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Amyloid-β deposition in mild cognitive impairment is associated with increased hippocampal activity, atrophy and clinical progression

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Cross-sectional functional magnetic resonance imaging studies using a memory task in patients with mild cognitive impairment have produced discordant results, with some studies reporting increased hippocampal activity—consistent with findings in genetic at-risk populations—and other studies reporting decreased hippocampal activity, relative to normal controls. However, previous studies in mild cognitive impairment have not included markers of amyloid-β, which may be particularly important in prediction of progression along the Alzheimer's disease continuum. Here, we examine the contribution of amyloid-β deposition to cross-sectional and longitudinal measures of hippocampal functional magnetic resonance imaging activity, hippocampal volume, global cognition and clinical progression over 36 months in 33 patients with mild cognitive impairment. Amyloid-β status was examined with positron emission tomography imaging using Pittsburg compound-B, hippocampal functional magnetic resonance imaging activity was assessed using an associative face-name memory encoding task, and hippocampal volume was quantified with structural magnetic resonance imaging. Finally global cognition was assessed using the Mini-Mental State Examination and clinical progression was assessed using the Clinical Dementia Rating (Sum of Boxes). At baseline, amyloid-β positive patients with mild cognitive impairment showed increased hippocampal activation, smaller hippocampal volumes, and a trend towards lower Mini-Mental State Examination scores and higher Clinical Dementia Ratings compared to amyloid-β negative patients with mild cognitive impairment. Longitudinally, amyloid-β positive patients with mild cognitive impairment continued to show high levels of hippocampal activity, despite increasing rates of hippocampal atrophy, decline on the Mini-Mental State Examination and faster progression on the Clinical Dementia Ratings. When entered simultaneously into the same linear mixed model, amyloid-β status, hippocampal activation, and hippocampal volume independently predicted clinical progression. These results indicate that amyloid-β positive patients with mild cognitive impairment are more likely on a path towards Alzheimer's disease dementia than amyloid-β negative patients. Increased hippocampal activity is discussed in relation to neuronal compensation and/or amyloid-β induced excitoxicity.

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Introduction

Alzheimer's disease is a progressive neurodegenerative disorder characterized by amyloid-β plaques and neurofibrillary tangles on neuropathology (Braak and Braak, 1991). Mild cognitive impairment (MCI) is often considered a prodromal stage of Alzheimer's disease, but patients with MCI are heterogeneous in their rates of progression towards Alzheimer's disease dementia. Similarly, both autopsy and biomarker studies using PET amyloid-β imaging or CSF assays suggest that a substantial proportion of patients with MCI do not have evidence of Alzheimer's disease pathology as the aetiology of their cognitive impairment (Jack *et al.*, 2008; Albert *et al.*, 2011). Interestingly, previous studies investigating the pattern of neural activity using task-related functional MRI in MCI have yielded discordant findings (Ewers *et al.*, 2011; Sperling, 2011), ranging from increased hippocampal activity concordant with findings in genetic-at-risk asymptomatic individuals, to decreased hippocampal activity, similar to patients with Alzheimer's disease dementia.

Several functional MRI studies have associated increased hippocampal activity with increased risk for Alzheimer's disease (Ewers *et al.*, 2011; Sperling, 2011). Cognitively normal older adults who carry the apolipoprotein E ε4 (APOE4) allele are more likely to harbour amyloid-β and demonstrate increased hippocampal activity during an associative memory task (Bookheimer *et al.*, 2000; Bondi *et al.*, 2005; Johnson, 2006; Trivedi *et al.*, 2008b; Dennis *et al.*, 2010). Also, autosomal dominant mutation carriers, who develop Alzheimer's disease with almost 100% certainty, show increased hippocampal activity during an associative memory task when compared to non-carrier family members who lack the mutation (Quiroz *et al.*, 2010; Reiman *et al.*, 2012). In MCI, increased hippocampal activity has also been linked to cortical thinning (Putcha *et al.*, 2011a) and clinical progression (O'Brien *et al.*, 2010). In animal models of Alzheimer's disease, release of soluble amyloid-β can trigger aberrant synaptic activity, resulting in hyperactivity (Palop and Mucke, 2010). Treatment of hyperactivity in human patients with MCI, or in mice with pathological levels of amyloid-β, using the anti-epileptic drug levetiracetam reduces hippocampal activity and improves memory (Bakker *et al.*, 2012;

Sanchez *et al.*, 2012). In cognitively normal older adults with high levels of amyloid-β deposition, increased functional MRI activity has often been linked to neuronal compensation for Alzheimer's disease pathology (Sperling *et al.*, 2009; Mormino *et al.*, 2012; Elman *et al.*, 2014). Together, these studies provide compelling evidence that increased hippocampal activity occurs transiently early in the course of Alzheimer's disease, but it remains unclear whether this aberrant activity represents a marker of neuronal compensation, and/or evidence of neuronal excitotoxicity. Here we investigate amyloid-β deposition, and longitudinal measures of hippocampal activity, hippocampal atrophy, cognition and clinical progression in MCI to elucidate these relationships.

Cross-sectional functional MRI studies have reported mixed results in MCI. Several studies have found increased hippocampal activity during an associative memory task (Dickerson *et al.*, 2004; Celone *et al.*, 2006; Hämäläinen *et al.*, 2007; Kircher *et al.*, 2007; Lenzi *et al.*, 2011), while several other studies have not (Small *et al.*, 1999; Machulda *et al.*, 2003; Petrella *et al.*, 2006; Trivedi *et al.*, 2008a; Hanseeuw *et al.*, 2011). Previously, increased hippocampal activity has been linked to clinical progression in MCI in the absence of known levels of amyloid-β deposition (O'Brien *et al.*, 2010). Interestingly, PET studies indicate that only half to two-thirds of patients with amnestic MCI show elevated amyloid-β deposition. Furthermore, patients with MCI with low levels of amyloid-β deposition are less likely to progress to Alzheimer's disease dementia when compared to patients with MCI with elevated levels of amyloid-β (Jack *et al.*, 2009; Wolk *et al.*, 2009; Petersen *et al.*, 2013). Therefore, we hypothesized that increased hippocampal activity contributes to clinical progression in MCI primarily in the presence of elevated levels of amyloid-β deposition.

