axmacher et al., 2007; Rissman, Gazzaley, & D'Esposito, 2008; Schon, Quiroz, Hasselmo, & Stern, 2009) and the N-back task has been used to investigate whether medial temporal lobe function is intact in stroke patients (Snaphaan, Rijpkema, Van Uden, Fernández, & De Leeuw, 2009). This task may, therefore, be a way to look at medial temporal function during an active cognitive task, without directly using an episodic or spatial memory test. Previous work using a working memory task in aMCI patients did not find significant group differences in hippocampal activity, although this did use a different working memory task (Kochan et al., 2010).

In the present study, we have used an N-back task at three levels of working memory load, as a replication of the design used by Rombouts et al (2005). We have included carefully selected aMCI patients, with corrections for multiple comparisons and using a potentially more sensitive scanning protocol using 3.0 Tesla (both previous N-back tasks using for fMRI with aMCI used 1.5 Tesla MRI scanners). Groups of aMCI patients and controls completed a visual letter N-back task with three levels; a baseline matching task and 1-back and 2-back conditions. The N-back task, particularly at the 2-back level, was expected to activate the well-established network of brain regions. Our patients

are early single-domain aMCI patients, with isolated memory deficits, rather than multi-domain aMCI. We therefore expect to see task-related hyperactivations since these are more commonly seen earlier in the dementia process (Celone et al., 2006; Sperling et al., 2010). MCI patients in general often show recruitment of additional brain regions or increased activity in regions associated with task performance in fMRI cognitive tasks (Pihlajamäki & Sperling, 2008). This could be a compensatory mechanism or an indication of neuronal damage, but the reasons remain unclear (see Sperling et al., 2010 for a review). Since patients should be able to perform as well as controls at both the 1-back and 2-back levels of the task, we did not have any strong predictions of interactions between group and working memory load. Given previous research, we expected any group differences to be seen in frontal and parietal cortex, potentially across widespread regions.

Methods

Participants

The patient group was composed of 10 single-domain aMCI patients, recruited from specialised memory clinics. All patients met the criteria for aMCI as defined in Petersen et al (2001) with subjective memory complaints, objective memory impairments, normal general cognitive function and intact activities of daily living. All scored 0.5 on the Clinical Dementia Rating Scale (CDR; J. C. Morris, 1993). A matched control group of 11 healthy volunteers were recruited from existing databases of research volunteers or in response to locally placed adverts. Patients and controls were selected to be matched on group measures of age, pre morbid IQ and years in education.

Participants aged under 60 and over 80 years of age were excluded, as were any participants who were not native English speakers. A full medical history and medical examination was carried out, with blood tests and urinalysis, to ensure that all participants were healthy with no history of head injury, alcoholism, or any psychiatric or neurological condition (other than MCI in the aMCI group). Informed written consent was obtained from all participants and the study was approved by the National Hospital for Neurology and Neurosurgery and Institute of Neurology Research Ethics Committee.

Neuropsychological assessment

All participants completed neuropsychological assessments of intelligence, executive function and memory. The memory assessment consisted of the Logical Memory and Visual Reproduction tests from the Wechsler Memory Scale Third Edition (WMS-III; Wechsler, 1998) and the California Verbal Learning Test (CVLT; Delis, Kramer, Kaplan, & Ober, 2000). Current intelligence was estimated by the short form of the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) and pre-morbid intelligence by the National Adult Reading Test (NART-Restandardised; Nelson & Willison, 1991). Executive function was assessed using the Hayling and Brixton Tasks (Burgess & Shallice, 1997) and the Trail Making Test (TMT; Delis, Kaplan, & Kramer, 2001) was used to assess processing speed and mental flexibility.

N-back task

Alphabet letters were presented in series in the centre of the screen. Participants responded by pressing a button using their index finger whenever they saw a target, defined by the working memory load for that section of the task. There were three levels of difficulty. In the 1-Back test, the target letter was an immediate repeat and in

the 2-Back test, the target letter was a repeated letter separated by one other letter. The 0-Back condition was a baseline task to control for attention, where participants were asked to respond whenever the letter 'X' was displayed (See Figure 1).

(Figure 1 about here)

Conditions were presented as separate blocks of trials, with 14 letters in each block, of which three were targets. Each type of block was presented three times, where the order was generated randomly but kept fixed across participants. Letters were presented for 1000ms each, with an inter-stimulus interval of 1000ms. Responses were collected if a button was pressed within this 2000ms period (stimulus presentation plus inter-stimulus interval). Instructions were presented for 3000ms at the start of each block.

