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Larger temporal volume in elderly with high versus low beta-amyloid deposition

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β-Amyloid deposition is one of the main hallmarks of Alzheimer's disease thought to eventually cause neuronal death. Post-mortem and neuroimaging studies have consistently reported cases with documented normal cognition despite high β-amyloid burden. It is of great interest to understand what differentiates these particular subjects from those without β-amyloid deposition or with both β-amyloid deposition and cognitive deficits, i.e. what allows these subjects to resist the damage of the pathological lesions. [11C]Pittsburgh compound B positron emission tomography and magnetic resonance brain scans were obtained in 149 participants including healthy controls and patients with subjective cognitive impairment, mild cognitive impairment and Alzheimer's disease. Magnetic resonance data were compared between high versus low-[11C]Pittsburgh compound B cases, and between high-[11C]Pittsburgh compound B cases with versus those without cognitive deficits. Larger temporal (including hippocampal) grey matter volume, associated with better episodic memory performance, was found in high- versus low-[11C]Pittsburgh compound B healthy controls. The same finding was obtained using different [11C]Pittsburgh compound B thresholds, correcting [11C]Pittsburgh compound B data for partial averaging, using age, education, Mini-Mental State Examination, apolipoprotein E4 and sex-matched subsamples, and using manual hippocampal delineation

instead of voxel-based analysis. By contrast, in participants with subjective cognitive impairment, significant grey matter atrophy was found in high-[11C]Pittsburgh compound B cases compared to low-[11C]Pittsburgh compound B cases, as well as in high-[11C]Pittsburgh compound B cases with subjective cognitive impairment, mild cognitive impairment and Alzheimer's disease compared to high-[11C]Pittsburgh compound B healthy controls. Larger grey matter volume in high-[11C]Pittsburgh compound B healthy controls may reflect either a tissue reactive response to β-amyloid or a combination of higher 'brain reserve' and under-representation of subjects with standard/low temporal volume in the high-[11C]Pittsburgh compound B healthy controls. Our complementary analyses tend to support the latter hypotheses. Overall, our findings suggest that the deleterious effects of β-amyloid on cognition may be delayed in those subjects with larger brain (temporal) volume.

Introduction

Alzheimer's disease is a progressive neurodegenerative disease characterized by synaptic and neuronal death associated with cognitive deterioration. β-Amyloid deposition is one of the main hallmarks of Alzheimer's disease and is thought to eventually cause neuronal death (Hardy and Selkoe, 2002; Masters et al., 2006). Post-mortem studies have consistently reported cases with documented normal cognition, while their brain autopsy demonstrated substantial levels of pathological lesions associated with Alzheimer's disease (Crystal et al., 1988; Katzman et al., 1988; Price and Morris, 1999; Schmitt et al., 2000). Similarly, studies using the recently developed [11C]Pittsburgh Compound B (PiB) PET radiotracer that binds to fibrillar β-amyloid plaques have reported a bimodal distribution of neocortical PiB values within elderly subjects with normal cognition, with a majority of them showing low PiB retention, but approximately one third showing distinctly elevated PiB retention (Archer et al., 2006; Mintun et al., 2006; Pike et al., 2007; Jack et al., 2008; Dickerson et al., 2009; Storandt et al., 2009; Bourgeat et al., 2010). These findings raise questions regarding the relationship between β-amyloid plaques, neurodegeneration and the clinical manifestation of Alzheimer's disease. It is also possible that some individuals have an idiosyncratic brain reserve that allows them to resist the damage of the pathological lesions (Katzman et al., 1989; Price and Morris, 1999; Stern, 2006).

Previous neuroimaging studies comparing regional brain volumes in normal elderly with high versus low PiB retention report discrepant findings; hippocampal atrophy in the elderly with high PiB has been found in some studies (Jack et al., 2008; Storandt et al., 2009) but not in others (Dickerson et al., 2009; Bourgeat et al., 2010) and has also been described in the temporal pole (Storandt et al., 2009) and in the cingulate cortex (Dickerson et al., 2009). Regarding the correlation between PiB-PET and atrophy in normal elderly, studies usually reported significant relationships with higher PiB being related to higher atrophy (Mormino et al., 2009; Bourgeat et al., 2010). In a previous study however, when separating elderly with subjective cognitive impairment from those with no subjective cognitive impairment (termed as healthy controls in what follows), we found that the correlation between atrophy and β-amyloid only occurs in participants with subjective cognitive impairment (Chételat et al., 2010).

The present study aims at further exploring the reasons why some particular elderly have no objective nor subjective cognitive deficits despite high β-amyloid deposition. We thus sought to identify what differentiates high versus low PiB cases within separate groups of healthy controls and subjective cognitive impairment, and what distinguishes—among participants with high PiB—the healthy controls from participants with subjective cognitive impairment, mild cognitive impairment or Alzheimer's disease, in terms of demographics, neuropsychological performances and global and regional brain volumes.