Here, we examined the contribution of amyloid-β deposition, assessed with PET amyloid imaging, to cross-sectional and longitudinal measures of hippocampal functional MRI activity, hippocampal atrophy and clinical progression over 36 months. We hypothesized that patients with MCI with elevated amyloid-β deposition (Aβ+ MCI) would show increased hippocampal activity during an associative memory encoding task (Sperling *et al.*, 2003) when compared to patients with MCI without evidence of amyloid-β deposition (Aβ− MCI). Additionally, we hypothesized that Aβ+
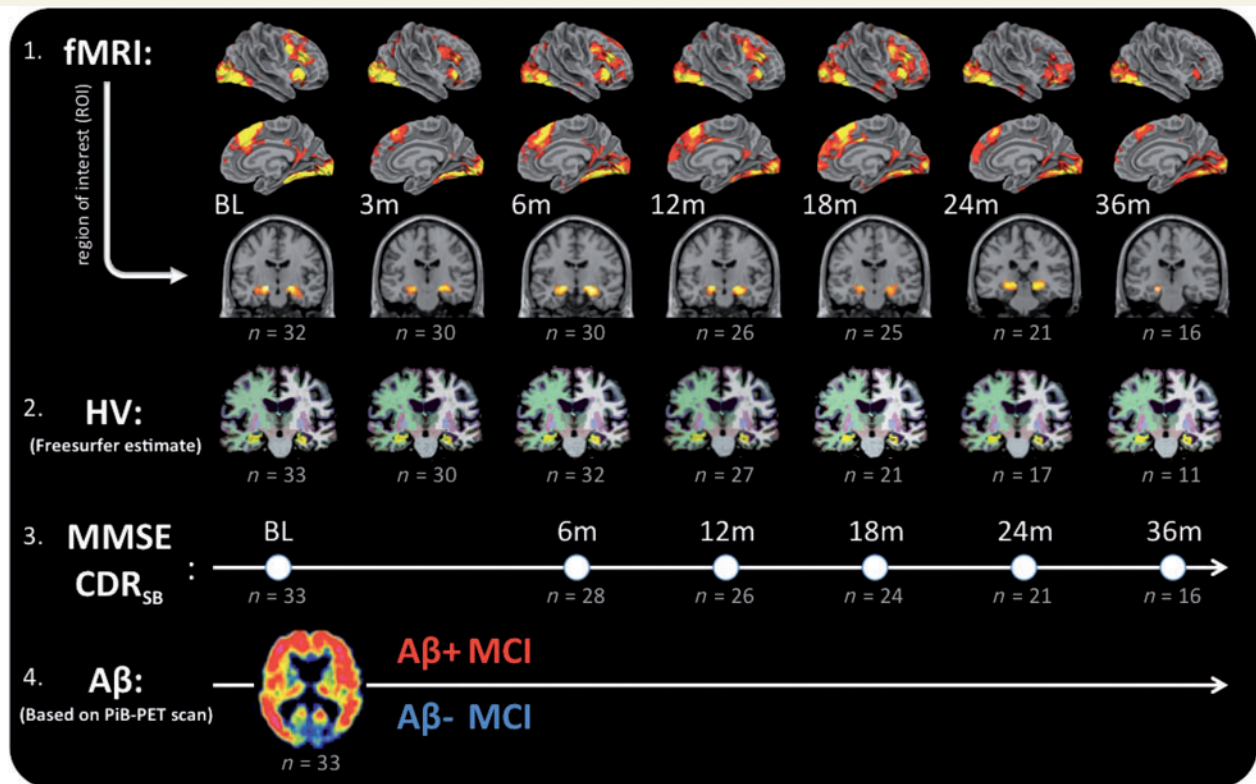
Figure 1 Schematic representation of data collected in patients with mild cognitive impairment. From *top to bottom*: (1) Functional MRI (fMRI) collected during an associative face-name encoding task; (2) hippocampal volume (HV), (3) Mini-Mental State Examination (MMSE) and Clinical Dementia Rating Sum of Boxes score (CDR$_{SB}$); and (4) amyloid-β PET imaging (Aβ). Functional MRI: shows brain activity depicted in yellow/red projected on an inflated brain for right hemisphere, midline and a sagittal slice with the hippocampus, shown at $P < 0.001$ (uncorrected) from the contrast novel-repeated at baseline (BL), 3, 6, 12, 18, 24 and 36 months (m). Hippocampal volume: shows the anatomical T$_1$-weighted scans of one MCI patient, analysed with the longitudinal FreeSurfer pipeline and used to estimate grey matter volume of the hippocampus (shown in yellow). MMSE/CDR$_{SB}$: shows time points of neuropsychological examination matched to the closest MRI visit. Amyloid-β: shows an example of an Aβ + PET scan, using PiB. PET data acquisition was collected near baseline (4 months) and used to identify A$\beta -$ and Aβ + patients with MCI. Below each visit number of observations (*n*) are shown in grey for at each visit separately for functional MRI, hippocampal volume, CDR$_{SB}$ and the amyloid-β PET scans.

MCIs would show faster rates of hippocampal atrophy (Reuter *et al.*, 2012), decline in global cognition (Folstein *et al.*, 1975) and clinical progression (Petersen, 2004) over time than A$\beta -$ MCIs.

Materials and methods

Thirty-three older adults with MCI with baseline amyloid PET and longitudinal clinical and structural and functional MRI data from an ongoing study on ageing and Alzheimer's disease were included in the current study (Fig. 1 and Table 1). We utilized criteria from the Alzheimer's Disease Neuroimaging Initiative (ADNI) to recruit a range of early and late patients with MCI. All patients with MCI met ADNI criteria for amnestic MCI, single or multiple domain. The Wechsler Memory Scale-Revised Logical Memory Test delayed recall (LMIIa) was used to assess objective memory, using an education adjusted cut-off, as previously described (Marshall *et al.*, 2013). The patient, and/or study partner, reported a memory complaint, but had essentially intact

activities of daily living, as assessed by the Functional Activities Questionnaire and Clinical Dementia Rating (CDR), and no evidence of dementia. Criteria included a Mini-Mental State Examination (MMSE) score between 24 and 30 (Folstein *et al.*, 1975), CDR global score of 0.5 (Petersen, 2004), and a Memory Box score 0.5. The LMIIa, MMSE and CDR scores are reported at baseline and final assessment (Table 1). Patients with MCI were medically stable, and did not have apparent cofounding neurological conditions, substance or alcohol abuse within the past 2 years, or primary psychiatric diagnoses (e.g. major depressive disorder) within the past 2 years. All MCI patients had a Modified Hachinski Ischaemic Score $\leqslant 4$ and a Geriatric Depression Scale (long form: 30 items) $\leqslant 10$ (Rosen *et al.*, 1980; Yesavage *et al.*, 1982). Written informed consent was obtained prior to experimental procedures and the study was approved, and conducted, in accordance with the Partners Human Research Committee at the Massachusetts General Hospital and Brigham and Women Hospital (Boston, MA). The patients with MCI were followed longitudinally for up to 3 years (36 months). MMSE was used to quantify global cognition and CDR Sum of Boxes score (CDR$_{SB}$) to quantify