Image acquisition

Images were acquired using a 3.0 Tesla, General Electric Medical Systems Excite II scanner (General Electric, Milwaukee, WI, USA). Image volumes (each consisting of 38 near-axial slices) were collected using a gradient-echo echo planar imaging sequence with a repetition time (TR) of 2000ms, echo time (TE) of 30ms and a 75 degree flip angle. The slices were positioned parallel to the AC-PC line. The body coil was used for radiofrequency (RF) transmission and an 8-channel head coil for RF reception. Each image slice was acquired using a 64x64 image matrix over a 24cm field of view. The resulting in-plane voxel size of the images was 3.75mm by 3.75mm. The images were 3.0mm thick with a 0.3mm gap. At the same session, a 60-slice high-resolution gradient-echo echo planar sequence was acquired in both the coronal and axial planes with the same acquisition parameters apart from a 128x128 matrix, giving 1.875x1.875 in-plane resolution. A semi-automated quality control procedure ensured consistent

image quality (Simmons, Moore, & Williams, 1999). A high resolution 3D T1 weighted SPGR image was acquired in the coronal plane, with 1.1mm isotropic voxels using a 256x256x196 matrix with a TI of 450ms, TR of 7.1ms, TE of 2.8ms and a flip angle of 20 degrees.

Image data processing

Functional imaging data were pre-processed and analysed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK; www.fil.ion.ucl.ac.uk/spm).

Functional data were spatially realigned to the mean image from the series and then resliced. Spatial normalisation into Montreal Neurological Institute (MNI) stereotactic space was carried out using DARTEL (Ashburner, 2007), using a sample template generated from all participants' structural scans. The functional images were resampled into 1.5mm³ voxels and spatially smoothed with an 8mm full-width half-maximum Gaussian kernel.

At the single-subject level, each block of each condition was modelled using a boxcar function convolved with a canonical model of the haemodynamic response. The design matrix therefore contained three task regressors and a regressor encoding the instruction periods. Additionally, six nuisance regressors encoding subject volume-to-volume head-movement (translations and rotations around the X, Y and Z axes) from the realignment stage of pre-processing were included. Following estimation of beta coefficients, contrasts of the parameter estimates were generated for each individual. The primary contrasts of interest compared both working memory conditions to the attentional control condition (i.e., responses during 1- and 2-back condition separately and also combined compared to those during the 0-back). These contrasts were taken forward to group-level-random effects-analysis. Group maps were generated using

one-sample t-tests, with age and current IQ (WASI) included as covariates of no interest to account for variance associated with these factors in both groups (analyses were repeated without the addition of these covariates but this did not alter the pattern of results seen). A mixed 2x2 factorial ANOVA analysis was carried out to investigate the simple main effect of group, the main effect of load and to test for evidence of the interaction between these two factors. Contrasts of 2-back>0-back and 1-back>0-back were used. The threshold of significance for these analyses was set at whole-brain multiple comparisons corrected threshold of $p=.05$ (family wise error correction on the basis of the spatial extent of the cluster) at a cluster forming threshold of $Z=3.1$.

For our regression analyses, we took the contrast of 2-back>0-back from the first level analysis forward to a second level model. This was regressed against performance as measured by reaction time to hits (since there were ceiling effects in the accuracy data, this could not be used). Age and current IQ (WASI) were included as covariates of no interest. We carried out separate whole brain analyses that included either all participants together, the aMCI and control groups separately as well as a test for the interaction between the groups. In the aMCI group, we completed an ROI analysis based on the regions that showed a significant hyperaction in the group contrast against controls. For all these analyses the threshold of significance was set at a whole-brain multiple comparisons corrected threshold of $p=.05$ (family wise error correction on the basis of the spatial extent of the cluster) at a cluster forming threshold of $Z=3.1$.

Structural data were processed for voxel based morphometry (VBM) analysis. Pre-processing and analysis were carried out using the VBM8 toolbox within SPM8 (<http://dbm.neuro.uni-jena.de/vbm/>). Default settings in VBM8 were used, which included normalisation via DARTEL and segmentation into grey matter (GM), white

matter (WM) and cerebrospinal fluid (CSF). Total intracranial volume (ICV) was calculated for each participant by summing global tissue volume (i.e., GM, WM and CSF) within VBM8. Images were smoothed with an 8mm full-width half-maximum Gaussian kernel. Statistical analysis used unpaired t-tests in SPM8 to compare GM differences between health controls and aMCI patients. Age and ICV were included as variables of no interest. The threshold of significance for this analysis was set to $p < .001$ (uncorrected) with an extent threshold of 100 voxels.

