Hippocampal dysfunction in patients with mild cognitive impairment: A functional neuroimaging study of a visuospatial paired associates learning task

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Mild cognitive impairment (MCI) patients report memory problems greater than those normally expected with ageing, but do not fulfil criteria for clinically probable Alzheimer’s disease. Accumulating evidence demonstrates that impaired performance on the Paired Associates Learning (PAL) test from the Cambridge Neuropsychological Test Automated Battery (CANTAB) may be sensitive and specific for early and differential diagnosis of Alzheimer’s disease. We adapted the basic CANTAB PAL task for functional magnetic resonance imaging (fMRI) in order to examine the functional brain deficits, at encoding and retrieval separately, in patients with MCI compared to healthy matched volunteers. As well as investigating the main effects of encoding and retrieval, we characterized neural responses in the two groups to increasing memory load. We focused on changes in BOLD response in the hippocampus and related structures, as an a priori region of interest based on what is known about the neuropathology of the early stages of Alzheimer’s disease and previous information on the neural substrates of the PAL task. We also used structural MRI in the same patients to assess accompanying structural brain abnormalities associated with MCI.

In terms of the BOLD response, the bilateral hippocampal activation in the MCI and control groups depended upon load, the MCI patients activating significantly more than controls at low loads and significantly less at higher loads. There were no other differences between MCI patients and controls in terms of the neural networks activated during either encoding or retrieval of the PAL task, including the prefrontal, cingulate and temporal cortex. The functional deficit in hippocampal activation in the MCI patients was accompanied by structural differences in the same location, suggesting that the decrease in hippocampal activation may be caused by a decrease in the amount of grey matter. This is one of the first studies to have used both encoding and retrieval phases of a memory paradigm for fMRI in MCI patients, and to have shown that the BOLD response in MCI patients can show both hyperactivation and hypoactivation in the same individuals as a function of memory load and encoding/retrieval. The findings suggest that performance on PAL might be a useful cognitive biomarker for early detection of Alzheimer’s disease, especially when used in conjunction with neuroimaging.

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1. Introduction

As populations age worldwide, prevalence rates of age-related cognitive disorders are rapidly increasing. Alzheimer’s disease (AD) accounts for about 60% of all dementia cases and thus the urgency of this problem is fast increasing (Beddington et al., 2008). Drugs that are hoped will modify disease progression are currently under development, creating an urgent need for the early detection of AD to enable preventative treatment to avoid any further neuropathology and ensuing cognitive decline (Geerts, 2007; Masters & Beyreuther, 2006; Vellas, Andrieu, Sampaio, & Wilcock, 2007). The focus to identify individuals in the prodrome of AD has primarily been on patients who report memory problems greater than...
Demographic details of the patients with mild cognitive impairment and controls.

<table>
<thead>
<tr>
<th></th>
<th>Controls Mean (SD)</th>
<th>MCI Mean (SD)</th>
<th>p-value</th>
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</thead>
<tbody>
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<td>n.s.</td>
</tr>
<tr>
<td>Gender (female:male)</td>
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<td>3/12</td>
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</tr>
<tr>
<td>MMSE</td>
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<td>25.93 (1.33)</td>
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<tr>
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<td>Range = 24–38</td>
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<tr>
<td>CDR sum of boxes</td>
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<td>1.0 (0.45)</td>
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</tr>
<tr>
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<td>Range = 0.3–3</td>
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<td></td>
</tr>
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<td>NART</td>
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<td>113.40 (5.79)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Range = 105–122</td>
<td>Range = 116–122</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geriatric Depression Scale</td>
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<td>2.27 (2.69)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Range = 0–7</td>
<td>Range = 0–9</td>
<td></td>
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</tr>
<tr>
<td>Digit span (combined forwards and backwards scores)</td>
<td>15.8 (2.7)</td>
<td>14.2 (3.0)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Range = 10–20</td>
<td>Range = 11–19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1

from the decline found in healthy ageing and in other neuropsychological disorders (Petersen et al., 1999).

However, AD can be very difficult to diagnose at the MCI stage because the associated cognitive decline is not easily discriminated from the decline found in healthy ageing and in other neuropsychological disorders (Petersen et al., 2004). Recently, important progress has been made using the Paired Associates Learning (PAL) test from the Cambridge Neuropsychological Test Automated Battery (CANTAB) in discriminating between those MCI patients who will go on to develop AD and those who will not (Blackwell et al., 2004; Swainson et al., 2001). Accumulating evidence demonstrates that impaired performance on the CANTAB PAL, which is a cued recall test of memory, may be sensitive and specific for the early and differential diagnosis of AD (Blackwell et al., 2004; de Jager, Lesk, Zhi, Marsico, & Chandler, 2008). Cited values for sensitivity and specificity indices were 0.94 and 0.91, respectively, and therefore CANTAB PAL may have value as a cognitive biomarker for early detection of AD (Beddington et al., 2008). CANTAB PAL may also play a role in evaluating new anti-dementia therapies (Greig et al., 2005) since it was shown to be sensitive to disease progression (Ahmed, Mitchell, Arnold, Nestor, & Hodges, 2008).