Table 1 Demographics, baseline and final assessment

		Aβ− MCI	Aβ+ MCI	
Baseline assessment	n	16	17	
	gender (% female)	19	24	chi = 0.57
	age	72 ± 1.91	74 ± 2.03	$P = 0.57$
	years of education	16 ± 0.64	17 ± 0.51	$P = 0.21$
	APOE (% ε4 carriers)	38	50	chi = 0.58[a]
	Amyloid-β (PiB/FLR)	1.1 ± 0.02	1.6 ± 0.05	$P < 0.001$[b]
	CDR	0.5	0.5	–
	LMIIa	7.6 ± 1.32	5.0 ± 0.89	$P = 0.12$
	MMSE	27.9 ± 0.33	26.9 ± 0.47	$P = 0.11$
	Hippocampal fMRI	0.15 ± 0.037	0.31 ± 0.058	$P = 0.024$
	Hippocampal volume	7197 ± 281	6287 ± 238	$P = 0.019$
	CDR_{SB}	1.3 ± 0.16	1.9 ± 0.25	$P = 0.055$
Final assessment	LMIIa	10.25 ± 1.48	6.1 ± 1.6	$P = 0.064$
	MMSE	27.9 ± 0.51	26.2 ± 0.54	$P = 0.037$
	Hippocampal fMRI	0.10 ± 0.038	0.27 ± 0.068	$P = 0.039$
	Hippocampal volume	7045 ± 285	6009 ± 273	$P = 0.013$
	CDR_{SB}	1.5 ± 0.22	3.2 ± 0.41	$P = 0.001$

APOE = carriers of apolipoprotein E (APOE) ε4 allele; LMIIa = the Wechsler Memory Scale-Revised Logical Memory Test delayed recall; MMSE = Mini-Mental State Examination; CDR_{SB} = Clinical Dementia Rating Sum of Boxes score; PiB = Pittsburg Compound-B (PiB) retention in neocortical regions of interest comprised of frontal, lateral temporal and retrosplenial (FLR) cortex.

P-values denote *t*-test or chi-squares test.

[a]APOE genotype available in 23 MCIs.

[b]Used to catogorize Aβ−/Aβ+ MCI groups.

clinical progression. Additionally, to assess clinical diagnosis, two experienced clinicians (R.A. and G.M.) held a consensus meeting to evaluate if a patient at a given time met criteria for Alzheimer's disease dementia, by reviewing performance on CDR, neuropsychological testing (LMIIa and MMSE) and the Functional Activities Questionnaire (instrumental activities of daily living). Clinicians were blinded to the neuroimaging data.

MRI acquisition

Thirty-three patients with MCI were included in our analyses. Figure 1 lists the number of anatomical MRIs (MPRAGE), functional MRI sessions and MMSE/CDR assessments included after quality control. MRI data were acquired at the Athinoula A. Martinos Centre for Biomedical Imaging at baseline, 3, 6, 12, 18, 24 and 36 months. At each visit, we acquired anatomical and functional MRI data using a Siemens Trio 3 T system with a 12-channel phased-array head coil. Foam pads restricted motion of the head. The anatomical MRI consisted of a T_1-weighted MPRAGE (isotropic 1 mm, repetition time = 2300 ms, echo time = 2.98 ms, inversion time = 900 ms, trigger delay = 600 ms, 9° flip angle, 160 sagittal contiguous slices, right to left). Functional MRI images were acquired using gradient T_2*-weighted echo-planar imaging (EPI) sequence with a time of repetition of 2000 ms, echo time of 30 ms, and a 90° flip angle. EPI scans included 30 oblique coronal slices, interleaved, 5 mm thick, with 1 mm gap between slices; slices oriented perpendicular to the AC–PC line, providing whole brain coverage extending from occipital to frontal poles. The in-plane resolution was 3.125 mm, resulting in an effective voxel size of 3.125 × 3.125 × 6.0 mm. Each functional run consisted of 127 repetition times. Prior to

each EPI run, five images were acquired and discarded to allow longitudinal magnetization to reach equilibrium. Each functional MRI visit consisted of a maximum of six functional runs (of 4:40 min each). Occasionally, due to time constrains or patient discomfort, we collected fewer runs. During preprocessing of the functional MRI data, we screened each individual run for head-motion and signal-to-noise and removed runs that did not meet quality criteria. Runs with translation > 5 mm, or rotation > 5°, where not included. Within a run, bad volumes were identified by a global signal difference > 2.5 standard deviations for the run, translational movement exceeding 0.75 mm or rotational movement exceeding 1.5° per repetition time. Runs with > 20 bad volumes were not included. In addition, we correlated the motion regressors with the SPM task regressors and removed runs with $R^2 > 0.25$. Previously, we demonstrated that functional MRI data using this exact paradigm are relatively reliable with only two runs (Putcha *et al.*, 2011*b*). No functional MRI visits with fewer than two functional runs of high quality data were included in the analysis. The quality control resulted in the complete exclusion of data from four patients with MCI and was performed prior to examination of neuropsychological and PET data.

Associative memory encoding task

Inside the MRI scanner, patients with MCI performed an associative memory task to probe associative encoding of face–name pairs, as previously described (Celone *et al.*, 2006; Sperling *et al.*, 2009; O'Brien *et al.*, 2010; Putcha *et al.*, 2011*b*). In brief, subjects completed up to six functional runs within each imaging visit. Each run consisted of two encoding blocks with seven novel face-name pairs (84 pairs

in total) and two blocks with repeated, alternating, face–name pairs and blocks of passive fixation. Faces were presented for 4500 ms on a black background with a fictional first name printed in white letters underneath. During the presentation of each face–name pair, subjects were asked to indicate, via a fibre-optic button box, whether the name was a 'good' name for the face or not, a subjective task designed to enhance associative memory encoding (Sperling *et al.*, 2003). Before each run, subjects were explicitly instructed to try to remember the name that was associated with each face. The intertrial interval consisted of visual fixation, jittered using OptSeq2 (Dale, 1999), varying in duration from 300–2200 ms. Between each block, a fixation cross was presented for 25 s. Stimuli were presented using MacStim 2.5 (WhiteAnt Occasional Publishing, Melbourne, Australia). Visual images were projected onto a screen at the end of the scanner bore and viewed in a mirror attached to the head coil.