Results

Neuropsychological assessment

Results from the background neuropsychological assessment are shown in Table 1. Data is presented using standardised scores for all tests except for the TMT, where absolute times are reported. aMCI patients and controls were well matched for intelligence and years of education, as well as executive function. Within the aMCI group, there was no significant difference between the NART and WASI IQ measures ($t(10) = .527, p = .611, d = .17$), indicating no difference between 'pre-morbid' IQ and current performance. The aMCI group were significantly impaired on all tests of recall memory regardless of material.

(Table 1 about here)

N-back task performance

Hit and false alarm rates were combined to give d' as an index of performance accuracy (Macmillan & Creelman, 2004); additionally the mean reaction time (RT) for hits was computed. Hit and false alarm rates were systematically corrected as recommended by Snodgrass and Corwin (1988) as some ceiling effects were present. This correction

adjusts scores so that proportions of zero or one are avoided, to allow conversion to a Z score. For every calculation of hit and false alarm rate, 0.5 was added to the numerator and 1 was added to the denominator. In this way, proportions of 0.5 are unchanged, but ceiling and floor proportions are avoided.

(Table 2 about here)

The RT data for Hits is shown in Figure 2. A two-way ANOVA of group (patients versus controls) and task difficulty (0-back versus 1-back versus 2-back) showed a main effect of task difficulty ($F(2,38)=24.91, p<.001$, partial $\eta^2=.567$), but no effect of group ($F(1,19)=2.173, p=.157$, partial $\eta^2=.103$) or interaction ($F(2,38)=.117, p=.890$, partial $\eta^2=.006$). Accuracy data (Figure 3) shows ceiling effects, with all control participants at ceiling at 0-back and 1-back. There were no significant differences between the groups, as indicated by separate Bonferroni corrected Mann-Whitney U tests (largest $Z=2.26$ for 0-back), with the lack of effects driven by the near ceiling performance associated with this task. Unadjusted hit and false alarm rate data are provided in Table 2, which presents median and interquartile range values since data was non-normally distributed.

(Figure 2 about here)

fMRI results

Within-group analysis

The activation maps in the control group indicate a network of activation typically associated with the N-back task (Owen et al., 2005), including the lateral premotor cortex bilaterally, the right inferior parietal lobule, the right DLPFC, the left precuneus and the left lateral cerebellum (Figure 3 and Table 3). Inspecting Figure 3 suggested

that this network was wider and more active with increased memory load, although no regions were significant in the direct contrast of 2-back>1-back. Three regions in the left hemisphere were significantly less active at 2-back compared to 0-back, see Table 3.

In the aMCI group, activations in Figure 3 appeared less widespread and less significant than observed in the controls. Some regions significantly activated in the control group were not significant in the aMCI patients, in particular the DLPFC and inferior parietal lobule (Table 3). The aMCI group showed some task deactivations (for contrasts of 1-back<0-back and 2-back<0-back) that were not seen in the control group, most notably in a large cluster with a peak in the left medial rostral prefrontal cortex for the comparison of 2-back<0-back, see Table 3. This region extended into Brodmann Areas 8, 9, 10 and 32, including the anterior cingulate and extending along the medial frontal gyrus.

(Figure 3 about here)

(Table 3 about here)

Between-group analysis

When the two groups were directly compared in a factorial analysis (group by working memory load), three clusters were significantly more active in the aMCI group compared to the control group (See Figure 4, Table 2). Two clusters were in the right insula and one was in the right lingual gyrus. This hyperactivation likely reflects the fact that controls showed a task related deactivation in all three regions, whereas in the patients, this deactivation was reduced or absent. No regions were significantly more active in the control group or were significant in the interaction between group and working memory load, or for any of the other comparisons within the factorial model.

We ran a region of interest (ROI) analysis to look for any group differences in the well specified network of brain regions known to be important for N-back performance.

Using 17 sets of co-ordinates reported in a meta-analysis of verbal N-back tasks (see Table II in Owen et al., 2005), we created a single mask with 5mm spheres centred on each co-ordinate. Within this mask, there were no regions which showed significant differences between aMCI patients and the control group.

Finally, given previous findings and our interest in medial temporal lobe function we carried out an ROI analysis of the parahippocampal/hippocampal region using an anatomically defined mask from the WFU Pickatlas (Wake Forest University, Winston-Salem, North Carolina). This showed one significant region with greater activity in the aMCI group compared to controls in the left posterior medial temporal lobes with a peak in the parahippocampal cortex, extending into the hippocampus proper ($x\ y\ z = 33, -33, -15$; 109 voxels; $Z=4.00$; $p_{FWE}=.050$). Again, this represents the absence of a task-related deactivation in the aMCI group compared to controls. To investigate structure-function correlations, we extracted the mean activity for this cluster for the contrast of 2-back>0-back, as well as the mean grey matter volume for the cluster for each participant using Marsbar (Brett, Anton, Valabregue, & Poline, 2002). These values were not significantly correlated within our patient group ($r=.388, p=.268$) or across all participants ($r=.294, p=.196$).