Materials and methods

Participants

All 149 subjects included in the present study were participants of the Australian Imaging Biomarkers and Lifestyle Study of Ageing (AIBL) (Ellis et al., 2009) who had both magnetic resonance (MRI) and PiB-PET scans at the Austin Hospital (Melbourne). The full methodology for the cohort recruitment and evaluation is detailed elsewhere (Ellis et al., 2009). All subjects underwent clinical and neuropsychological examination, including the Mini-Mental State Examination, Wechsler Test of Adult Reading, California Verbal Learning Test—second edition, Rey Complex Figure Test, 30-item Boston Naming Test, Digit Span subtest of the Wechsler Adult Intelligence Scale—third edition, verbal category fluency (animals and boy's names) and Stroop tests. The present study focuses on a group of 44 healthy elderly without memory complaints (determined by a 'no' response to the question: 'Do you have any difficulty with your memory?'). For the sake of comparison, participants with subjective cognitive impairment as well as high-PiB (see below), patients with mild cognitive impairment or with Alzheimer's disease were also included. Allocation of individuals to a diagnostic group and exclusion of ineligible individuals were performed by a clinical review panel based on the screening interview and neuropsychological assessment and according to internationally agreed criteria: patients with mild cognitive impairment met Petersen's consensus criteria for amnestic mild cognitive impairment (Petersen et al., 2005) while patients with Alzheimer's disease met standard NINCDS-ADRDA clinical criteria for

probable Alzheimer's disease (McKhann et al., 1984). Participant' demographics, percentage of subjects with at least one apolipoprotein E4 (ApoE4) allele, and neuropsychological scores for each group are reported in Table 1. Approval for the study was obtained from the Austin Health Human Research Ethics Committee, and written informed consent for participation was obtained for each subject prior to the scans.

Neuroimaging data acquisition

Sagittal T_1-weighted magnetic resonance images were acquired using a standard 3D-magnetization prepared rapid gradient echo sequence at 3 T, with in-plane resolution 1 mm × 1 mm, slice thickness 1.2 mm, repetition time/echo time/ inversion time = 2300/2.98/900 ms, flip angle 9° and field of view 240 × 256 and 160 slices.

The PiB-PET scans were acquired using a Phillips Allegro™ PET camera. Each participant was injected with 370 MBq of PiB and a 30 min acquisition in 3D mode was performed starting 40 min after injection of PiB. A transmission scan was performed for attenuation correction. PET images were reconstructed using a 3D row-action maximum likelihood algorithm (RAMLA). Summed images for the 40–70 min time frame were used in this study.

Neuropsychological and neuroimaging evaluations were usually performed within two months (mean interval between the first and the last examination was 60 ± 63 days; all participants with an interval longer than 6 months were excluded from the study).

Neuroimaging data processing

The procedure for neuroimaging data handling and transformation is fully detailed elsewhere (Chételat et al., 2010). Briefly, MRI data were spatially normalized and segmented onto grey matter, white matter and cerebrospinal fluid partitions using the voxel-based morphometry 5 toolbox implemented in Statistical Parametric Mapping 5 (Ashburner

et al., 2000; Good et al., 2001). The grey matter segment was used as an estimate of total grey matter volume and the sum of the three compartments (grey matter, white matter and cerebrospinal fluid) obtained from the segmentation step with voxel-based morphometry was used as an estimate of the total intracranial volume. Grey matter and white matter partitions were modulated to correct for non-linear warping only so that values in resultant images are expressed as volume corrected for brain size. Images were then masked to remove remaining non-grey matter or non-white matter voxels and smoothed (13 mm full-width at half-maximum). PiB-PET data were co-registered to their corresponding MRI, spatially normalized applying the parameters defined from their corresponding MRI and scaled using the mean PiB value in the cerebellum grey matter. The resulting PiB-PET data—expressed as standardized uptake value ratios—were used to obtain the individual mean global neocortical PiB value used to classify participants as high-PiB versus low-PiB using a cut-off of 1.4, determined through a cluster analysis on the controls and consistent with cut-off values usually used in PiB-PET studies (Archer et al., 2006; Pike et al., 2007; Jack et al., 2008; Bourgeat et al., 2010). Spatially normalized PiB-PET data were also smoothed (12 mm full-width at half-maximum) for the sake of complementary analyses. Six groups of participants were included in the present study: low-PiB healthy controls, high-PiB healthy controls, low-PiB subjective cognitive impairment, high-PiB subjective cognitive impairment, high-PiB mild cognitive impairment and high-PiB Alzheimer's disease.

Statistical analyses

The main analyses, corresponding to the main objectives of the present studies, consisted of the comparison of demographical, neuropsychological and grey matter data in high-PiB healthy controls versus low-PiB healthy controls, high-PiB subjective cognitive impairment versus low-PiB subjective cognitive impairment and high-PiB Alzheimer's disease, high-PiB mild cognitive impairment and high-PiB