Although neuropsychological damage underlying AD eventually affects the entire brain volume (Fox, Warrington, & Rosser, 1999), brain regions are differentially affected in histological terms. Neuroradiological tangles, made up of hyperphosphorylated tau, first deposit pathology in the transentorhinal cortex, spreading early to the entorhinal cortex and hippocampus proper (Braak & Braak, 1991). This observation accords with numerous imaging studies that have found mesial temporal atrophy in early AD including at the MCI stage (Jack et al., 2008; Pennanen et al., 2005). Hypometabolism and atrophy of the posterior cingulate cortex are also present in the MCI-stage of AD (Minoshima et al., 1997; Nestor, Fryer, Smielewski, & Hodges, 2003; Pengas, Hodges, Watson, & Nestor, 2010); the recent development of in vivo amyloid ligand imaging has highlighted this region as one of the most intense sites of amyloid deposition in early AD (Rowe et al., 2007; Ziolko et al., 2006).

CANTAB PAL is a visuospatial associative learning test that is sensitive and specific for the diagnosis of AD (Sahakian et al., 1988), and is also sensitive to the MCI-stage (Swainson et al., 2001). Memorizing objects in space is a function well-known to engage the hippocampus (Maguire, Frith, Burgess, Donnett, & O’Keeffe, 1998; O’Keeffe & Nadal, 1978; Smith & Milner, 1981), presumably because it is a site at which spatial and object processing converges (Jones & Powell, 1970). Lesions of the hippocampus and adjacent regions including the parahippocampal gyrus in rhesus monkeys
Fig. 1. CANTAB PAL task adapted for fMRI. (a) Structure of the experiment. Per subject there are 2 fMRI sessions with a short break in between during which the subject rests but does not leave the scanner. Both sessions contain 2 sets of patterns for each memory load (2 times PAL4, 2 times PAL6 and 2 times PAL8) and 1 set of each control condition (1 control condition for PAL4, 1 for PAL6 and 1 control condition for PAL8). These nine blocks appear in pseudorandom order. Block order was counterbalanced between subject-groups. Each block consists of 4 phases: the encoding phase of the first repetition, the retrieval phase of the first repetition, the encoding phase of the second repetition and the retrieval phase of the second repetition. There is an 8.1 s delay after every phase. (b) Structure of an encoding phase. Example stimuli from the encoding phase of the task. 4, 6 or 8 patterns are displayed (2 example patterns are shown) sequentially in the available boxes (in the 8-pattern stage 2 more boxes are added). (c) Structure of a retrieval phase. In the retrieval phase each pattern is then presented in the centre of the display in random order and the subject is required to press a button to move a red square around over the available boxes and then press another button to confirm the choice for the box in which the pattern was previously seen. Regardless of the number of errors all the patterns will be redisplayed in their original locations, followed by another retrieval phase (repetition 2, see Fig. 1a). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)
ulated in the functional imaging task by varying the number of item-location pairings that participants were required to encode and retrieve.

2. Materials and methods

2.1. Participants

Twenty MCI patients were recruited from the Memory Clinic at Addenbrooke’s Hospital in Cambridge (UK). The selection procedure for MCI patients was based upon Petersen criteria (Petersen et al., 1999) as follows: subjective memory symptoms corroborated by an informant; objective memory impairment (lower than 1.5 standard deviations relative to controls on measures of delayed free recall on the Rey Auditory Verbal Learning Test; preserved Activities of Daily Living as ascertained by the Cambridge Behavioural Inventory (Nagahama, Okina, Suzuki, & Matsuda, 2006); and no evidence of dementia (based on Mini-Mental State Examination (MMSE), Folstein, Folstein, & McHugh, 1975) score of at least 24 and Clinical Dementia Rating (Morris, 1993) score of 0.5. Seven of the MCI sample have proceeded to a diagnosis of Alzheimer’s disease (after an average of about 2 yr following these scans) and the others had stable MCI, although some were lost to follow up at 12 months. Importantly, none of these patients improved over time. Twenty healthy elderly volunteers were recruited from the general public and matched by age and pre-morbid IQ to the MCI patients (Table 1). Exclusion criteria were restricted to exclusions for MRI imaging (metallic foreign body, claustrophobia, etc.), concurrent medication targeting the central nervous system and psychiatric diagnosis. Vision was normal or corrected-to-normal in every participant. All participants gave written informed consent according to the Helsinki Declaration and the study was approved by the Ethics Committee of the University of Cambridge. Control subjects were excluded if they had had a psychiatric illness, epilepsy, a serious head injury, loss of consciousness, surgery requiring extended hospitalisation, medication that would interfere with cognitive performance or medication for depression or anxiety. In the control group two participants were excluded because of equipment malfunction, one participant was excluded because of excessive movement, and one participant was excluded because his recall performance was not significantly different from chance level. In the MCI group two participants were excluded because they were unable to finish the task and three participants were excluded because their recall performance was not significantly different from chance level. Sixteen volunteers were finally included in the control group and fifteen volunteers were included in the MCI group (age of the control group: range 60–76; age of the MCI group: range 63–77; n.s.).