(Figure 4 about here)

Regression analyses

When including all participants in a single model to look for any brain regions that were significantly related to task performance, we found one significant cluster in the left

anterior cingulate that was significantly positively associated with RT ($x y z = -14, 36, 13$; 389 voxels; $Z=3.89$; $p_{FWE}=.042$). No regions were significant for the reverse association. We then carried out separate analyses for the control group and the aMCI group. For the whole brain results, there were no significant results for the control group, but in the aMCI group, two regions were significantly positively associated with performance; the anterior cingulate bilaterally ($L x y z = -10, 35, 7$; 365 voxels; $Z=4.99$; $p_{FWE}=.002$, $R x y z = 21, 45, 15$; 562 voxels; $Z=4.00$; $p_{FWE}<.001$). No regions were significant for the reverse contrast. When we looked for significant performance interactions between the groups, no regions were significant. Finally, when we used an ROI based on the regions showing significant group differences in the between-groups analysis reported above (i.e., the regions in the right lingual gyrus and insula), no significant results were found for any of the regression analyses.

VBM Analysis

Comparing aMCI patients against controls revealed multiple regions of reduced grey matter in the patient group (Table 4). This included a cluster of 1172 voxels covering the left hippocampus and extending into the left entorhinal cortex, perirhinal cortex and amygdala. Another cluster covered the bilateral thalamus. None of the significant regions in this analysis overlapped with regions from the fMRI results. No regions showed significantly higher grey matter in the aMCI patients compared to controls.

(Table 4 about here)

Discussion

In this paper, aMCI patients and matched controls completed a three-level N-back task in an fMRI experiment. There was no evidence of significant performance differences

between the groups. The N-back task, particularly at the 2-back level, activated a well-established network of brain regions (Owen et al., 2005) including the DLPFC, lateral premotor cortex and inferior parietal lobule. Within the aMCI group, the lateral rostral prefrontal cortex (BA10) was significantly activated during the task, whereas the medial rostral prefrontal cortex showed task-related deactivation. Previous work with aMCI patients performing the N-back task has reported within-group activations of BA10 (Döhnell et al., 2008). The frontal region of deactivation at 2-back versus 0-back seen in our patients also matches previous results using the N-back task, where Rombouts et al (2005) reported deactivation in the anterior cingulate as well as the inferior, medial and superior frontal gyrus in their patient group.

When directly comparing results from the two groups, greater activations in the aMCI group compared to controls were seen in the right insula and lingual gyri. These group differences are driven by a task-related deactivation being present in controls, but not in patients. The previous study targeting aMCI using the letter N-back task did not find any group differences in standard whole brain analyses (Rombouts et al., 2005). Similarly, using an emotional pictures 2-back task, no group differences were found (Döhnell et al., 2008). Where significant group differences have been reported in N-back tasks, one study used a broader MCI group and did not correct for multiple comparisons (Yetkin et al., 2006), leaving a risk of a type one error. Saykin et al (2004), using an auditory N-back task, reported a cluster of three voxels in the frontoparietal cortex that were significantly less activated in aMCI patients compared to controls. Our results therefore were unexpected from the previous literature.

Previous work in N-back and other working memory tasks would suggest that group differences in activation would be found in frontal and parietal regions. We did not find

any group differences when using a ROI based on the established network of regions engaged in the N-back tasks. Instead, we found differences in the insula and the lingual gyrus after a stringent whole brain correction. The insula is known to be recruited in working memory tasks, particularly verbal tasks (Cabeza & Nyberg, 2000; Kurth, Zilles, Fox, Laird, & Eickhoff, 2010), albeit usually more anteriorly. The default mode network (DMN) describes a number of regions which are consistently deactivated during cognitive tasks (Raichle et al., 2001). Changes in the DMN in MCI/aMCI have been reliably reported in the literature (Bai et al., 2008; Celone et al., 2006; Rombouts et al., 2005). Although not usually considered to be part of the DMN, there has been some evidence that task-induced deactivation can extend more widely, including in the posterior insula (Harrison et al., 2011), albeit when tasks are demanding. Changes in BOLD activity in this region in aMCI remain slightly puzzling, but an explanation relating to disruption of normal task-related deactivation seems plausible. One recent fMRI study using a complex working span task has shown increased activity in MCI patients versus controls in a region overlapping with this right insula region (Faraco et al., 2013). This converging result suggests that our insula between-group BOLD differences reflect some meaningful neural changes that occur in MCI.