Functional MRI analysis

The functional MRI images were preprocessed and analysed using SPM8 (UCL, http://www.fil.ion.ucl.ac.uk/spm). The functional images for each visit were slice time-corrected, realigned and resized to $3 \times 3 \times 3$ mm isotropic voxels. A within-subject average was created for each subject using the realigned scans across all visits. This within-subject average was normalized to the standard SPM MNI template, and the resulting normalization parameters were subsequently applied to all scans from all visits, in the same manner as the FreeSurfer longitudinal pipeline for anatomical data (Reuter *et al.*, 2012) to avoid a normalization bias towards a single visit. Next, the normalized time-series were smoothed with an 8 mm full-width half-maximum Gaussian kernel and high-pass filtered (1/260 Hz).

Using the general linear model (GLM), as implemented in SPM8, we modelled novel and repeated blocks by convolving the onset and block duration with the canonical haemodynamic response function. Individual beta-maps were calculated for novel versus repeated blocks. Next, we used SPM8 in combination with in-house MATLAB scripts to create group-level maps (GLM-Flex, http://mrtools.mgh.harvard.edu/, Harvard Aging Brain Study, Martinos Centre, MGH, USA). Regions of interest were defined in a manner consistent with our previous work (O'Brien *et al.*, 2010). First, we used a one-sample *t*-test across baseline functional MRI data to identify activity peaks within the medial temporal lobe that showed the greatest difference between novel and repeated items (left hippocampus: $T_{max} = 6.27$, $MNI_{x,y,z} = -18, -13, -13$ and right hippocampus: $T_{max} = 5.66$, $MNI_{x,y,z} = 21, -16, -13$). At these locations, we extracted the beta-estimates for each subject's visit using 5-mm radius spheres. Next, we calculated a single hippocampal estimate, by averaging across hemispheres. These hippocampal estimates were used to quantify baseline functional MRI activity and change in longitudinal functional MRI activity.

Anatomical MRI analysis

The anatomical MRI data were analysed using FreeSurfer v5.1 longitudinal pipeline (Dale *et al.*, 1999; Reuter *et al.*, 2012). Briefly, each anatomical scan was normalized to an individual within-subject template based on all data and therefore not biased to a single visit (Reuter *et al.*, 2012). Anatomic regions

of interest were then defined in template space, and warped to subject space at each visit. The white matter and pial surface segmentation was examined visually for quality assessment using FreeSurfer tools. In cases where dura or skull influenced the segmentation, voxels were either manually edited or corrected by adjusting the watershed threshold. The preprocessing steps were subsequently re-run on the edited files and re-evaluated. This cumulative quality assessment was iterated until the segmentation results were deemed either sufficient or irreparable for cases with poor T_1-weighted MPRAGE images. From each of these FreeSurfer parcellations, we extracted hippocampal volume and normalized it by estimated total intracranial volume using simple regression (Mathalon *et al.*, 1993; Buckner *et al.*, 2004).

Amyloid PET imaging

Amyloid PET imaging data were acquired on average 4.2 ± 1.4 months after the baseline clinical visit. Deposition of amyloid-β was measured by PET using Pittsburg compound-B (N-methyl-$[^{11}C]$-2(4-methylaminophenyl)-6-hydroxy-benzothiazole) according to previously described methods (Johnson *et al.*, 2007). Briefly, we acquired 60 min of dynamic PET data following intravenous administration of ^{11}C-PiB (Pittsburg compound-B) using an HR+ PET camera (Siemens) operating in 3D mode (63 image planes; 15.2-cm axial field of view; 5.6-mm transaxial resolution; 2.4-mm slice interval; 69 frames: 12×15 s, 57×60 s). PET data were reconstructed and corrected for attenuation using standard Siemens software. Each frame was evaluated for head motion and adequate count statistics. Using Logan's graphical analysis method, we calculated PiB retention expressed as a distribution volume ratio (DVR) using a grey matter cerebellum reference region (Price *et al.*, 2005). Neocortical amyloid-β deposition was quantified using an aggregate DVR from a set of regions that comprised most of the association cortex, including frontal, lateral parietal, lateral temporal and retrosplenial cortex. MCIs were classified as amyloid positive (Aβ+) or amyloid negative (Aβ−) using a DVR threshold of 1.20. This threshold was determined by a Gaussian mixture modelling approach (Mormino *et al.*, 2014) on an independent data sample with an identical amyloid PiB-PET protocol (Johnson *et al.*, 2007) collected in the Harvard Aging Brain study.

Statistical analysis

The cross-sectional baseline results were modelled using two-sample *t*-tests (two-sided). Longitudinal effects were estimated using linear mixed models, as implemented in R v3.0.1 and the companion to Applied Regression Toolbox (Fox and Weisberg, 2011). For each dependent variable (functional MRI, hippocampal volume, MMSE, CDR$_{SB}$), we conducted three separate models (Table 2). The first model included only time. The second model included time, amyloid-β and the interaction Time × Amyloid-β. The third model included time, amyloid-β, age, sex and the interactions Time × Amyloid-β, Time × Age and Time × Sex. Amyloid-β and sex were modelled dichotomously. Age, functional MRI, hippocampal volume, CDR$_{SB}$ and MMSE were modelled continuously. Amyloid-β and sex are modelled dichotomously. Aβ−MCIs were used as the reference group for amyloid-β.

Table 2 Predictive models of longitudinal change in functional MRI, hippocampal volume, MMSE and CDR

Dependent variable:	Functional MRI		Hippocampal volume		MMSE		CDR$_{SB}$	
Number of observations:	180		171		147		148	
	Estimate	P-value	Estimate	P-value	Estimate	P-value	Estimate	P-value
I LM models with only Time:	−0.029	0.062[#]	−115.440	<0.001***	−0.267	0.028*	0.303	<0.001***
II LM models with amyloid-β:								
Time	−0.016	0.539	−190.980	<0.001***	−0.604	0.001**	0.62	<0.001***
Amyloid-β	0.144	0.044*	−885.696	0.024*	−1.198	0.079[#]	0.60	0.012*
Amyloid-β × Time	0.019	0.543	−125.880	<0.001***	−0.565	0.019*	0.56	<0.001***
III LM models with amyloid-β, age, sex:								
Time	−0.013	0.572	−224.995	<0.001***	−0.384	0.755	0.654	0.085[#]
Amyloid-β	0.153	0.042*	−744.046	0.029*	−1.244	0.079[#]	0.648	0.092[#]
Age	−0.003	0.482	−50.623	0.023*	0.027	0.549	−0.034	0.177
Sex	0.035	0.698	1242.721	0.004**	−0.279	0.745	−0.082	0.860
Amyloid-β × Time	0.014	0.689	−127.572	<0.001***	−0.613	0.017*	0.575	<0.001***
Age × Time	0.001	0.616	1.271	0.473	0.000	0.997	−0.004	0.571
Sex × Time	−0.007	0.850	41.070	0.202	−0.314	0.299	−0.035	0.802