Table 1 Demographics and cognitive scores for each group

	HC− (n = 31)	HC+ (n = 13)	SCI− (n = 30)	SCI+ (n = 19)	MCI+ (n = 22)	AD+ (n = 34)
Male	35%	69%	57%	37%	50%	51%
ApoE4 positive	35%	54%	3%	63%	68%	80%
Age (years)	73.1 ± 7.1	78.8 ± 5.5	72.1 ± 7.1	76.7 ± 6.5	75.8 ± 7.1	75 ± 7.9
Education (years)	14 ± 3.3	14.3 ± 3	13.7 ± 3.5	12.7 ± 3.4	11.5 ± 2.7	11.5 ± 3
WTAR IQ[a]	111.5 ± 7.3	114.6 ± 4.7	111 ± 6.9	112 ± 4.6	108.1 ± 6.4	106.9 ± 8.5
MMSE score	29.3 ± 0.9	28.8 ± 1	28.8 ± 1.3	29.2 ± 1.2	26.2 ± 1.9	21.6 ± 5.3
CVLT-II delayed free recall score	11.8 ± 2.7	11.5 ± 1.7	11.4 ± 2.6	10.7 ± 3.7	2.9 ± 2.2	0.9 ± 1.9
Rey-3′ recall	17.4 ± 5.4	18.9 ± 5.5	19 ± 5.4	14.2 ± 5.3	10.2 ± 5.3	4.3 ± 3.6
Rey-30′ recall	16.9 ± 4.9	18 ± 4.6	18.3 ± 4.8	14.3 ± 6.5	8.9 ± 5.8	3.3 ± 3.4
Rey copy	32.2 ± 2.7	32.5 ± 2.2	31.9 ± 2.5	29.9 ± 4.9	28.6 ± 6.4	24.6 ± 8.8
Digit span[b]	17.8 ± 4	19.1 ± 4.5	18.1 ± 3.2	17.7 ± 4.1	15.3 ± 3	13.9 ± 3.9
Fluency[c]	41.5 ± 8.9	42.1 ± 7.3	38.9 ± 7.2	36.7 ± 6.5	30.9 ± 8.9	22.7 ± 8.5
Boston[d]	28.5 ± 1.6	28.5 ± 1.3	28.4 ± 1.5	27.7 ± 2.1	24.5 ± 5.7	22.3 ± 6.8
Stroop[e]	34.3 ± 10.1	34.6 ± 10.1	32.9 ± 13	35.4 ± 11.4	46.1 ± 29.8	55.4 ± 23

For each variable, mean and SD are indicated, except for 'male' and 'ApoE4 positive' where the percentage of males in the group and the percentage of subjects in the group having at least one ε4 allele, respectively, are indicated. Significant between-group differences are all indicated in the text. HC − = low-PiB healthy controls; HC+ = high-PiB healthy controls; SCI− = low-PiB subjective cognitive impairment; SCI+ = high-PiB subjective cognitive impairment; MCI+ = high-PiB mild cognitive impairment; AD+ = high-PiB Alzheimer's disease; MMSE = Mini-Mental State Examination; CVLT-II = California Verbal Learning Test—second edition.
a Predicted intellectual quotient calculated from the Wechsler Test of Adult Reading (WTAR) and adjusted for age.
b Total score from the digit span subtest of the Wechsler Adult Intelligence Scale—third edition.
c Sum of animals and boy's names category fluency scores.
d Score at the 30-item version of the Boston naming test.
e Time taken for the incongruence condition of the Victoria version of the Stroop.

subjective cognitive impairment versus high-PiB healthy controls. Two sets of complementary analyses were then conducted, the first one to verify the validity of the findings obtained in the main analyses, and the second to support their intepretation (see the Results section).

For all comparisons of demographic and neuropsychological data between high versus low PiB cases (within the healthy controls and within the subjective cognitive impairment), contingency chi-squares were performed for gender and ApoE4 status, two-sample *t*-tests for independent samples were used for other demographic variables and ANOVAs were used to compare neuropsychological performances introducing age, gender and years of education as covariates. When comparing high-PiB healthy controls to high-PiB subjective cognitive impairment, high-PiB mild cognitive impairment and high-PiB Alzheimer's disease, ANOVAs were performed to assess the main effect of the clinical group on demographic and neuropsychological variables (except gender and ApoE status where contingency chi-squares were performed), introducing age, gender and years of education as covariates for neuropsychological variables only; *post hoc* 2×2 group comparisons were then performed when the main effect of group was significant. MRI data were analysed with Statistical Parametric Mapping 5 using ANOVAs for group comparisons and including age, gender and years of education as covariates. A P(uncorrected) < 0.001 threshold was used for all voxel-based analyses.

Results

Main analyses

First, demographic, neuropsychological and grey matter data of high-PiB healthy controls were compared to those of low-PiB healthy controls. High-PiB healthy controls were significantly older ($P = 0.008$) and comprised more males ($P = 0.04$) than low-PiB healthy controls with no differences on other demographic variables (Table 1). Controlling for the effects of age, gender and years of education, high-PiB healthy controls had higher scores at the long-delay recall of the California Verbal Learning Test compared to low-PiB healthy controls ($P = 0.05$). Note that the same results were obtained with performances expressed as z-scores (using normative data adjusted for age and gender) and only introducing years of education as a covariate (low-PiB healthy controls mean = 0.8; high-PiB healthy controls mean = 1.4; $P = 0.01$). No significant differences were found for the other neuropsychological measures. Regarding MRI data, global grey matter volume did not significantly differ between high-PiB healthy controls and low-PiB healthy controls when controlling for the effects of age, gender and years of education, but a trend for larger volume in high-PiB healthy controls was observed ($P = 0.07$). The voxel-based comparison of grey matter data between high versus low-PiB healthy controls did not reveal any area of significant atrophy in high-PiB healthy controls, but higher grey matter volume was found in high-PiB healthy controls compared to low-PiB healthy controls in the temporal lobe, including the bilateral parahippocampal and temporopolar cortices and hippocampus (subiculum), as well as right middle and superior temporal cortex and left inferior temporal cortex (Fig. 1 and Table 2).

