Sequential relationships between grey matter and white matter atrophy and brain metabolic abnormalities in early Alzheimer’s disease

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Hippocampal atrophy, posterior cingulate and frontal glucose hypometabolism, and white-matter tract disruption are well described early macroscopic events in Alzheimer’s disease. The relationships between these three types of alterations have been documented in previous studies, but their chronology still remains to be established. The present study used multi-modal fluorodeoxyglucose-positron emission tomography and magnetic resonance imaging longitudinal data to address this question in patients with amnestic mild cognitive impairment. We found unidirectional, specific sequential relationships between: (i) baseline hippocampal atrophy and both cingulum bundle ($r=0.70; P=3 \times 10^{-3}$) and uncinate fasciculus ($r=0.75; P=7 \times 10^{-4}$) rate of atrophy; (ii) baseline cingulum bundle atrophy and rate of decline of posterior ($r=0.72; P=2 \times 10^{-3}$); and anterior ($r=0.74; P=1 \times 10^{-3}$) cingulate metabolism; and (iii) baseline uncinate white matter atrophy and subgenual metabolism rate of change ($r=0.65; P=6 \times 10^{-3}$). Baseline local grey matter atrophy was not found to contribute to hypometabolism progression within the posterior and anterior cingulate as well as subgenual cortices. These findings suggest that hippocampal atrophy progressively leads to disruption of the cingulum bundle and uncinate fasciculus, which in turn leads to glucose hypometabolism of the cingulate and subgenual cortices, respectively. This study reinforces the relevance of remote mechanisms above local interactions to account for the pattern of metabolic brain alteration observed in amnestic mild cognitive impairment, and provides new avenues to assess the sequence of events in complex diseases characterized by multiple manifestations.

Keywords: Alzheimer’s disease; magnetic resonance imaging/functional magnetic resonance imaging; positron emission tomography imaging; white matter; hippocampus

Abbreviations: $^{18}$FDG = fluorodeoxyglucose; MCI = mild cognitive impairment
Introduction

Alzheimer’s disease is characterized by distinct brain alterations highlighted in vivo using imaging techniques including grey matter atrophy, resting-state glucose hypometabolism and disruption of some white matter tracts. More specifically, studies using T1-weighted MRI have shown that the hippocampus is the brain region of highest and earliest atrophy in Alzheimer’s disease (Whitwell et al., 2007; Risacher et al., 2009). Fluorodeoxyglucose (18FDG)-PET studies have highlighted early hypometabolism in posterior associative cortical areas, especially in the posterior cingulate cortex and subsequently in prefrontal areas (Morbelli et al., 2010). Finally, studies using voxel-based morphometry or diffusion tensor imaging have pointed to the alteration of several white matter tracts in Alzheimer’s disease, particularly the corpus callosum, cingulum bundle, fornix and uncinate fasciculus (Li et al., 2008; Bai et al., 2009b; Kiuchi et al., 2009; Stricker et al., 2009). The discrepancies between structural and functional changes, especially in the posterior cingulate cortex showing early hypometabolism despite non-predominant atrophy (Alsop et al., 2008; Chételat et al., 2008), have led to the disconnection hypothesis according to which grey matter atrophy would disrupt white matter tracts and then induce distant hypometabolism (Jobst et al., 1992; Mielke et al., 1996; Matsuda, 2001; Smith, 2002; Nestor et al., 2003). Recently, several functional MRI studies have demonstrated alterations of functional connectivity in Alzheimer’s disease (Wang et al., 2006; Allen et al., 2007; Sorg et al., 2007; Zhang et al., 2009) arguing for the view that Alzheimer’s disease is, at least in part, a disconnection syndrome targeting cerebral regions of a same brain network (Seeley et al., 2009).