The top row shows estimates from four linear mixed-effects (LM) models (Model I) with only time across all patients with MCI. The middle row shows estimates from four linear mixed models (Model II) with time (in years) and amyloid-β group as predictors without covariates. The bottom row shows the estimate from four linear mixed models (Model III) with additional covariates for age, gender and their effect by time. The first column shows models with hippocampal functional MRI activity as the dependent variable. The second column shows models with adjusted hippocampal volume, the third column shows models with Mini-Mental State Exam (MMSE) scores and the fourth column with Clinical Dementia Ratings Sum of Boxes scores (CDR$_{SB}$).
Amyloid-β and sex are a dichotomous variable and age, functional MRI and hippocampal volume are continuous. Aβ− MCIs were used as the reference group for amyloid-β. Females are used as the reference group for sex. Age, as a control variable, was centred at the group mean. Significant at [#]$P < 0.10$ (trending), *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Females are used as the reference group for sex. Age, as a control variable, was centred at the group mean. All models included a random intercept. To visualize the slopes (Fig. 3), we interpolated missing time points in the longitudinal data using a linear estimate as implemented in the R package zoo (Zeileis and Grothendieck, 2005). The interpolated values were not included in the linear mixed models or any of the statistical analyses.
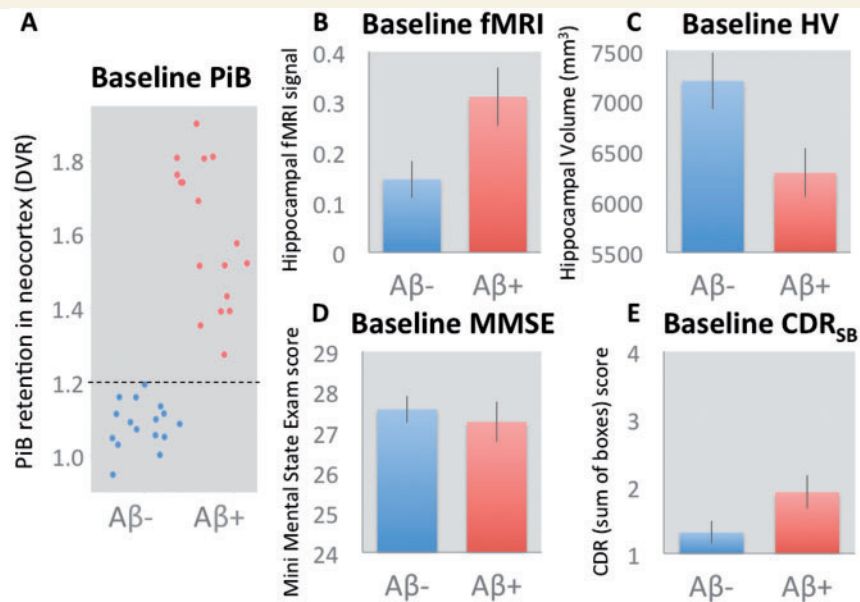
Results

The Aβ− and Aβ+ MCI groups did not differ in terms of age, sex and years of education (Table 2). Note that a global CDR score of 0.5 was a study inclusion criterion to define MCI (Petersen, 2004). At baseline (Fig. 2), Aβ+ MCIs, showed greater hippocampal functional MRI activity ($P = 0.024$), smaller hippocampal volume ($P = 0.019$), and a trend towards lower MMSE scores ($P = 0.11$) and worse CDR$_{SB}$ ($P = 0.055$), as indicated by two-sample *t*-tests. Consistent with the MMSE/CDR$_{SB}$ findings, Aβ + MCIs also trended towards worse performance on LMIIa ($P = 0.12$).

Next, we examined longitudinal change in hippocampal functional MRI activity, hippocampal volume, MMSE and CDR$_{SB}$ over 36 months using linear mixed models (Fig. 3). We examined whether amyloid-β status was associated with different rates of change for hippocampal functional MRI, hippocampal volume, MMSE and CDR$_{SB}$ (Table 2). Models were conducted without covariates (Model I), models with amyloid-β group (Model II) and models with amyloid-β group including covariates controlling for age and sex (Model III). There was a trend towards

longitudinal hippocampal functional MRI activity to decrease over time across all subjects ($P = 0.062$), similar to a previous report that did not include amyloid-β PET imaging (O'Brien *et al.*, 2010). We found a main effect of baseline amyloid-β, consistent with the cross-sectional baseline results, such that Aβ + MCIs had increased activity in the hippocampus ($P = 0.044$) compared to Aβ−. We did not observe an interaction between time and amyloid-β ($P = 0.543$), suggesting that the change in functional MRI activity over time was not different across amyloid-β groups. Longitudinal measures of hippocampal volume also significantly decreased over time across all subjects ($P < 0.001$). We observed an interaction between time and baseline amyloid-β ($P < 0.001$), such that Aβ + MCIs showed a higher rate of hippocampal atrophy over time. Longitudinal measures of MMSE decreased over time across all subjects ($P = 0.028$). We observed an interaction between time and baseline amyloid-β ($P = 0.019$), such that Aβ + MCIs showed greater cognitive decline on the MMSE over time. A model that included covariates for age and sex showed a similar pattern (Table 2). Longitudinal measures of CDR$_{SB}$ increased over time across all subjects ($P < 0.001$). We observed an interaction between time and baseline amyloid-β ($P < 0.001$), such that Aβ + MCIs showed a greater progression on CDR$_{SB}$ over time. Similar patterns were observed across all models when age and sex were included as covariates (Table 2).