2.2. Experimental procedure

In the CANTAB PAL test, visual abstract, coloured patterns (‘objects’) are presented (and extinguished) randomly and sequentially, one by one in six or eight boxes around the edge of a touch screen computer. After a brief delay, the same patterns are presented in the middle of the screen in random order and the subject is required to touch the box in which they saw that pattern appear (www.cantab.com). We converted the CANTAB PAL for use in neuroimaging studies, but made as few changes as possible to maintain the comparability between the neuroimaging and the CANTAB PAL. Thus, in the neuroimaging PAL: 4 (PAL4), 6 (PAL6) or 8 (PAL8) abstract coloured patterns were presented one-by-one in random order in six or eight boxes (encoding phase, Fig. 1a and b) back-projected via an LCD-projector on the Rey Auditory Verbal Learning Test; preserved Activities of Daily Living as ascertained by the Cambridge Behavioural Inventory (Nagahama, Okina, Suzuki, & Matsuda, 2006); and no evidence of dementia (based on Mini-Mental State Examination (MMSE), Folstein, Folstein, & McHugh, 1975) score of at least 24 and Clinical Dementia Rating (Morris, 1993) score of 0.5. Seven of the MCI sample have proceeded to a diagnosis of Alzheimer’s disease (after an average of about 2 yr following these scans) and the others had stable MCI, although some were lost to follow up at 12 months. Importantly, none of these patients improved over time. Twenty healthy elderly volunteers were recruited from the general public and matched by age and pre-morbid IQ to the MCI patients (Table 1). Exclusion criteria were restricted to exclusions for MRI imaging (metallic foreign body, claustrophobia, etc.), concurrent medication targeting the central nervous system and psychiatric diagnosis. Vision was normal or corrected-to-normal in every participant. All participants gave written informed consent according to the Helsinki Declaration and the study was approved by the Ethics Committee of the University of Cambridge. Control subjects were excluded if they had had a psychiatric illness, epilepsy, a serious head injury, loss of consciousness, surgery requiring extended hospitalisation, medication that would interfere with cognitive performance or medication for depression or anxiety. In the control group two participants were excluded because of equipment malfunction, one participant was excluded because of excessive movement, and one participant was excluded because his recall performance was not significantly different from chance level. In the MCI group two participants were excluded because they were unable to finish the task and three participants were excluded because their recall performance was not significantly different from chance level. Sixteen volunteers were finally included in the control group and fifteen volunteers were included in the MCI group (age of the control group: range 60–76; age of the MCI group: range 63–77; n.s.).

2.3. MRI data acquisition

MRI data were acquired at the MRC-Cognition and Brain Sciences Unit in Cambridge. Whole head T2*-weighted EPI-BOLD fMRI data were acquired with a Siemens Trio 3T MR scanner with Tim (total imaging matrix technology) using a descending slice acquisition sequence (EPI; volume TR = 2000 ms, TE = 30 ms, 78° flip-angle, 32 axial slices, slice-matrix size = 64 × 64, slice thickness = 3 mm, 0.75 mm gap, voxel-size = 3 mm × 3 mm × 3 mm). Task presentation and recording of the behavioural responses was performed using Visual Basic 2005. Functional data were acquired in two runs of 20 m each. Additionally, high-resolution structural MR images were acquired with a T1-weighted MP-RAGE sequence (volume TR = 2.25 s, TE = 3.93 ms, 15° flip-angle, 176 sagittal slices, slice-matrix size = 256 × 256, slice thickness = 1 mm, no slice gap, voxel-size = 1 mm × 1 mm × 1 mm).