Several recent studies have directly assessed the relationship between some of these brain alterations in Alzheimer’s disease or amnestic mild cognitive impairment (MCI). These studies showed a relationship between hippocampal atrophy and distant alterations including posterior cingulate hypometabolism (Guedj et al., 2009), posterior cingulate activity during a memory task (Garrido et al., 2002; Rémy et al., 2005) and disruption of the cingulum bundle (Firbank et al., 2007; Villain et al., 2008; Choo et al., 2010). Thanks to a multi-modal correlative approach, we previously demonstrated a significant relationship between hippocampal atrophy, cingulum bundle disruption and posterior cingulate hypometabolism in patients with Alzheimer’s disease (Villain et al., 2008). Moreover, in another study on amnestic MCI, metabolic decrease in two frontal areas, namely the anterior cingulate cortex and the subgenual area, correlated with posterior cingulate and hippocampal metabolic changes, respectively (Fouquet et al., 2009). The hippocampus is connected to both the posterior and the anterior cingulate cortices via the cingulum bundle, and to the subgenual region via the uncinate fasciculus (Mufson and Pandya, 1984; Morris et al., 1999; Schmahmann and Pandya, 2006; Zhong et al., 2006); the metabolic alteration in these regions was interpreted as reflecting the disruption of these two major hippocampofrontal pathways as a result of hippocampal atrophy.

There are, however, several alternatives to this hypothesis. For instance, these inter-related brain alterations may occur in parallel as distinct effects of a common upstream pathological process. Even if they reflect a cascade of sequential events, several mechanisms are still possible: grey matter atrophy or hypometabolism could be responsible for disruption of distant connected white matter tracts and/or white matter tract disruption could induce distant hypometabolism and grey matter atrophy as a result of Wallerian and retrograde degeneration. Local interactions should also be considered, i.e. grey matter atrophy may induce local hypometabolism and, conversely, hypometabolism may lead to local grey matter atrophy. Determining the initial event responsible for the others is of importance as it may help target the key component for therapeutic interventions.

The aim of the present study was therefore to take advantage of repeated multi-modal (18FDG-PET and T1-MRI) acquisitions obtained in patients with amnestic MCI to directly assess the sequential relationships between the main brain alterations observed in early Alzheimer’s disease, namely hippocampal atrophy, cingulate and uncinate fasciculi disruption and posterior cingulate and medial prefrontal hypometabolism. To this end, we assessed the local and distant relationships between baseline and rates of change measurements; an alteration A was considered as the starting and preceding event of B if the correlation between baseline A and rate of change was both statistically significant and significantly higher than the correlation between rate of change and baseline B or baseline A and baseline B.

Material and methods

Subjects

Seventeen unmedicated patients with amnestic MCI were recruited through a memory clinic; all complained of memory impairment. They were right-handed, aged >55 years and had at least 7 years of education (see Table 1 for demographic and clinical characteristics). They underwent medical, neurological, neuropsychological and radiological examinations and were screened for the lack of cerebrovascular risk factors, substance abuse, head trauma and significant MRI (other than cortical atrophy) or biological abnormality. They were selected according to current criteria of single domain amnestic MCI (Petersen and Negash, 2008), i.e. isolated episodic memory

<table>
<thead>
<tr>
<th>Table 1 Demographic and clinical characteristics of participants</th>
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<tr>
<td><strong>Healthy controls</strong></td>
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<td>Number</td>
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<td>Age at baseline (years)</td>
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Baseline = entry in the protocol; follow-up = 18 months after baseline. MMSE = Mini mental state examination.

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deficits (>1.5 SD of the normal mean for age and education), normal performance in other areas of cognition and in global cognition (assessed with the Mini Mental State Examination and Mattis scales) and clinical criteria for probable Alzheimer’s disease (McKhann et al., 1984) not met (for more details see Chételat et al., 2005). All patients with amnestic MCI were evaluated every 6 months over an 18-month follow-up period to assess whether they converted to probable Alzheimer’s disease. Over the 18-month follow-up period, seven of the 17 patients with amnestic MCI met National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria for probable Alzheimer’s disease. Only patients who had both T1-weighted MRI and 18FDG-PET examinations at baseline and follow-up were included in the present study. The patients were the same as those reported in Fouquet et al. (2009). Seventeen unmedicated right-handed healthy aged controls with at least 7 years of education also underwent both MRI and 18FDG-PET examinations at inclusion. They were screened for the absence of cerebrovascular risk factors, mental disorder, substance abuse, head trauma and significant MRI or biological abnormality. The two groups were matched for age and sex (Table 1). All subjects were fully cooperative and free from behavioural disturbances. They all gave their consent to the study after detailed information was provided to them and the PET procedure was approved by the Ethical Committee of the University of Caen. The study was carried out in line with the Declaration of Helsinki (1964).