The VBM analysis indicated reduced grey matter volume in our aMCI patients in expected regions such as the left medial temporal lobe and bilateral thalamus (Yang et al., 2012). Volume reduction in the hippocampus and entorhinal cortex is one of the most reliable results seen in aMCI volumetric studies (Ferreira, Diniz, Forlenza, Busatto, & Zanetti, 2011; Yang et al., 2012). Our results therefore match previous findings and also help to interpret our main fMRI results; there was no evidence of grey matter loss in the regions showing group differences in task-related activations from our whole

brain analyses. If these differences do indicate neuronal dysfunction, this must be early in the process before grey matter volume changes are seen.

Atrophy of the insula is well established in MCI (Fan, Batmanghelich, Clark, & Davatzikos, 2008), particularly in the left hemisphere, and in at least one study the degree of atrophy there was at least as severe as found in medial temporal lobe structures (Karas et al., 2004). Grey matter loss in the insula is particularly associated with aMCI versus other MCI types (Whitwell et al., 2007) and in patients with aMCI with increased memory impairments (Barbeau et al., 2008). However, this region does not show up in meta-analysis of grey matter loss in MCI patients (Yang et al., 2012) or grey matter loss predictive of aMCI patients who convert to AD (Ferreira et al., 2011).

A recent longitudinal study of grey matter changes in the progression from aMCI to AD could help to understand the differences in the literature. Spulber and colleagues (2012) conducted structural MRI scans on aMCI patients at three time points and could therefore compare grey matter changes in patients who progressed to AD versus those who did not. In the patients who did progress to AD, grey matter changes in the right posterior insula were present only in the year immediately preceding AD diagnosis. This result indicates the importance of longitudinal studies or longitudinal follow ups to stratify patients.

fMRI studies of aMCI patients performing other cognitive tasks have shown increased left insula activation compared to controls, such as in an associative memory task (Hämäläinen et al., 2007) and in a numerical Stroop task (Kaufmann et al., 2008). Increased activity in the right insula compared to controls has also been shown in APOE $\epsilon 4$ carriers, a genetic risk factor for AD (Corder et al., 1993), during a mental rotation task (Yassa, Verduzco, Cristinzio, & Bassett, 2008). These tasks reflect a wide variety of

cognitive function. Resting state connectivity is also disrupted in the posterior insula in aMCI patients (Xie et al., 2012).

The study also indicated increased right lingual gyrus activity. There is some evidence that structural changes in this region are particularly important in the conversion between MCI and AD (Spulber et al., 2012). Meta analysis of fMRI and PET studies of episodic memory has also shown BA19 within the lingual gyrus to be an area consistently showing hypoactivations in AD (Browndyke et al., 2013), suggesting its involvement in later stages of pathological aging. This region is not typically recruited for working memory tasks or seen in group differences in fMRI tasks with aMCI patients versus controls.

Because insula and lingual gyrus activation is not normally found in working memory processing, the finding in aMCI patients could represent some additional recruitment of neural regions that somehow compensate for less efficient processing elsewhere (e.g., see Faraco et al., 2013 for this proposal relating to working memory tasks). However, an alternative explanation is that this region is usually deactivated during tasks but this task-related deactivation is disrupted in aMCI. This difference therefore may reflect subtle disruption of normal coordination of network interactions rather than some form of compensation. Although the reasons for the group differences are not fully clear, they certainly indicate that expanding the focus of fMRI tasks away from purely episodic and spatial memory tasks may be able to help understand the nature of changes in brain networks in aMCI more fully.

Our regression analyses demonstrated that, across all participants, the left anterior cingulate (BA32) was associated with performance and separate analysis of the groups separately indicates that this result was particularly driven by the aMCI patients. The

involvement of the anterior cingulate is potentially consistent with its purported role in selective attention in working memory tasks (Wager & Smith, 2003). Other fMRI work using a visuospatial associative working memory span task found increased activity in this region in aMCI patients at lower task loads (Kochan et al., 2010). Alternatively, the anterior cingulate has been linked to change in cognitive demands and cognitive effort (e.g., Engström, Landtblom, & Karlsson, 2013; Mulert, Menzinger, Leicht, Pogarell, & Hegerl, 2005). The fact that slower RTs were linked to increased anterior cingulate activity in patients may be consistent with this interpretation, although the ceiling performance found in patients and controls at 2-back suggests that participants did not find the tasks so challenging that they could not complete it successfully. No significant differences between the two groups was seen in the regression analysis, perhaps due to our relatively small numbers.