Second, high versus low PiB cases were compared within the subjective cognitive impairment group. Compared to low-PiB cases with subjective cognitive impairment, high-PiB cases with
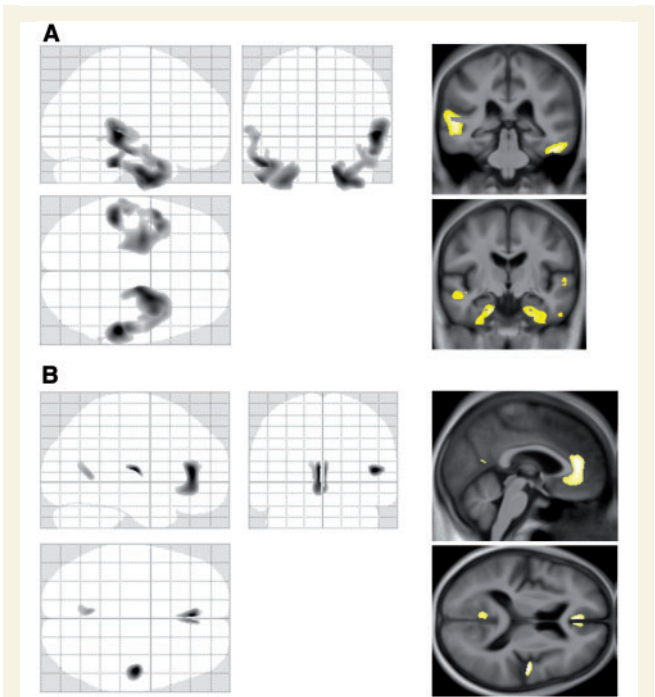

Figure 1 Brain areas of higher grey matter volume in high versus low-PiB healthy controls [**A** P(uncorrected) < 0.001 and cluster size $k > 5000$] and brain areas of lower grey matter volume in high versus low cases with subjective cognitive impairment [**B** P(uncorrected) < 0.001 and cluster size $k > 500$], displayed as Statistical Parametric Mapping 'glass brain' views and superimposed onto selected sections of the template MRI. Details on the peaks are provided in Table 2.

Table 2 Details on the voxel-based findings of the comparison between high-PiB versus low-PiB cases within the healthy controls and the subjective cognitive impairment groups

Montreal Neurological Institute Coordinates	Cluster size K	P (family wise error-corrected)	t-value	P (uncorrected)
Peaks of significantly higher grey matter volume in high versus low-PiB healthy controls				
54, −30, 3	18 481	0.007	5.23	5.8e−07
−48, −33, −25	15 672	0.03	4.85	2.7e−06
Peaks of significant grey matter atrophy in high versus low-PiB subjective cognitive impairment				
49, −14, 11	677	0.2	4.1	4.1e−05
−3, 38, 10	2740	0.3	4.1	4.2e−05
−7, −56, 8	619	0.8	3.5	3.8e−04

Size, coordinates and statistics for each cluster peak of significantly larger grey matter volume in high-PiB healthy controls compared to low-PiB healthy controls and of significant atrophy in high-PiB subjective cognitive impairment compared to low-PiB subjective cognitive impairment (main analyses).

subjective cognitive impairment were older ($P = 0.02$) and had a higher prevalence of ApoE4 allele ($P = 0.00006$—only 1/30 cases of low-PiB subjective cognitive impairment had at least one ApoE4 allele compared with 63% of the high-PiB subjective cognitive

impairment group). Controlling for the effects of age, years of education and gender, high-PiB subjective cognitive impairment cases had lower performances than those with low-PiB subjective cognitive impairment on the Mini-Mental State Examination ($P = 0.03$), Rey-3′ recall ($P = 0.01$) and trends in the same direction were observed for the longer delay (Rey-30′ recall; $P = 0.07$). Global grey matter volume was not significantly different between high-PiB subjective cognitive impairment cases and those with low-PiB subjective cognitive impairment when age, years of education and gender were accounted for, although a trend was observed for lower grey matter volume in high-PiB subjective cognitive impairment compared to low-PiB subjective cognitive impairment ($P = 0.09$). The voxel-based comparison of grey matter data revealed areas of significant atrophy in high-PiB subjective cognitive impairment compared to low-PiB subjective cognitive impairment located in the anterior and posterior cingulate cortex and temporoparietal regions (Fig. 1 and Table 2), while there was no area showing significantly higher grey matter volume in the high-PiB subjective cognitive impairment compared to the low-PiB subjective cognitive impairment.

Third, high-PiB healthy controls were compared to high-PiB subjective cognitive impairment, high-PiB mild cognitive impairment, and high-PiB Alzheimer's disease to assess what differentiates, among cases with β-amyloid deposition, those with subjective or objective cognitive deficits from those without cognitive deficits. There were no differences between high-PiB healthy controls and those with high-PiB subjective cognitive impairment, high-PiB mild cognitive impairment or high-PiB Alzheimer's disease in terms of age, gender and ApoE4 status. There was a main effect of group on years of education ($P = 0.03$) and intellectual quotient ($P = 0.002$), with *post hoc* pairwise comparisons revealing higher education ($P = 0.04$) and higher intellectual quotient (0.005) in