2.4. MR image preprocessing and statistical analysis

Image preprocessing and statistical analysis was executed with the Statistical Parametric Mapping 5 software (SPM5, Wellcome Department of Imaging Neuroscience, University College London, London, UK; www.fil.ion.ucl.ac.uk). The first six images were discarded in order to reduce T1 saturation effects. The functional EPI-BOLD images were realigned and the subject-mean functional MR images were co-registered with the corresponding structural MR images using mutual information optimization. The structural MR images were processed with SPM5, which enables spatial normalization – i.e. warping to MN152 standard space (Montreal Neurological Institute and Hospital, McGill University, Montreal, Canada) – tissue classification and radio-frequency bias correction to be combined within its model (Ashburner & Friston, 2005). Preprocessing steps such as skull-stripping and bias-field correction are known to improve the performance of warping algorithms (Acosta-Cabronero, Williams, Pereira, Pangas, & Nestor, 2008; Fein et al., 2006); hence raw whole-head scans were pre-processed prior to SPM5 with the automated pipeline of registered elsewhere (Acosta-Cabronero et al., 2008; Pereira et al., 2010). Briefly, skull-stripping was performed using the hybrid watershed algorithm, HWA (Sergonne et al., 2004). Stripped volumes were then bias-corrected using the non-parametric uniform intensity normalization or N3 v.1.10 (Sled, Zijdenbos, & Evans, 1998) and finally, fine brain extractions were performed using BET v.2.1 (Smith, 2002) to evaluate brain morphometry using voxel-based morphometry (VBM: Ashburner & Friston, 2000), grey matter segments were modulated to preserve the total amount of tissue, and spatially filtered with an isotropic, 8-mm FWHM Gaussian kernel (Hayasaka & Nichols, 2003; Petersen et al., 1999). A general linear model (GLM) was then fit at each voxel, with one variable of interest, and a nuisance covariate: total intracranial volume (TIV) calculated using a previously described method (Pengas, Pereira, Williams, & Nestor, 2009). Grey matter differences between groups were assessed using a two population t-test (patients worse than controls); the resulting statistical parametric map was thresholded at uncorrected p < 0.001. We found no differences in total intracranial volume (TIV) or in overall grey matter density between our healthy control group and our MCI group (average TIV in the control group: 1.7 ± 0.2 in the MCI group: 1.8 ± 0.2; t-test p > 0.05). A post hoc analysis of the reverse contrast (patients better than controls) showed no brain matter density loss in controls relative to patients (p > 0.05).

To spatially normalize the functional MR images, the transformation matrices generated from the unified segmentation process were applied; the resulting warped images were then smoothed with an 8-mm FWHM kernel. The fMRI data were proportionally scaled to account for global effects and analyzed statistically using a general linear model and statistical parametric mapping (Friston, Holmes, Worsley, Poline, & Frackowiak, 1995). The linear model included convolved explanatory variables (box-car regressors) modeling the experimental conditions in a blocked fMRI design. The 24 experimental conditions modeled included a factorial design of memory load (PAL4, PAL6, PAL8) by memory stage (encoding, delay, retrieval) (Fig. 1c). The retrieval phase was followed by another brief delay (8.1 s) after which the next encoding phase (i.e. for 6 or 8 item loads) started. Because of the 8.1 s delay, each phase started at a different point on the BOLD response. No further jittering was included because the fMRI data were analyzed in a block design. The following phase started at a different point on the BOLD response. No further jittering was included because the fMRI data were analyzed in a block design. The following phase started at a different point on the BOLD response. No further jittering was included because the fMRI data were analyzed in a block design. The following phase started at a different point on the BOLD response. No further jittering was included because the fMRI data were analyzed in a block design. The following phase started at a different point on the BOLD response. No further jittering was included because the fMRI data were analyzed in a block design.
effects of no-interest: session/subject-effects, realignment parameters, and a temporal high-pass filter to account for various low-frequency effects. In the statistical analysis, all relevant contrasts were set up: every memory-related condition minus the corresponding control condition as a baseline (for example PAL4 encoding minus PAL4 encoding control condition); and used to generate contrast images for each subject, which were subsequently subjected to a second-level random effect analysis, using ANOVA. The terms of activation and deactivation are used as synonyms for a relative increase and decrease in BOLD signal respectively.

To test for the specific role of the hippocampus and parahippocampal gyrus during encoding and retrieval, based on the findings reviewed in the Introduction, the hippocampus and the parahippocampal gyrus were a priori defined as regions of interest (ROI) using the pick atlas tool (version 2.4: http://fmri.wfubmc.edu/cms/software)(Maldjian, Laurienti, Kraft, & Burdette, 2003). To test null hypotheses in ROI analysis, a small volume correction for multiple testing was applied using the false discovery rate (FDR) method (Genovese, Lazar, & Nichols, 2002). For whole brain random effect analysis, correction for multiple non-independent comparisons was based on the family-wise error (FWE) rate (Worsley et al., 1996). In the second-level random effect analysis, the following contrasts were made both for the encoding and the retrieval phase: the main effects irrespective of load, group differences in these main effects, the main effect of load and group differences in load, as well as the load x group interactions, and their subsequent post hoc tests.

We investigated deactivations in the contrast ‘control condition versus PAL4’ and checked for group effects. To investigate regional attenuated deactivations in patients we masked this contrast with the main effect of deactivation in the control group and then looked for brain areas in which the deactivations were larger in the control than in the MCI group. To investigate regional augmented deactivations in patients we masked this contrast with the main effect of deactivation in the MCI group and then looked for brain areas in which the deactivations were larger in the MCI than in the control group.

3. Results

The MCI and control groups were well matched for age, pre-morbid verbal IQ and gender distribution. There was also no significant difference in scores on the Geriatric Depression Scale or digit span although, as expected, they differed significantly in MMSE scores \(t(29) = 4.52, p < 0.001\) (Table 1). In addition, MCI patients were given the Addenbrooke’s Cognitive Examination Revised (ACE-R, Mioshi, Dawson, Mitchell, Arnold, & Hodges, 2006).

Table 2
Addenbrooke’s Cognitive Examination Revised (ACE-R) scores of MCI patients and control population.