Imaging data acquisition

All MRI data sets were acquired on the same scanner (1.5 T Signa Advantage echospeed; General Electric, Milwaukee, WI) using the same acquisition parameters. MRI consisted of a set of 128 adjacent axial slices parallel to the anterior commissure–posterior commissure line with slice thickness 1.5 mm and pixel size 0.9375 × 0.9375 mm² using a spoiled gradient echo sequence (repetition time = 10.3 ms; echo time = 2.1 ms; field of view = 240 × 180 mm²; matrix = 256 × 192).

18FDG-PET data were collected using the Siemens ECAT Exact HR+ PET device with resolution of 4.2 × 4.2 × 4.6 mm³ (x y z) (axial field of view = 158 mm). Patients fasted for at least 4 h before scanning. To minimize anxiety, the PET procedure was explained in detail beforehand. The head was positioned on a headrest according to the centromental line and gently restrained with straps. 18FDG uptake was measured in the resting condition, with eyes closed, in a quiet and dark environment. A catheter was introduced into a vein of the arm to inject the radiotracer. Following 68Ga transmission scans, 3–5 mCi of 18FDG were injected as a bolus at Time 0, and a 10 min PET data acquisition started at 50 min post-injection. Sixty-three planes were acquired with septa out (3D acquisition), using a voxel size of 2.2 × 2.2 × 2.43 mm³ (x y z).

Imaging data handling and transformation

Magnetic resonance imaging data

MRI data sets were analysed using Statistical Parametric Mapping 5 (SPM5; http://www.fil.ioi.ucl.ac.uk/spm). In order to reduce noise differences between baseline and follow-up MRI data and to improve the accuracy of baseline to follow-up change measurements, we used a procedure derived from our previous longitudinal voxel-based morphometry procedure (Chételat et al., 2005): (i) pairs of raw baseline and follow-up MRIs were first rigidly coregistered and a high dimensional warping was undertaken to calculate volumetric deformations from baseline to follow-up MRI and from follow-up to baseline MRI (Ashburner and Friston, 2004); (ii) warps from baseline to follow-up MRI were then applied to the baseline MRI data sets and warps from follow-up to baseline MRI were applied to the follow-up MRI data set; and (iii) a softmean MRI was calculated for each baseline (baseline plus warped follow-up) and follow-up (follow-up plus warped baseline) MRI data set.

These baseline and follow-up softmean MRI data were then segmented using VBM5.1 (http://dbm.neuro.uni-jena.de/vbm/) and imported into the diffeomorphic DARTEL pipeline (Ashburner, 2007). Using this pipeline, 17 ‘single-subject baseline-follow-up templates’ were created and then averaged to create a ‘group template’. For each subject, baseline and follow-up softmean grey matter, white matter and CSF data sets were normalized to the group template by applying a combination of the deformation parameters from both warps (i.e. the warp to the single-subject baseline-follow-up template and the warp to the group template). Resultant images were modulated by Jacobian determinants, smoothed at 9 mm full-width-at-half-maximum and masked (see below).

For the sake of group comparison, baseline MRI data from healthy controls were segmented using VBM5.1 and warped to the same group template as that used for patients with amnestic MCI using the DARTEL pipeline.