We also investigated whether this N-back task could identify differences in hippocampal activity in aMCI in a non-episodic or spatial memory task. Using an ROI analysis, we found a region in the left parahippocampus/hippocampus that showed a reduced task-related deactivation in the patient compared to the control group. This was not seen using a different working memory task (Kochan et al., 2010), perhaps due to having more power in our blocked N-back design. Our structure-function correlation within this cluster was not significant. In fMRI studies which have used episodic memory tasks in MCI, increased activity in the medial temporal lobes in patients is one of the most consistently reported results (e.g., Browndyke et al., 2013; Pihlajamäki et al., 2009). There is increasing evidence that the medial temporal lobes play a role in working memory (Ranganath & Blumenfeld, 2005) although this is usually restricted to associative or demanding tasks (Axmacher et al., 2007; Olson et al., 2006; Rissman et al.,

2008; Schon et al., 2009). Our patients performed as well as the control group, with near ceiling performance even at the 2-back level. Work with other patient groups has used the N-back task to look for medial temporal fMRI differences (Caseras et al., 2006; Snaphaan et al., 2009), highlighting how working memory tasks can provide insight more widely into abnormal brain function in MCI.

The N-back task is a continuous recognition task and differs from some standard neuropsychological measures of working memory. Typically, these tests assess working memory capacity, e.g., digit span, or involve additional manipulation of the information that is being remembered, e.g., letter-number-sequencing (see Conway, Kane, & Engle, 2003 for a review of working memory tasks). In interpreting the results, it is important to know whether this task reliably indexes working memory. As a task, it has received little attention with respect to its validity as a working memory measure, largely due to its development for neuroimaging rather than as a clinical indicator. Concerns about the N-back task have been raised by studies showing a lack of convergent validity with other established measures of working memory (e.g., Kane, Conway, Miura, & Colflesh, 2007; Miller, Price, Okun, Montijo, & Bowers, 2009; but see also Schmiedek, Hildebrandt, Lovden, Wilhelm, & Lindenberger, 2009). A potential lack of reliability in the task has also been highlighted (e.g., Jaeggi, Buschkuhl, Perrig, & Meier, 2010) meaning that the N-back task is not appropriate as a diagnostic task. However, here it is not being used to assess possible deficits and give a clinical assessment; indeed our aMCI patients were not impaired on the task as measured by accuracy or reaction time.

Other more demanding working memory tasks have been used with MCI patients in fMRI studies, such as visuospatial associative working memory tasks (Alichniewicz et al., 2012; Kochan et al., 2010; Kochan et al., 2011), complex working memory span tasks

(Faraco et al., 2013) and verbal delayed matching to sample tasks (Bokde et al., 2010). These tasks have shown varied results. Faraco et al (2013) found that patients recruited more widespread regions than controls, covering the medial temporal, frontal and parietal lobes. Similarly, Bodke et al (2010) found increased activity in their patient group in the frontal and parietal lobes. Conversely, Alichniewicz et al (2012) found hypoactivations, and no hyperactivations, in the patient group, in the middle and superior frontal gyri. The reasons behind these levels of variation are unclear, but could be due to differences in working memory load/task difficulty. Kochan et al (2010) reported aMCI hyperactivations in the anterior cingulate and precuneus at low working loads, but hypoactivations of the same regions at higher working memory loads. These results were found in the context of relatively matched performance between aMCI patients and controls, even at the most demanding level of the task (Kochan et al., 2010). Task differences may be particularly important in understanding how results from different studies converge and diverge. Patient performance levels can give an indication of how difficult the task is, with hypoactivations in patients also being reported using a task where patients had impaired performance (Alichniewicz et al., 2012).

The lack of a significant performance difference in our study could be attributable to our small sample size, although N-back performance has been shown to be unimpaired in multiple other studies with aMCI patients (Döhnell et al., 2008; Nordahl et al., 2005; Rombouts et al., 2005; Yetkin et al., 2006). It is also worth noting that this sample size was sufficient to show significant group differences on the standardised episodic memory tests used. This lack of performance differences can also be seen as strength since we find significant BOLD differences which are not confounded by significant

differences in task performance (e.g., see Nagel et al., 2009; Price & Friston, 1999). As a task to engage working memory processing, the N-back task remains important, particularly in the neuroimaging literature where the engagement of brain regions across studies is reliable and robust (Owen et al., 2005). Future work with the N-back task could try to replicate the working memory load dependent results reported by Kochan et al (2010), by increasing the load to higher levels. Increased levels of working memory load within the N-back task beyond the 2-back level used here might allow group differences to be seen, since ceiling effects are clear in the performance of both groups at these levels.