<table>
<thead>
<tr>
<th></th>
<th>Controls Mean (SD)</th>
<th>MCI Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orientation/attention (Max = 18)</td>
<td>17.87 (0.34)</td>
<td>17.20 (1.08)</td>
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<tr>
<td>Memory (Max = 26)</td>
<td>23.73 (2.31)</td>
<td>16.40 (4.39)</td>
</tr>
<tr>
<td>Verbal fluency (Max = 14)</td>
<td>11.58 (1.86)</td>
<td>9.73 (2.09)</td>
</tr>
<tr>
<td>Language (Max = 26)</td>
<td>24.65 (1.37)</td>
<td>24.47 (1.30)</td>
</tr>
<tr>
<td>Visuospatial (Max = 16)</td>
<td>15.59 (0.83)</td>
<td>15.33 (0.90)</td>
</tr>
<tr>
<td>Total (Max = 100)</td>
<td>93.42 (4.20)</td>
<td>83.13 (6.08)</td>
</tr>
</tbody>
</table>

As can be seen in Fig. 2, at all three difficulty levels the percentage of correctly recalled object-location associations was significantly lower in the MCI group compared to the control group [PAL4: \(t(29) = 6.33, p < 0.001\); PAL6: \(t(29) = 3.74, p < 0.001\); PAL8: \(t(29) = 5.32, p < 0.001\)]. The effect sizes were 2.28, 1.34 and 1.92 for PAL4, PAL6 and PAL8, respectively. In addition to the main effect of group, an Analysis of Variance (ANOVA) with group, difficulty and repetition as independent factors revealed main effects of difficulty \(F(2,58) = 29.00, p < 0.001\) and repetition \(F(1,29) = 150.10, p < 0.001\). The interaction between difficulty and group did not reach significance, but was marginal \(F(2,58) = 2.88, p < 0.05\). Their scores are reported in Table 2 along with the scores of a reference control population.

3.1. Behavioural results

Fig. 2. Overall cued recall performance (percentage correct answers ± SEM) in the control and the MCI group. White bar Con = control group, \(n = 16\). Grey bar MCI = MCI group, \(n = 15\). Healthy volunteers (Con) performed significantly better than MCI patients (MCI) in every PAL-stage tested (PAL4, PAL6 and PAL8: \(p < 0.001\)). **\(p < 0.001\).

Fig. 3. ROI analysis revealed significant bilateral activations in the hippocampus in all participants during encoding. Activations are shown superimposed onto selected slices of an individual high-resolution T1-weighted volume. Only significant clusters are shown \((p < 0.05\) FDR corrected).
p < 0.07], which stemmed from a slightly smaller group difference in PAL6 compared to PAL4 and PAL8. Cued recall performance was significantly above chance at all three levels of difficulty in both groups (Control group: PAL4: t(15) = 16.50, p < 0.001; PAL6: t(15) = 12.73, p < 0.001; PAL8: t(15) = 10.14, p < 0.001; MCI group: PAL4: t(14) = 4.26, p < 0.001; PAL6: t(14) = 6.23, p < 0.001; PAL8: t(14) = 5.72, p < 0.001). For PAL4 and PAL6, chance level is 16.7% and for PAL8, chance level is 12.5%. No other interactions approached significance (p > 0.05). The number of non-responses was low in both groups (total average: MCI group: 2.47, (range = 0–8); control group: 0.93 (range = 0–7)) but was nonetheless significantly different [F(1,29) = 12.6, p < 0.01].

3.2. Neuroimaging results

3.2.1. Functional imaging

3.2.1.1. Encoding stage. ROI analyses in the hippocampus for all participants examining the effects of encoding of the object-location associations as compared to the visual control conditions, revealed significant bilateral activations (Fig. 3, p < 0.05, FDR corrected). Interrogating this finding further revealed that this effect was primarily due to significant bilateral activations in the hippocampus in control participants (p < 0.05, FDR corrected) as no significant effect was found for the MCI group alone. No significant effects were found for the parahippocampal gyrus ROI (p > 0.05 FDR corrected).

ROI analyses found no significant main effects of memory load, although there was a significant group by load interaction for the hippocampus bilaterally. As shown in Fig. 4 this is driven by an increase in hippocampal activation with increasing load in the control group and a decreasing hippocampal activation with increasing memory load in the MCI group (Fig. 5, p < 0.05 FDR corrected, MNI coordinates were −30, −14, −20 and 32, −12, −20 for left and right hippocampus respectively). Post hoc analyses revealed a significant difference between the control group and the MCI group during the PAL8, t(29) = 2.8, p = 0.009; a trend toward significant results in the PAL4, t(29) = −1.942, p = 0.062; and no significant difference during the PAL6, t(29) = 0.603, p = 0.551.