high-PiB healthy controls compared to high-PiB Alzheimer's disease, and higher intellectual quotient (0.05) and a trend for higher education ($P = 0.06$) in high-PiB healthy controls compared to high-PiB mild cognitive impairment. A significant main effect of Group was found for all neuropsychological variables with *post hoc* analyses showing lower performances compared to high-PiB healthy controls in all the tests for the high-PiB Alzheimer's disease, and in the long-delay recall of the California Verbal Learning Test, Rey 3′ and 30′ recall as well as category fluency for the high-PiB mild cognitive impairment; there were no significant differences in high-PiB subjective cognitive impairment compared to high-PiB healthy controls. The effect of group on total grey matter volume was highly significant ($P < 0.0001$), with a trend for high-PiB healthy controls > high-PiB subjective cognitive impairment > high-PiB mild cognitive impairment > high-PiB Alzheimer's disease, and *post hoc* group comparisons reaching statistical significance for high-PiB healthy controls > high-PiB Alzheimer's disease ($P = 0.0002$) and high-PiB healthy controls > high-PiB mild cognitive impairment ($P = 0.007$). The voxel-based analysis of volume compared to high-PiB healthy controls revealed significant atrophy, mainly located in the temporal lobe in high-PiB subjective cognitive impairment, extending to temporo-occipital, temporoparietal and frontal areas in high-PiB mild cognitive impairment, and involving almost the whole grey matter in high-PiB Alzheimer's disease (Fig. 2).

Complementary analyses

A first set of complementary analyses was performed to ensure the finding of larger (temporal) grey matter volume in high-PiB healthy controls versus low-PiB healthy controls was not due to methodological issues, such as the selected PiB threshold, partial
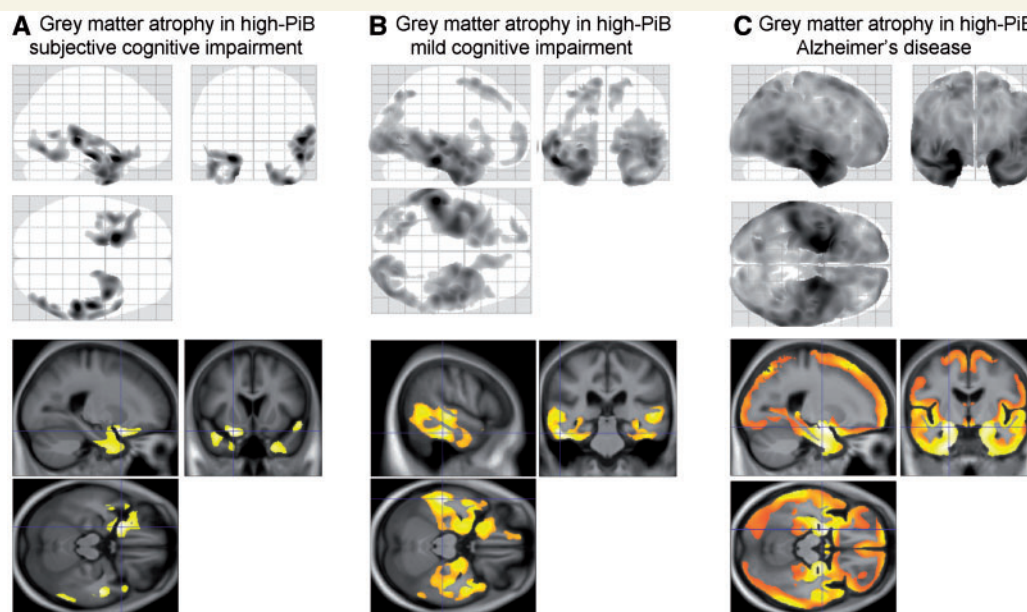
Figure 2 Brain pattern of atrophy in high-PiB cases with subjective cognitive impairment (**A**), mild cognitive impairment (**B**) and Alzheimer's disease (**C**) compared to high-PiB healthy controls, displayed as Statistical Parametric Mapping 'glass brain' views and superimposed onto coronal, sagittal and axial sections of the template MRI. Findings are displayed at P(uncorrected) < 0.001 and cluster size $k > 5000$.

volume effects, mismatches between groups (in term of age, education, ApoE4, Mini-Mental State Examination or gender) or the use of an automated voxel-based morphometry method.

First, the same analysis was performed in a restricted subsample (Subsample 1; *n* = 10 high-PiB healthy controls and 27 low-PiB healthy controls; Supplementary Table 1) excluding participants showing intermediate neocortical PiB values (i.e. values between 1.25 and 1.55) as well as those with a different PiB-status when using partial volume effect-corrected PiB values. As illustrated in Supplementary Fig. 1, the results were highly similar, showing larger temporal grey matter volume in the high-PiB healthy controls.