Our data has directly replicated a design used previously with aMCI patients and healthy controls (Rombouts et al., 2005). We did not find performance differences between the two groups on the task. The small sample size may also have left us underpowered to see some significant differences in BOLD activity. We have therefore used strict statistical thresholds to try to limit the possibility that our results reflect a type one error. This small number of participants will be a particular concern for the regression analyses. There have been legitimate concerns in the literature over the heterogeneity of MCI/aMCI patients and how this can make it difficult to directly compare studies (Stephan, Matthews, McKeith, Bond, & Brayne, 2007; Stephan et al., 2013; Ward, Arrighi, Michels, & Cedarbaum, 2012). Our relatively small group all underwent a full neuropsychological assessment and physical examination to allow us to be sure of the specificity of their diagnosis. Reporting details of scores on full neuropsychological batteries, as opposed to relying on a single brief index such as the MMSE, should allow similarities and differences between our patients and those of other studies to be easily identifiable. For example, our group shows a relatively high IQ

and education level. Our patients performed well on all but the episodic memory tasks in our battery. This suggests that these are relatively mild aMCI patients which may help to explain our hyperactivations/reduced deactivations. Certainly in the medial temporal lobe, hyperactivations in fMRI are usually seen more prominently in early rather than late stage MCI (Dickerson & Sperling, 2008).

Conclusion

Much focus on fMRI in aMCI patients has centred on episodic long-term memory and spatial memory tasks. There have been limited reports of fMRI tasks of working memory in aMCI patients specifically using the N-back task (Döhnelt et al., 2008; Rombouts et al., 2005; Yetkin et al., 2006), one focussed on emotional stimuli (Döhnelt et al., 2008) and one with auditory presentation (Yetkin et al., 2006). Here we have presented data from a working memory task, where differences in activation were seen in the absence of gross performance deficits. The significant effects in the insula converge with studies of other aspects of cognition in similar populations, with the additional finding of lingual gyrus activation. These types of task, with different patterns of BOLD activation in the absence of performance differences, may be important to fully understand the neural and cognitive changes associated with aMCI.

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Figure Captions

Figure 1 - The N-back task conditions. The target letter for each level is indicated by the red arrow.

Figure 2 - Performance on N-back task. Top panel shows RT data, with means and standard error of the mean presented. Bottom panel shows accuracy data, with median and interquartile range presented (data not normally distributed).

Figure 3 – Activation maps for contrasts of separate groups. Clusters are corrected for multiple comparisons on the basis of cluster extent ($p < .05$) with a voxel threshold of $p < .001$.

Figure 4 – Significant clusters for aMCI > Control in factorial model. See also Table 3. Clusters are corrected for multiple comparisons on the basis of cluster extent ($p < .05$) with a voxel threshold of $p < .001$

Table 1 – Performance by aMCI group and controls on standardised

neuropsychological tests. Data represents mean (SEM) with p(difference) showing results from t tests, except for tests of executive function which display median (IQR), with Mann-Whitney U test used to assess group differences. NART=National Adult Reading Test, WASI=Wechsler Abbreviated Scale of Intelligence, CVLT=California Verbal Learning Test, WMS-III=Wechsler Memory Scale Third Edition, VR=Visual Reproduction, LM=Logical Memory, TMT=Trail Making Task. CVLT scores are presented as T or Z scores (as standardly reported), WMS-II scores are presented as scaled scores (i.e. range 0 to 20), Hayling and Brixton scores are presented using the standardised scores defined in the test (i.e. range 0 to 10).

		Control	aMCI	p(difference)
N		11	10	-
Age		70.27 (6.20)	71.40 (6.35)	.685
No. Male		7	5	-
Intelligence	Yrs in education	15.64 (4.13)	16.00 (4.30)	.845
	NART IQ	121.55 (6.04)	120.10 (8.24)	.650
	WASI IQ	123.73 (15.74)	117.90 (16.20)	.414
Memory: CVLT	T Score	56.55 (11.34)	38.80 (15.11)	.006
	Short Delay	.227 (.848)	-1.350 (1.334)	.004
	Long Delay	.409 (.769)	-1.250 (1.670)	.014
Memory: WMS-III	LM Immediate	11.82 (2.86)	7.80 (3.71)	.011
	LM Delay	13.00 (2.00)	9.20 (3.33)	.005
	VR Immediate	13.09 (3.08)	8.90 (4.25)	.017
	VR Delay	14.82 (2.60)	9.10 (4.18)	.002
Executive Function: Hayling and Brixton	Hayling	6.00 (1.00)	6.00 (3.00)	.214
	Brixton	6.00 (5.00)	2.00 (4.00)	.368
Executive Function: TMT	A Time	34.00 (20.00)	40.00 (19.00)	.136
	B Time	61.00 (48.00)	92.00 (49.00)	.119

Table 2 – Performance by aMCI group and controls on N-back test, as indicated by hit and false alarm rates. Data represents median (IQR).