In a whole brain, random effect analysis, we investigated whether there were effects of encoding of the object-location associations in other parts of the brain. We examined encoding as compared to the visual control conditions and found significant activation of several brain areas, including the inferior and middle frontal gyri, superior temporal gyrus, extending into parietal cortex, middle occipital and cingulate gyri (p < 0.05, FWE corrected, Fig. 5 and Table 3). There were no significant effects of group, memory load or repetition in this analysis. The whole brain contrast of

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Brain regions differentially activated during the encoding and during the retrieval phases of the PAL task. Not including extranuclear local maxima and brain stem areas.</th>
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<tr>
<td>Side (L/R)</td>
<td>Z-score</td>
</tr>
<tr>
<td>Encoding</td>
<td></td>
</tr>
<tr>
<td>Middle occipital gyrus</td>
<td>R</td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
<td>L</td>
</tr>
<tr>
<td>Middle cingulate gyrus</td>
<td>R</td>
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<tr>
<td>Middle frontal gyrus</td>
<td>R</td>
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</tr>
<tr>
<td>Lingual gyrus</td>
<td>L</td>
</tr>
<tr>
<td>Precuneus</td>
<td>R</td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>R</td>
</tr>
</tbody>
</table>

| Retrieval |                                      |
| Posterior cingulate | L | 5.09 | −8 | −54 | 12 |
| Cuneus | R | 4.57 | 2 | −78 | 30 |
Fig. 6. ROI analysis revealed significant bilateral activations in the parahippocampal gyrus in all participants during retrieval. Activations are shown superimposed onto selected slices of an individual high-resolution T1-weighted volume. Only significant clusters are shown (p < 0.05 FDR corrected).

Fig. 7. Brain regions activated in a whole brain random effect analysis comparing the retrieval phases versus the visuo-motor control conditions in all participants. Activations are shown superimposed onto selected slices of an individual high-resolution T1-weighted volume. Only significant clusters are shown (p < 0.05: FWE corrected).

load x group showed a significant cluster in the left hippocampus (p < 0.001, uncorrected; MNI coordinates: −30, −14, −20). There were no regional deactivations in the control group that are significantly attenuated or augmented in the patients.

3.2.1.2. Retrieval stage. ROI analyses of the effects of retrieval of the pattern-location associations as compared to the visuomotor control conditions in all participants revealed significant bilateral activations in the parahippocampal gyrus (Fig. 6, p < 0.05, FDR corrected) but no effect was found in the hippocampus. Investigating this finding further suggested that it was driven by the control group, given significant bilateral activations in the parahippocampal gyrus for the controls but not for the MCI group (p < 0.05, FDR corrected; MNI coordinates were −18, −40, −8 and 16, −40, −6 for the left and right parahippocampal gyrus respectively). No significant effects were found for the hippocampus ROI (p > 0.05 FDR corrected). ROI analyses examining the interaction between memory load and group did not reveal any significant results.

In a whole brain random effect analysis, we investigated whether there were effects of retrieval of the object-location associations in other parts of the brain. We examined retrieval as compared to the visuomotor control condition. There was significant activation of the posterior cingulate and left cuneus (Fig. 7, p < 0.05, FWE corrected, Table 3). There were no significant effects of group, memory load or repetition in this analysis. The whole brain contrast of load x group showed significant activation in the left hippocampus (p < 0.001, uncorrected; MNI co-ordinates: −18, −8, −16).

3.2.2. Structural imaging: voxel-based morphometry

Whole brain analysis showed that, compared with the healthy control group, the MCI group had significant reductions of grey matter density in the left and right hippocampus (MNI coordinates: −26, −14, −12 and 28, −10, −12 respectively) and in the left precuneus (MNI coordinates: −4, −74, 54) (Fig. 8).

4. Discussion

This study has shown that the neural network activated by a commonly used and highly sensitive neuropsychological test for MCI and early AD, requiring visuospatial paired associates learning, includes the hippocampus during encoding and the parahippocampal gyrus during retrieval. MCI patients performing the task exhibited both hyper-activation and hypo-activation of the hippocampus as a function of memory load during encoding. In addition, we found corresponding structural deficits in the hippocampus of MCI patients compared to healthy controls.

Medial temporal lobe degeneration has long been assumed to be the main neural substrate for the early memory deficits observed in Alzheimer’s disease, including prodromal amnestic MCI-stage AD, as revealed by structural or metabolic neuroimaging (Apostolova et al., 2006; Chetelat et al., 2003; Jack et al., 2008, 2009; Whitwell...
et al., 2007). In contrast, fMRI has been less employed in the analysis of memory decline in MCI, although its use is growing (Bai et al., 2009; Dannhauser et al., 2008; Dickerson et al., 2004, 2005; Dickerson & Sperling, 2008; Machulda et al., 2003; Small, Perera, DeLaPaz, Mayeux, & Stern, 1999; Trivedi et al., 2008).

The early fMRI studies tended in general to reflect the findings of other modalities. Thus, Small et al. (1999), Machulda et al. (2003) and Johnson et al. (2004) all found that patients with MCI or early Alzheimer’s disease exhibited less activation during a memory test than healthy controls. Bai et al. (2009) found altered patterns of hippocampal connectivity during episodic memory retrieval. By contrast, a number of more recent studies have found greater hippocampal activation in MCI during fMRI (Celone et al., 2006; Dickerson et al., 2004, 2005; Hamalainen et al., 2006) under a variety of testing conditions. A recent example of this is the study by Trivedi et al. (2008) which found greater right hippocampal activation (but less frontal cortex activation) relative to controls during certain test conditions, specifically the intentional encoding of items subsequently recognized.