Finally, to determine the relative contributions of these markers to prediction of longitudinal clinical progression, baseline amyloid-β group, baseline hippocampal functional MRI, baseline hippocampal volume, baseline age and baseline sex, as well as their interactions with time, were all

Figure 2 Baseline results. (**A**) A scatter plot of PiB retention values at baseline. The y-axis contains the neocortical PiB retention values, normalized by a DVR. The black line indicates the 1.20 cut-off used to classify Aβ + and Aβ− MCIs. (**B**) Baseline functional MRI (fMRI) activity. The y-axis contains the average beta-estimate from both left and right hippocampus. (**C**) Average baseline hippocampal volume (HV). The y-axis contains the total hippocampal volume in mm³ of both left and right hippocampus. (**D**) Baseline Mini-Mental State Examination scores (MMSE). (**E**) Baseline Clinical Dementia Rating Sum of Boxes scores (CDR$_{SB}$). The Aβ− MCI group is shown in blue and Aβ+ MCI in red. Error bars denote the standard error of the mean.
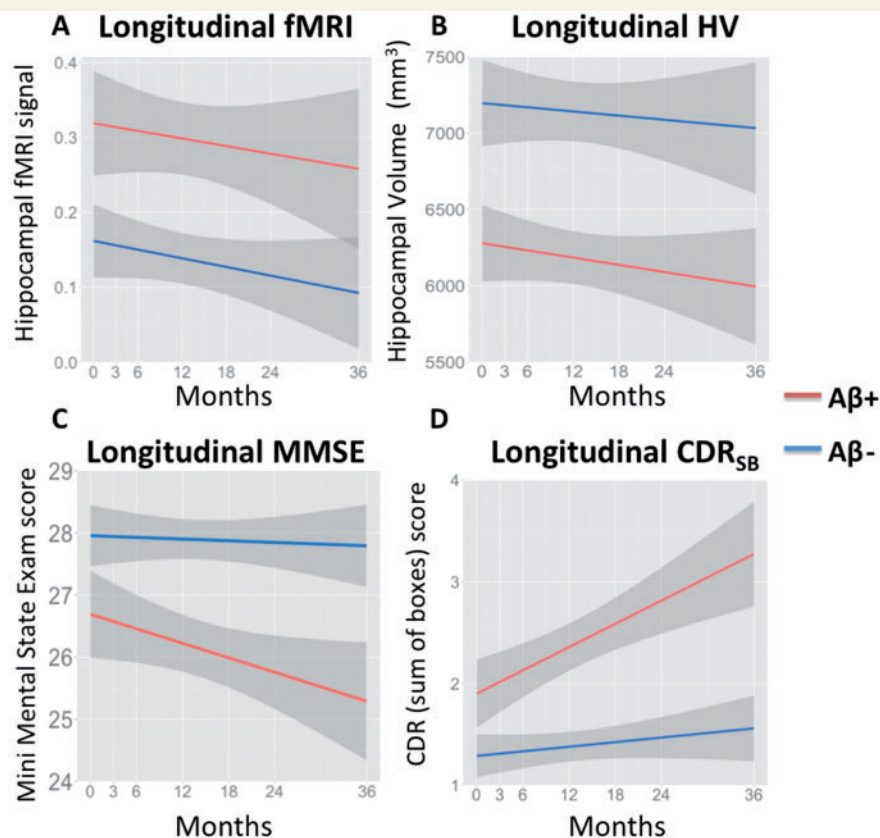
Figure 3 Longitudinal results. (**A**) Estimates from linear mixed models in functional MRI (fMRI) activity. The y-axis contains the average beta-estimates from both left and right hippocampus. (**B**) Estimates in hippocampal volume (HV). The y-axis contains total hippocampal volume in mm³ of both left and right hippocampus. (**C**) Estimates in Mini-Mental State Examination (MMSE), with lower scores representing lower cognition. (D) Estimates in Clinical Dementia Rating Sum of Boxes score (CDR$_{SB}$), with higher score representing greater clinical deficits. The x-axis shows time in months. The Aβ− MCI group is shown in blue and Aβ+ MCI in red. Grey area denotes the standard error of the mean.

Table 3 Predictive model of clinical progression

Dependent variable:	CDR_{SB}	
Number of observations:	143	
	t-value	*P*-value
Time	0.408	0.684
Amyloid-β	0.590	0.560
Functional MRI	0.818	0.421
Hippocampal volume	2.399	0.024*
Age	2.712	0.012*
Sex	0.923	0.365
Amyloid-β × Time	4.740	<0.001***
Functional MRI × Time	2.249	0.027*
Hippocampal volume × Time	0.831	0.408
Age × Time	0.254	0.800
Sex × Time	0.011	0.991

Table shows estimates from a linear mixed model with Clinical Dementia Ratings Sum of Boxes score (CDR_{SB}) as the dependent variable. Predictors include time (in years), amyloid-β group, hippocampal functional MRI activity, hippocampal volume, age, gender and their effects by time.
Amyloid-β and sex are a dichotomous variable and age, functional MRI and hippocampal volume are continuous.
Significant at *$P < 0.05$ and ***$P < 0.001$.

modelled as simultaneous predictors of change in CDR_{SB} over time. We found main effects of baseline hippocampal volume ($P = 0.025$) and baseline age ($P = 0.012$), such that a smaller hippocampus and higher age where associated with worse CDR_{SB} (Table 3). Importantly, we observed an interaction between time and amyloid-β group ($P < 0.001$), such that Aβ+ MCIs showed a greater progression on CDR_{SB} over time. These results demonstrate that amyloid-β group was the strongest independent predictor of clinical progression (Table 3). In addition, we also observed an interaction between time and baseline functional MRI ($P = 0.027$), such that baseline hippocampal activation was associated with greater clinical progression on CDR_{SB}. These latter results demonstrate that functional MRI showed an independent contribution to clinical progression, even with amyloid-β in the model.

During the 3-year longitudinal study, 9 of 33 patients with MCI progressed to meet clinical criteria for the diagnosis of Alzheimer's disease dementia. From enrolment, the average time of clinical progression to dementia was 19.3 ± 3.9 months. Seven of 9 of the MCI patients who progressed were Aβ+ at baseline. The demographics for stable MCI and progressive MCIs, baseline data and data obtained at final assessment are included in Supplementary Table 1.

Discussion

In this study, we first examined the cross-sectional relationship of amyloid-β deposition with hippocampal functional MRI activity in patients with MCI. Secondly, we examined the influence of amyloid-β deposition on 3-year longitudinal measures of hippocampal functional MRI activity,

hippocampal atrophy, cognition, and functional clinical progression. At baseline, we found that patients with MCI with high amyloid-β deposition showed increased hippocampal activity, smaller hippocampal volumes and slightly greater functional impairment compared to MCI with low amyloid-β deposition. Longitudinally, we found that Aβ+ MCIs showed persistent greater functional MRI activity, higher rates of hippocampal atrophy, and greater cognitive decline on the MMSE and faster clinical progression on CDR_{SB}. Finally, we investigated the contribution of all of these imaging markers to the prediction of clinical progression. We found that amyloid-β deposition was the strongest predictor. However, we found an independent contribution of functional MRI, such that greater hippocampal activity was associated with faster clinical progression, even when accounting for amyloid and hippocampal volume. Below we discuss our findings in the context of other cross-sectional and longitudinal studies.