Second, each of the ten high-PiB healthy controls cases from Subsample 1 was carefully matched to a low-PiB healthy control case in terms of age, gender, ApoE4 status, education and Mini-Mental State Examination (Subsample 2; *n* = 10 high-PiB healthy controls and 10 low-PiB healthy controls; Supplementary Table 1). Region of interest analyses of the hippocampus were then performed on this subsample of subjects. Although the cluster of larger grey matter volume in high-PiB healthy controls versus low-PiB healthy controls from the main analysis did not include the whole hippocampus (but mainly concerned the subiculum; Fig. 1) it was selected here for the region of interest analysis because it is the easiest and most reliable structure to delineate. Manual delination of the hippocampus was performed on the right and left hemispheres of each of the 20 participants from Subsample 2. Hippocampal anatomic boundaries were drawn on each of the contiguous coronal slices of each individual scan, from anterior to posterior, by the same experienced observer (G.C.) according to previously published anatomical guidelines (Mevel *et al.*, 2007), blinded to PiB status and using the publicly available 'Anatomist/BrainVISA' software (http://www.brainvisa .info). Hippocampal volumes were then normalized to the total intracranial volume (see above) and right and left hippocampal volumes were averaged for each individual. Two-sample *t*-tests were used to compare demographic, neuropsychological and MRI data between high versus low-PiB healthy controls. As expected, high-PiB healthy controls and low-PiB healthy controls from Subsample 2 were similar in terms of age, years of education, gender, Mini-Mental State Examination and ApoE4 status (all *P*-values > 0.5). Compared to low-PiB healthy controls, high-PiB healthy controls performed better on the long-delay recall of the California Verbal Learning Test (*P* = 0.01) and on category fluency (*P* = 0.05; Fig. 3). Higher global grey matter volume was found in the 10 high-PiB healthy controls compared to the 10 low-PiB healthy controls (*P* = 0.01; Fig. 3), and the voxel-based analysis revealed the same pattern of larger temporal grey matter volume in high versus low-PiB healthy controls as that reported for the whole sample, although with lower statistical significance. The comparison of hippocampal volumes obtained by manual delineation also consistently revealed a statistically significant difference between both groups, with high-PiB healthy controls showing greater hippocampal volume than low-PiB healthy controls (*P* = 0.02; Fig. 3).

A second set of complementary analyses was performed to support the interpretation of the findings, i.e. to assess whether larger temporal volume in high versus low-PiB healthy controls would rather reflect a pathological or a protective process and to further define the nature of the process. First the correlation between temporal volume and memory performances was assessed in the healthy controls, as a positive versus negative relationship would rather support the protective versus pathological process hypothesis, respectively. Individual measures of temporal volume in the cluster of most significant difference in high versus low-PiB healthy controls (from the main analysis) was extracted and correlated to performances on the long-delay recall of the California Verbal Learning Test, controlling for age, education and gender in the whole group of healthy controls. A significant positive relationship was found, with larger medial temporal volume being associated with better episodic memory performances (*P* = 0.008).

Secondly, we also compared white matter data in high versus low-PiB healthy controls to assess whether this larger temporal volume was confined to the grey matter where the neuronal bodies reside, or if it was paralleled by larger white matter volume, which would rather suggest an increase in the number or size of neurons instead of an hypertrophy of the neuronal nuclei, cell bodies and nucleoli as previously reported (Riudavets *et al.*, 2007; Iacono *et al.*, 2009; see Supplementary material). There were no areas of significantly larger white matter volume in high versus low-PiB healthy controls (even when lowering the statistical threshold to *P* < 0.005).

Thirdly, we assessed whether higher atrophy in high-PiB mild cognitive impairment and high-PiB Alzheimer's disease compared to high-PiB healthy controls was due to higher degree of β-amyloid deposition in the former groups (see Supplementary material). We thus compared grey matter images between groups as performed in the main analyses, but introducing global neocortical PiB as a supplementary covariate. The findings were almost unchanged, indicating that differences in regional volumes are not related to the progressive increase in PiB from high-PiB healthy controls/high-PiB subjective cognitive impairment to high-PiB mild cognitive impairment and from high-PiB mild cognitive impairment to high-PiB Alzheimer's disease.