In their review of fMRI studies of MCI, Dickerson and Sperling (2008) conclude that early in the course of this disorder, when memory deficits and hippocampal atrophy are less prominent, hyperactivation of medial temporal lobe circuits possibly represents compensatory activity in response to the task employed. This compensatory hyperactivity may be analogous to that reported in several other brain disorders or disease, although Dickerson and Sperling discuss alternative possibilities linked more specifically to the Alzheimer disease process. Later in the course of the transition from MCI to clinical Alzheimer’s disease, functioning of the MTL deteriorates further to an extent that such compensatory activity is no longer possible. The hyperactivity in early MCI might then represent a possible predictor or biomarker of the progression to Alzheimer’s disease.

The present study confirmed the importance of medial temporal lobe structures, either when specifically focusing on the hippocampus (during encoding, Fig. 4a) and parahippocampal gyrus (during retrieval, Fig. 4b) as regions of interest, specified either a priori or emerging from the whole brain, random effect analysis (for encoding, the left hippocampus, as a function of memory load). Whereas control subjects exhibited increases in activity from the lighter to the heavier loads during the encoding stage of the visual object-location memory task, MCI patients exhibited the opposite trend: significant increases over control subjects at light memory loads, but significant reductions for the heaviest memory load (Fig. 4a). These changes were not directly reflected in memory performance, which was significantly impaired at all levels of load, although there was a marginally significant tendency for the deficit in MCI patients to be less at the intermediate memory load. One possible interpretation of this finding is that the MCI patients attempted to recruit additional processing resources within the hippocampus when the task was still tractable for them, but could not do so when the task was at its most challenging, perhaps because of their failure to encode efficiently. In the present study we thus saw both hippocampal hyperactivity and hypoactivity in the same patients as a function of memory load. By comparison with the MCI patients tested by Dickerson and Sperling (e.g. 2005) our MCI patients were not particularly mild, with MMSE average scores of about 26 (compared with 29.6 ± 0.5SD); this might explain why we were able to observe both hypoactivity and hyperactivity, as a function of task difficulty. O’Brien et al. (2010) have noted that hippocampal hyperactivity may herald the prodromal stages of Alzheimer’s disease and be an indicator of impending hippocampal failure.

The memory material for the present study has most in common with that used by Gould et al. (2005), Gould, Arroyo, et al. (2006) and Gould, Brown, et al. (2006) who adopted the CANTAB PAL design and mode of stimulus presentation, but used drawings of everyday objects rather than abstract stimuli and tested patients with mild Alzheimer’s disease rather than MCI. In these studies hippocampal deficits were not found but may have been underestimated because their use of everyday objects may have enabled strategies for encoding that changed the nature of the task. Moreover, they
used a force choice recognition paradigm to test retrieval, which is clearly very different from the standard PAL approach in which the participant is presented with a stimulus and must recollect its prior position.

Previous neuropsychological studies using PAL have shown impairments in MCI patients that predict the future diagnosis of Alzheimer’s disease up to 32 months later, indicating that the CANTAB PAL task may be a neuropsychological “marker” of preclinical AD (Blackwell et al., 2004; Fowler, Salig, Conway, Semple, & Louis, 1995, 1997, 2002; Swainson et al., 2001). The deficits in behavioural performance of the present version of PAL adapted for fMRI were quite profound and found at easier stages of the task than in the previous neuropsychological studies. However, this probably reflected the fact that these MCI patients had already received objective memory tests such as the Rey Auditory-Verbal Learning Task and therefore had been previously screened for memory impairment rather than in the earlier studies when PAL effectively discriminated patients with subjective memory disorders presenting for the first time at a memory clinic.

It is a possible limitation of the present study that we were unable to define a level of performance of the PAL task that was equivalent between MCI patients and controls, although this has proven difficult (though not impossible) to achieve in other studies also (Dickerson & Sperling, 2008). Careful matching of behavioural test memory scores would have provided the most powerful test of whether a functional neuroimaging biomarker could be a more sensitive index of deficit than a behavioural or cognitive one. Future studies may achieve behavioural matching with a PAL2 or PAL3 version of the task. It is nevertheless quite controversial whether a functional neuroimaging biomarker would be more sensitive, as a recent meta-analysis (Schmand, Huizenga, & van Gool, 2010) concluded that memory impairment is a more accurate early predictor of AD than atrophy of the medial temporal lobe on magnetic resonance imaging (whereas CSF abnormalities and memory impairment are about equally predictive). This means that the possibility of a cognitive biomarker for the detection of early Alzheimer’s disease may be as plausible as a biochemical or neuroimaging biomarker, although it is the case that such a biomarker may well have problems of specificity, unless tied to other evidence, e.g. progressive decline.