Increased functional MRI activity in mild cognitive impairment

At baseline, we found increased hippocampal activity in Aβ+ MCIs compared to Aβ− MCIs. It is possible that amyloid status may at least partially explain the discrepancies between several cross-sectional functional MRI studies that found hyperactivity in MCI (Dickerson *et al.*, 2004; Johnson *et al.*, 2004; Celone *et al.*, 2006; Hämäläinen *et al.*, 2007; Kircher *et al.*, 2007; Lenzi *et al.*, 2011), and those studies that did not (Small *et al.*, 1999; Machulda *et al.*, 2003; Johnson *et al.*, 2006; Petrella *et al.*, 2006; Trivedi *et al.*, 2008b; Hanseeuw *et al.*, 2011). These previous studies did not measure amyloid-β deposition, and therefore might differ in their relative number of Aβ+ and Aβ− MCIs included in their cohorts. Previous studies have found that APOE ε4 carriers show relative hyperactivity in the hippocampus (Johnson, 2006; Bookheimer *et al.*, 2000; Bondi *et al.*, 2005; Filippini *et al.*, 2009; Dennis *et al.*, 2010) and APOE ε4 is known to increase the likelihood of amyloid positivity (Johnson *et al.*, 2013). The association between high levels of amyloid-β deposition and increased hippocampal activity is consistent with reports from autosomal dominant genetic at-risk populations. Asymptomatic carriers of the PSEN1 mutation also demonstrate relative hyperactivity in the hippocampus and adjacent cortices during an associative memory task (Quiroz *et al.*, 2010; Braskie *et al.*, 2012; Reiman *et al.*, 2012). Nonetheless, our observational data only provide an associative link between amyloid-β deposition and increased hippocampal activity, since other pathological processes, for example tau accumulation in the medial temporal lobe, also start before the stage of MCI (Nelson *et al.*, 2012) and might be more closely linked to either amyloid-β or increased hippocampal activity. Taken together, our cross-sectional and longitudinal data suggest that amyloid-β deposition may be an important factor that explains

at least some of the discrepant findings reported in functional MRI studies in MCI.

In addition to the influence of amyloid-β, the degree of cognitive impairment may influence the presence of increased hippocampal activity. Our cohort includes some MCIs with very mild impairment (early MCI by ADNI criteria), and we have previously speculated that early MCIs may demonstrate increased activity that diminishes with disease progression and loss of hippocampal neurons (Sperling et al., 2010; Ewers et al., 2011). Consistent with a previous longitudinal functional MRI study in a different set of MCI subjects (O'Brien et al., 2010), we reported that greater hippocampal activity at baseline was associated with decreases activity and faster cognitive decline over time. In the present study, we did not observe that rate of decline in functional MRI activity was a predictor of clinical progression. Instead we found that amyloid-β deposition is a key factor and is linked to both increased hippocampal activity and clinical progression.

At baseline, we found that Aβ+ MCIs had smaller hippocampal volumes compared to Aβ− MCIs, a finding consistent with cross-sectional studies (Jack et al., 2008; Mormino et al., 2009; Wolk et al., 2009) and the link between increased risk for Alzheimer's disease progression in MCIs with smaller hippocampal volumes (Visser et al., 2002; Landau et al., 2010). Consistent with other longitudinal studies, we also found faster rates of hippocampal atrophy in Aβ+ MCIs (Jack et al., 2010; Chetelat et al., 2012; Villemagne et al., 2013; Ossenkoppele et al., 2014). These anatomical results support the view that Aβ+ MCIs are more likely to develop dementia due to Alzheimer's disease.

The cross-sectional association of smaller hippocampal volume with increased functional MRI activity suggests that grey matter loss alone does not account for the functional MRI results. Both hippocampal activity and volume are influenced by amyloid-β deposition, but in opposite directions. In addition, it is likely that hippocampal volume has an independent influence on hippocampal activity. At baseline across all subjects, smaller hippocampal volume is associated with decreased activity. Longitudinal atrophy of the hippocampus, present in both amyloid-β groups, may partially explain the trend towards decreased hippocampal activity also present in both amyloid-β groups. Thus, we hypothesize that while amyloid-β deposition contributes to increased hippocampal activity, likely beginning prior to the clinical stage of MCI, hippocampal atrophy, increasing over the course of MCI, contributes to decreasing hippocampal activity.

Increased hippocampal activity predicts cognitive decline in the setting of hippocampal atrophy and amyloid-β

The increased hippocampal activity in Aβ+ MCIs support the view that increased neuronal activity and amyloid-β are associated (Palop and Mucke, 2010; Jagust, 2013). Yet, our longitudinal data do not provide evidence of whether initial amyloid-β deposition induces increased hippocampal activity (Palop and Mucke, 2010; Jack et al., 2013) or whether initial hyperactive neurons drive deposition of amyloid-β (Bero et al., 2011; Jagust and Mormino, 2011). In the context of recent longitudinal studies that estimated the rate of amyloid-β accumulation, it is likely that amyloid-β continues to accumulate in Aβ+ MCIs (Vlassenko et al., 2011; Jack et al., 2013; Villemagne et al., 2013). As we only acquired amyloid PET data at baseline, it remains unclear if the rate of amyloid-β accumulation is directly linked to the level of aberrant functional MRI activity. Based on longitudinal studies, it is also likely that Aβ+ MCI began to accumulate amyloid-β years before the start of our study (Morris et al., 2009; Vlassenko et al., 2011; Jack et al., 2013; Roe et al., 2013; Villemagne et al., 2013) and showed increased hippocampal activity, possibly before the clinical stage of MCI. Studies in Aβ+ cognitively normal older adults typically found increased functional MRI activity in the default network (manifesting as failure of deactivation), including the entorhinal cortex, while demonstrating normal levels of activity in the hippocampus (Sperling et al., 2009; Kennedy et al., 2012; Oh and Jagust, 2013; Elman et al., 2014; Huijbers et al., 2014). In early stages of amyloid-β deposition, the default network, including the functionally connected entorhinal cortices, might demonstrate the earliest signs of relative hyperactivity, prior to change in the hippocampus, consistent with our recent cross-sectional observation using a different functional MRI task in a group of clinically normal older individuals (Huijbers et al., 2014). In a previous cross-sectional study (Sperling et al. 2009), we only observed evidence of amyloid-β associated aberrant activity in the default network of clinically normal older individuals (CDR 0) but increases in hippocampal activity were only seen in a small group of individuals with subtle memory impairment (CDR 0.5). Thus, we postulate that increased hippocampal activity, in the setting of amyloid-β deposition, may mark the onset of memory impairment and predict more rapid cognitive decline. Future longitudinal studies in preclinical Alzheimer's disease (Sperling et al., 2011) will test this hypothesized anatomic progression of aberrant activity, with initial aberrant activity (decreased deactivation) in the default network followed by increased activity in the hippocampus.