	Control		aMCI	
	Hit rate	False alarm rate	Hit rate	False alarm rate
0-back	1.00 (.00)	.00 (.00)	1.00 (.08)	.00 (.03)
1-back	1.00 (.00)	.00 (.00)	1.00 (.11)	.00 (.03)
2-back	1.00 (.00)	.00 (.00)	.89 (.31)	.00 (.00)

Table 3 – Significant clusters for fMRI results.

Region	BA Area	Peak MNI Co-ordinates	Cluster Size	Z	p(FWE-corr)
Controls 1-back > 0-back					
R Lateral Premotor	6	36 -6 48	1256	4.66	<.001
R Fusiform Gyrus	19	40 -82 4	545	4.21	<.001
L Fusiform Gyrus	19	-40 -67 -12	268	3.94	.016
L Lateral Premotor	6	-40 3 30	639	4.10	<.001
L Lateral Premotor	6	-28 -16 45	873	4.00	<.001
L Precuneus	7	-26 -64 31	884	3.76	<.001
Controls 2-back > 0-back					
L Cerebellum	-	-28 -73 -26	4158	5.52	<.001
R Inferior Parietal Lobule	40	40 -54 36	9726	5.44	<.001
R DLPFC	46	39 36 30	12010	5.29	<.001
R Cerebellum	-	27 -64 -32	469	4.63	<.001
L Caudate	-	-24 -7 24	427	4.53	<.001
R Midbrain	-	6 -27 -14	406	4.40	.001
R Fusiform Gyrus	37	48 -63 -18	278	4.09	.007
R Frontal Pole	10	29 59 16	213	3.87	.026
Controls 2 back < 0-back					
L Posterior Cingulate	23	-8 -57 12	3155	5.51	<.001
R Insula	-	51 -19 9	1273	4.85	<.001
L Superior Temporal Gyrus	39	-63 -51 25	228	4.25	.019
R Anterior Cingulate	24	4 -15 40	353	4.05	.002
Controls 1-back > 2-back					
L Frontal Eye Fields	8	-12 38 40	303	4.31	.011
L Dorsal Posterior Cingulate	31	9 -52 27	403	4.11	.002
L Middle Temporal Gyrus	39	-44 -61 24	414	4.08	.002
aMCI 1-back > 0-back					
R Precuneus	7	22 -51 55	292	4.56	.032
aMCI 1-back < 0-back					
L Medial Rostral Prefrontal Cortex	10	-9 54 1	1644	5.63	<.001
L Posterior Cingulate	31	-12 -63 21	758	5.11	<.001
aMCI 2-back > 0-back					
R Lateral Premotor	6	47 -15 24	6620	4.67	<.001

L Precuneus	7	-18 -57 52	2892	4.56	<.001
L Pons	-	-10 -22 -30	245	4.24	.035
L Lateral Premotor	6	-52 -1 19	2685	4.15	<.001
R Cerebellum	-	4 -48 -27	588	4.07	<.001
R Lingual Gyrus	18	30 -82 -8	407	4.04	.003
R Lateral Rostral Prefrontal Cortex	10	44 48 19	375	3.93	<.001
aMCI 2 back < 0-back					
L Medial Rostral Prefrontal Cortex	10	-9 60 -8	4873	5.05	<.001
L Precuneus/Posterior Cingulate	23/31	-2 -66 -19	606	4.16	<.001
aMCI > Control					
R Lingual Gryus	17	20 -85 4	565	5.11	.017
R Insula	13	42 -15 19	965	4.85	.001
R Insula	13	44 -36 18	477	4.13	.033

Table 4 – Significant clusters for VBM results where reduced grey matter was found in aMCI patients compared to controls.

Region	BA Area	Peak MNI Co-ordinates	Cluster Size	Z	p _(uncorrected)
L Amygdala/Entorhinal Cortex	34	-21 2 -18	1173	4.30	<.001
L Cerebellum	-	-4 -37 -3	853	4.69	<.001
L Middle Temporal Area	21	-54 -55 19	248	3.82	<.001
L Inferior Temporal Area	20	-52 -33 -23	1462	5.02	<.001
R Inferior Temporal Area	20	66 -39 -15	142	3.75	<.001
R Frontal Orbital Cortex	11	21 32 -24	305	4.43	<.001