The current study confirmed that different neural networks are implicated in encoding and retrieval, at least for this visuospatial cued recall task. The encoding stage involved an extensive neural network including different sectors of the prefrontal cortex, anterior cingulate, temporal–parietal and occipital cortex, consistent with many other studies (Fletcher & Henson, 2001; Lee et al., 2000), most of which however utilised verbal rather than visual memory material. However, we could find no evidence to support a differential hemispheric asymmetry in encoding (nor indeed in retrieval) as suggested by the hemispheric encoding/retrieval asymmetry hypothesis (Tulving et al., 1994) – possibly because of the non-verbal nature of the memory material used in this study in contrast to much of the literature (Lee et al., 2000). By contrast, retrieval implicated a much more restricted network comprising mainly the cuneus and posterior cingulate, consistent with numerous studies highlighting activation in this region with memory retrieval (Cabeza & Nyberg, 2000; Svoboda, McKinnon, & Levine, 2006). It was interesting to see that the hippocampus proper was implicated in the encoding network, but the parahippocampal gyrus in retrieval. Presumably, this difference reflects the changing roles of the hippocampus during encoding and retrieval processes. Of particular significance for this study is that differences between MCI patients and controls were limited to the hippocampal formation, there being no evidence of differential activation of the other structures, such as the prefrontal cortex and posterior cingulate cortex, in these memory networks.

The posterior cingulate cortex is often described as part of a so-called “resting state network”, exhibiting ongoing activity during rest and deactivation during task performance (Greicius, Krasnow, Reiss, & Menon, 2003; Gusnard, Raichle, & Raichle, 2001; Mazoyer et al., 2001; McKiernan, Kaufman, Kucera-Thompson, & Binder, 2002; Raichle et al., 2001). Severe hypometabolism and focal atrophy in the posterior cingulate cortex have been previously shown to be associated with MCI, suggesting that the posterior cingulate cortex is as vulnerable to neurodegeneration as the hippocampus (Nestor et al., 2003; Pengas et al., 2010). It is thus significant that we found the posterior cingulate cortex to be activated during the retrieval phase of the CANTAB PAL task. However, we did not find any differences in this activation between MCI patients and healthy controls. Further, although recent studies have shown that the posterior cingulate cortex is highly atrophic in incipient Alzheimer’s disease (Choo et al., 2010; Pengas et al., 2010) our VBM analysis did not reveal any significant structural differences in the posterior cingulate area between this sample of MCI patients and healthy controls.

There were no significant differences in intra-cranial volume between the MCI patients and controls and grey matter density reductions were detected only in the hippocampus, bilaterally and the left precuneus. Given the previous evidence of hippocampal involvement of PAL performance (Owen et al., 1995) we assume that the present hippocampal structural deficit underlies, at least in part, the deficient performance of these patients on the PAL task. However, it is apparent that this structural deficit can be associated with both hyper- and hypo-activation of this structure, as measured using the BOLD response. The use of fMRI thus provides important ancillary information as to the functional status of this compromised structure in MCI and the present study underscores the importance of using several imaging modalities, in association with neuropsychological tests, to assess MCI with a view to predicting the progression to Alzheimer’s disease.

It is important to note that we observed both structural and functional changes in the patient group, but a limitation of the study is that the group size precludes detailed analysis of interactions between these variables. The relationship, for instance, between areas of functional activation and atrophic areas is likely to be important. Although localised differently (the functional group difference in the posterior hippocampal/parahippocampal region, the structural change maximal in the anterior hippocampus), it remains possible that the two observations are intimately related but that the limited numbers studied here do not allow us to elucidate this relationship further.

This study provides new information about the neural basis of the deficits in MCI patients on the CANTAB PAL task. As the CANTAB PAL task is evidently a sensitive test for predicting progression to Alzheimer’s disease in patients with MCI it could also be potentially useful for evaluating its treatment. The PAL task is, moreover, a cued recall task, as specifically recommended for early detection in a recent influential revision of diagnostic criteria for Alzheimer’s disease (Dubois et al., 2007), albeit of delayed visuospatial, as distinct from delayed verbal, recall. One important translational advantage of the human PAL task, with its visuospatial characteristics is that, unlike the many verbal memory paradigms commonly used to assess MCI, there are analogues of the test that can be used in drug development in experimental animals, whether non-human primates (Parkinson et al., 1988; Taffe, Weed, Gutierrez, Davis, & Gold, 2002; Weed et al., 1999) or rodents (Talpos, Winters, Dias, Saksida, & Bussey, 2009).

Ultimately, it is anticipated that a better characterization of MCI will contribute to a sensitive and specific early detection of AD and enable preventative treatment. Most importantly, this study has shown that hippocampal dysfunction can be detected at a very early stage in MCI patients at least 2 yr (on average, thus far) prior to AD.
diagnosis. This emphasizes the importance of sensitive cognitive and imaging measures that can be used in screening healthy elderly people so that preventative treatments, including neuroprotective drugs, can be administered before there is a significant decline in mental capital and well-being (Beddington et al., 2008).

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