Discussion

The main finding of this study is a larger temporal (including hippocampal/parahippocampal area) volume in high-PiB healthy controls compared to low-PiB healthy controls. This finding may appear surprising as amyloid deposition is thought to be associated with atrophy, as already reported in some, though not all, studies (Jack *et al.*, 2008; Dickerson *et al.*, 2009; Storandt *et al.*, 2009; Bourgeat *et al.*, 2010). In a previous study, however, we demonstrated that the relationship between PiB and atrophy differs when separating healthy controls from individuals with subjective cognitive impairment, a significant correlation only being observed in the latter (Chételat *et al.*, 2010). The lack of correlation in the healthy controls could have reflected the lack of statistical power due to the limited number of cases with high-PiB in this group. The findings in the present study make this explanation unlikely, as high-PiB healthy controls instead had significantly larger (temporal) grey matter volume than low-PiB healthy controls. Controlling for several methodological factors that may have
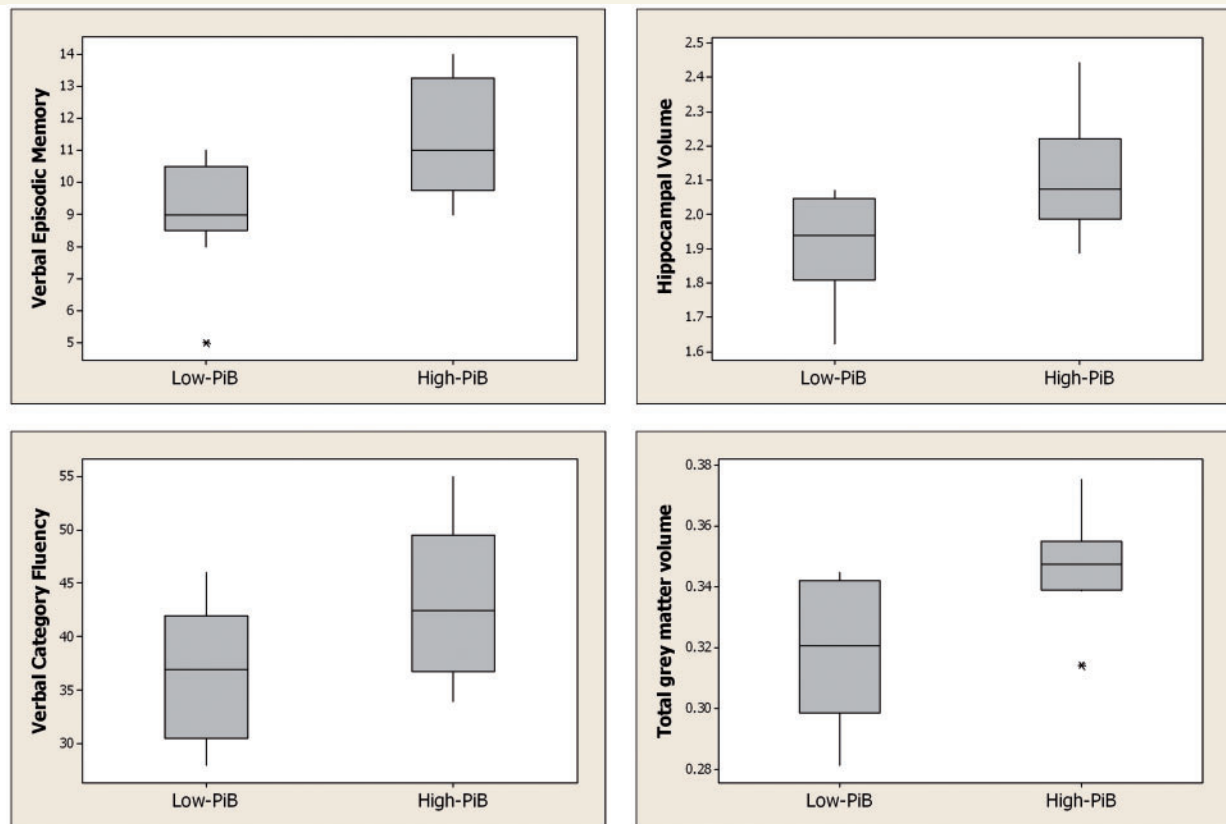
Figure 3 Comparison of verbal memory and category fluency performances, as well as manually delineated hippocampal volume (average of right and left hippocampi divided by the total intracranial volume and multiplied by 10^5 for display) and global grey matter volume (divided by the total intracranial volume) in the 10 high-PiB healthy controls compared to the 10 matched low-PiB healthy controls (Subsample 2). All data are represented as boxplots indicating (from top to down) the largest observation, upper quartile, median, lower quartile and smallest observation (and outliers if any). All differences were significant ($P < 0.05$) and showed higher values for low-PiB healthy controls.

explained this result, such as partial-volume averaging (that may lead to decreased PiB values in subjects with lower brain volume), the use of a voxel-based method, a potential mismatch between high versus low PiB subgroups or the use of a specific threshold for defining the PiB status, confirmed that none of these factors accounted for the findings. Interestingly, a previous study reported that cognitively intact individuals with a high burden of Alzheimer's disease pathology had larger hippocampal and total brain volume than individuals with overt Alzheimer's disease dementia and a similar amount of Alzheimer's disease pathological change (Erten-Lyons et al., 2009). The findings presented here are consistent with that report, as high-PiB healthy controls were found to have larger global and regional grey matter volumes than high-PiB mild cognitive impairment and high-PiB Alzheimer's disease. Additionally, the temporal volume of high-PiB healthy controls was also larger than that of low-PiB healthy controls, suggesting that the results reflect larger volume compared to the standard volume, rather than a lack of atrophic process in healthy controls compared to mild cognitive impairment or Alzheimer's disease.

This larger (temporal) volume in the high-PiB healthy controls may reflect oedema or other tissue reactive responses to β-amyloid deposition. Indeed, in vivo studies in Alzheimer's

disease show evidence of glial activation in temporal and parietal (Cagnin et al., 2001) as well as frontal and occipital (Edison et al., 2008) cortices, and activated microglia were found to cluster around sites of β-amyloid in transgenic mouse models of Alzheimer's disease (Meyer-Luehmann et al., 2008). Furthermore, anti-amyloid immunotherapy was found to be associated with increased brain volume losses (despite cognitive improvement) thought to reflect β-amyloid removal and associated cerebral fluid shifts (Fox et al., 2005). The regions of larger volume in high-PiB healthy controls compared to low-PiB healthy controls evidenced in the current study however, namely anterior medial and lateral temporal cortices, did not match those of highest β-amyloid deposition (i.e. posterior and anterior cingulate, medial frontal and temporoparietal cortices). Although there might be region-specific differences in the reactivity of microglial populations, our findings are thus unlikely to reflect a direct reaction of tissue to β-amyloid deposition. Moreover, neuroimaging studies assessing both β-amyloid deposition and microglial activation in vivo in the same subjects with mild cognitive impairment (Okello et al., 2009) or Alzheimer's disease (Edison et al., 2008) did not find any correlation between regional β-amyloid burden and microglial activation, suggesting that these pathological changes can develop independently. Lastly, the finding that

temporal grey matter volume was positively correlated to verbal episodic memory performances in the healthy controls, and that better memory performances were found in the high-PiB healthy controls compared to the low-PiB healthy controls, argue against the hypotheses of neuroinflammation or β-amyloid deposition *per se*.