Several studies have interpreted increased functional MRI activity in Aβ+ adults as a marker for neuronal compensation (Sperling et al., 2009; Mormino et al., 2012; Oh and Jagust, 2013). A recent study demonstrated compelling evidence for compensation by increased functional MRI activity in Aβ+ cognitively normal older adults (Elman et al., 2014). Elman and colleagues (2014) found increased functional MRI activity in task-positive brain regions and this coincided with more vivid memory encoding, suggesting increased functional MRI activity is linked to maintaining performance in the presence of Alzheimer's disease

pathology. At the stage of MCI, increased hippocampal activity might reflect compensatory activity that occurs relatively late in the Alzheimer's disease trajectory and could coincide with increased activity in the default network and task-positive regions. Wide-spread co-activation in the hippocampus, default-network and task-positive brain regions is also consistent with de-differentiation theories of ageing (Cabeza, 2002; Park and Reuter-Lorenz, 2009), as brain regions become less specialized. Previous functional MRI studies in MCI have suggested that increased hippocampal activity is associated with increased rates of cortical atrophy and more rapid cognitive decline (O'Brien *et al.*, 2010; Putcha *et al.*, 2011a). Whether age-related changes in activity reflect aberrant or compensatory processes remain a topic of debate (Stern, 2012; Jagust, 2013). The causal association of hyperactivity and amyloid accumulation in the evolution of sporadic Alzheimer's disease remains to be elucidated. The interpretation of increased hippocampal activity, as compensatory neuronal activity, versus evidence of excitotoxicity is not mutually exclusive. Early in the trajectory of Alzheimer's disease, as pathology starts to accumulate, increased neuronal activity might be compensatory and benefit cognition. However, these overactive neurons may produce greater amounts of soluble amyloid-β (Cirrito *et al.*, 2005), which might further perpetuate the cycle and contribute to disease progression.

Limitations

A foremost limitation of this study is the modest cohort size; this study was designed for analysis of longitudinal data obtained at multiple visits, which limited the total number of patients who could be included. Also, we have a limited number of observations at 36 months (Table 1), as several MCIs did not complete their 36-month visit due to increased impairment or enrolment into clinical trials. In this cohort, males were over-represented (Table 2), consistent with the higher number of males diagnosed with MCI (Petersen *et al.*, 2010), which has been suggested to reflect a faster rate of Alzheimer's disease progression in females with MCI. Yet, we did not detect interactions with sex and merely observed a main effect of baseline hippocampal volume. In addition, we found that longitudinal changes in hippocampal functional MRI activity were not different between Aβ + and Aβ − MCI groups and did not predict clinical progression. A second potential limitation is the PET acquisition times ($>60\,\text{min}$). Longer acquisition or qualitative assessment of the PET scans (Ossenkoppele *et al.*, 2013), might help discriminate between MCIs with low and intermediate levels of amyloid-β deposition (Villemagne *et al.*, 2013). Thus, the acquisition method and the DVR threshold applied here (1.2) might slightly underestimate the number of MCIs with clinically relevant amyloid-β deposition. However, this likely would have decreased our ability to detect amyloid-β related differences between the MCI groups. Finally, we did not find evidence that learning or habituation of the associative memory task could account for the longitudinal changes in hippocampal functional MRI activity. Nevertheless, we hypothesize that as the Aβ + MCI group continues to progress towards dementia, memory performance and increased hippocampal activity will diminish, in accordance with cross-sectional observations in patients with Alzheimer's disease (Sperling *et al.*, 2003; Trivedi *et al.*, 2008a; Ewers *et al.*, 2011).

Implications for future clinical research

The longitudinal models demonstrate that these neuroimaging markers provide information above and beyond clinical status at baseline. About half of the early MCIs (17 of 33) in this study, as defined in accordance with ADNI criteria (Aisen *et al.*, 2010), showed high levels of amyloid-β deposition and slightly more impaired cognition. This percentage of Aβ + MCIs is roughly consistent with previous studies (Jack *et al.*, 2009; Wolk *et al.*, 2009; Petersen *et al.*, 2013), and is markedly higher than the 30% Aβ + typically observed in clinically normal older adults (Sperling *et al.*, 2011). Our findings provide further support for the role of biomarkers in patients with a clinical diagnosis of MCI to define a prodromal stage of Alzheimer's disease (Albert *et al.*, 2011; Hinrichs *et al.*, 2011; Dubois *et al.*, 2014). The MMSE scores and CDR_{SB}, in combination with the neuroimaging data, demonstrate that Aβ − MCIs are more stable, consistent with a trajectory of suspected non-Alzheimer's disease pathology (Petersen *et al.*, 2013). In contrast, the Aβ + MCI group in our study progressed clinically and are more likely on the path to Alzheimer's disease dementia, consistent with other recent longitudinal studies in MCI (Jack *et al.*, 2010; Nordberg *et al.*, 2012; Hatashita and Yamasaki, 2013).

Currently, there is no effective treatment for Alzheimer's disease (Selkoe, 2012). The contributions of both amyloid-β deposition and increased hippocampal activity to clinical progression suggest two potential targets for clinical intervention: amyloid-β and excitotoxicity. The current findings provide support for an initial study that used anti-epileptic drugs to reduce hippocampal activity and demonstrated some cognitive benefit in MCI (Bakker *et al.*, 2012). In individuals who are already experiencing symptoms due to Alzheimer's disease with manifest evidence of neuronal dysfunction and neurodegeneration, we will likely need to pursue other mechanisms beyond amyloid-β (Sperling *et al.*, 2014), and consider combination therapies of anti-amyloid agents with drugs to mitigate excitotoxicity.

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Supplementary material

Supplementary material is available at *Brain* online.

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