There are alternative hypotheses to account for the finding of larger temporal brain volume in high versus low-PiB healthy controls. This finding is likely to reflect, at least in part, the fact that individuals with both β-amyloid deposition and temporal atrophy are more likely to have subjective or objective cognitive decline, so that high-PiB healthy controls are only those subjects with large temporal volume. Variability in the temporal volume could reflect age-related processes independent from β-amyloid deposition, such as neurofibrillary degeneration, but also higher 'brain reserve' or other protective/compensatory processes in the high-PiB healthy controls. Both the brain reserve and the compensatory hypotheses do have experimental support from previous studies. Thus, regarding brain reserve, increase of cortical thickness was found in high versus normal performing elderly (Fjell *et al.*, 2006) and larger brain volume was reported in more educated compared to less educated healthy elderly (Solé-Padullés *et al.*, 2009). Moreover, education was also found to modify the relation of plaques to cognition so that highly educated subjects have less susceptibility to amyloid-related cognitive impairment than those with lower education (Bennett *et al.*, 2003; Rentz *et al.*, 2010). It is thus possible that the high-PiB healthy controls in the present study represent those subjects with particularly high brain reserve reflected by larger brain (temporal) volume and cognitive-integrity, while high-PiB subjects with lower reserve would be found in the subjective cognitive impairment, mild cognitive impairment or Alzheimer's disease groups. Proxies of brain/cognitive reserve include years of education, intellectual quotient and total intracranial volume (Mori *et al.*, 1997; Stern *et al.*, 2006), but the findings reported here regarding these indices do not allow clear-cut conclusions. Indeed, on the one hand no significant difference was found in total intracranial volume or education in high-PiB healthy controls compared to low-PiB healthy controls, and there was no correlation between temporal volume and years of education in the healthy controls (data not shown), suggesting that larger volume is not directly linked with higher education. On the other hand, high-PiB healthy controls have higher memory score than low-PiB healthy controls, and also have significantly more years of education and higher intellectual quotient compared to high-PiB mild cognitive impairment and high-PiB Alzheimer's disease, which would argue for the hypothesis of brain reserve. These findings are consistent with previous studies showing a significantly lower education (or other reserve proxies) in Alzheimer's disease and mild cognitive impairment compared to controls (Katzman *et al.*, 1989), and even compared to controls with Alzheimer's disease pathologic changes (Iacono *et al.*, 2009). Note that more sophisticated measures of social, physical and intellectual occupation or environmental/lifestyle factors may allow a better understanding of these findings. Plasma vitamin B12 measurement was also found to be a significant determinant of brain atrophy in the normal elderly (Vogiatzoglou *et al.*, 2008), but it was not associated with larger temporal volume in the high-PiB healthy controls in the present study (data not shown). Alternatively, larger (temporal) volume in high-PiB healthy controls compared to low-PiB healthy controls may reflect a compensatory response resulting from β-amyloid deposition. Interestingly, hippocampal hypertrophy of the neuronal nuclei (Riudavets *et al.*, 2007; Iacono *et al.*, 2009), cell bodies and nucleoli (Iacono *et al.*, 2009) has been evidenced at autopsy in the brains of normal elderly with β-amyloid plaques compared to normal elderly without β-amyloid plaques and patients with mild cognitive impairment or Alzheimer's disease. These findings were interpreted as reflecting an early (compensatory) cellular response to injury allowing the brain to function normally despite the presence of Alzheimer's disease lesions. Our complementary analysis on the white matter suggesting that changes were confined to the grey matter where the neuronal-bodies reside would be consistent with these previous post-mortem reports, although it is not possible to establish whether this larger temporal volume was present before β-amyloid deposition, reflecting brain reserve, or is a reaction to β-amyloid deposition.

Some other findings of the present study also deserve comment. High-PiB cases were older than low-PiB ones, in both the healthy controls and the subjective cognitive impairment groups. This probably reflects the increased risk of β-amyloid deposition with increasing age. Also, all but one low-PiB participants with subjective cognitive impairment were ApoE4-negative. This finding suggests that individuals with both an ApoE4 allele and memory complaint are very likely to have high-PiB, which is consistent with recent evidence of an association between fibrillar β-amyloid burden and ApoE4 gene dose in cognitively normal older people (Reiman *et al.*, 2009). Finally, consistent with a previous study (Chételat *et al.*, 2010), our findings suggest that the separation between complainers and non-complainers within the elderly is especially relevant when assessing the relationship between PiB and atrophy. However, the definition of subjective cognitive impairment deserves comment. There are no consensual criteria to date, and the types of questions used to determine subjective cognitive impairment include simple questions with yes/no responses, questions with graded responses, scales and self-report questionnaires (Abdulrab and Heun, 2008). Based on a single question in the present study, subjective cognitive impairment is likely to encompass heterogeneous aetiologies, including cognitive deterioration (due to various different underlying processes) still undetectable using cognitive tests as well as various psychological factors. Note that using objective criteria (i.e. memory performances), the same findings of larger temporal volume in high-PiB compared to low-PiB was found in the high-performers only, although the effect was less clear-cut than when using subjective criteria (data not shown). This probably reflects the fact that subjective memory deficits are only imperfectly associated with objective measures of memory capacity. Further studies are needed to define consensual criteria for subjective cognitive impairment.

The present study provides strong evidence for larger temporal volume in high-PiB healthy controls compared to low-PiB healthy controls, suggesting that people with larger temporal grey matter better and/or longer tolerate the presence of β-amyloid deposition. Critical questions raised by the present study include what genetic or environmental factors, if any, enable individuals to have

preserved/higher cognition and brain volume despite β-amyloid deposition.

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Supplementary material

Supplementary material is available at *Brain* online.

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