18FDG positron emission tomography data

18FDG-PET data were first corrected for partial volume effect due to both CSF and white matter using the optimal voxel-by-voxel method originally proposed by Muller-Gartner et al. (1992) and slightly modified as proposed by Roussel et al. (1998). This method, referred to as ‘modified Muller-Gartner’, is described in detail in Quarantelli et al. (2004) and has been widely applied in our laboratory (Chételat et al., 2003b, 2008; Mevel et al., 2007; Villain et al., 2008; Fouquet et al., 2009). All image processing steps for partial volume effect correction were carried out using the ‘PVE-lab’ software (Quarantelli et al., 2004). 18FDG-PET data were then scaled using a metabolically preserved brain region, namely the cerebellar vermis, to control for inter- and intra-individual global variations in 18FDG-PET signal (Mevel et al., 2007). Using SPM5, baseline partial volume effect-corrected scaled 18FDG-PET data of controls and baseline and follow-up partial volume effect-corrected scaled 18FDG-PET data of patients with amnestic MCI were then coregistered onto their respective MRIs and spatially normalized onto the same group template as that used for the spatial normalization of MRI data, by reapplying the combined warping parameters estimated from the DARTEL pipeline. The normalized scaled 18FDG-PET data sets were then smoothed (8 mm full-width-at-half-maximum; see below) and masked using the same grey matter mask as that used for the grey matter partition obtained from MRI data (see below).

Differential smoothing

To blur individual variations in gyral anatomy and increase the signal-to-noise ratio, the spatially normalized grey and white matter partitions and the partial volume effect-corrected scaled spatially normalized 18FDG-PET data sets were smoothed. We used a Gaussian kernel of 8 mm full-width-at-half-maximum for the 18FDG-PET data. Since 18FDG-PET and MRI data had different original spatial resolutions, differential smoothing was applied in order to obtain images of equivalent effective smoothness and thus of identical resultant resolution (Richardson et al., 1997; Van Laere and Dierckx, 2001). To this end, we used a Gaussian kernel of 9 mm
full-width-at-half-maximum for the MRI grey and white matter data, resulting in an effective smoothness identical to $^{18}$FDG-PET images smoothed at 8 mm full-width-at-half-maximum (Poline et al., 1995).

Masking
The grey matter, white matter and $^{18}$FDG-PET images obtained following the steps above were masked so as to include only grey or white matter voxels of interest and to prevent any overlap between voxels included in analyses with grey matter and those with white matter. The grey matter mask corresponded to the voxels of the group template with a grey matter probability higher than both white matter and CSF probabilities (grey matter > white matter > CSF) and the white matter mask to the voxels of the group template with a white matter probability higher than both grey matter and CSF probabilities (white matter > grey matter > CSF), thus avoiding any overlap between voxels included in analyses with grey matter and those with white matter.

Percent change maps
For each patient with amnestic MCI, maps of percent annual change were created for each imaging modality (grey matter, white matter, and $^{18}$FDG-PET). The percent change maps represent the voxel-wise calculation of percent change in one imaging modality over the 18-month follow-up period expressed as annual percent:

$$\text{percent change} = \left( \frac{\text{Follow-up map} - \text{Baseline map}}{\text{Baseline map}} \right) \times 100 \times \frac{12}{\text{Follow-up duration (months)}}$$

(Fouquet et al., 2009). Briefly, percent change maps were calculated from baseline and follow-up partial volume effect-corrected scaled images warped to the single-subject template and smoothed at 3 mm full-width-at-half-maximum (baseline and follow-up $^{18}$FDG-PET data) or at 5.15 mm full-width-at-half-maximum (baseline and follow-up grey and white matter data). Percent change maps were then warped onto the group template, smoothed at 7.4 mm full-width-at-half-maximum resulting in an effective smoothness identical to baseline or follow-up $^{18}$FDG-PET images smoothed at 8 mm full-width-at-half-maximum and masked using the grey or white matter mask described above.

Statistical analyses

Intramodality analyses
For the sake of completeness, we first assessed the baseline patterns of grey and white matter atrophy by comparing the smoothed and masked grey or white matter baseline data sets of patients with amnestic MCI with those of controls using a two-sample $t$-test in SPM5 with total intracranial volume as a covariate. Patterns of grey and white matter atrophy evolution within the amnestic MCI sample were then assessed using a paired $t$-test in SPM5 with total intracranial volume as a covariate. The baseline pattern of grey matter hypometabolism and its evolution were assessed performing the same statistical analyses with $^{18}$FDG-PET data set except that total intracranial volume was not used as a covariate.

Intermodality analyses

Voxel-to-voxel intermodality analyses
To assess whether baseline grey matter atrophy induces local hypometabolism, or conversely whether baseline hypometabolism induces local grey matter atrophy (Fig. 1I and II), we performed two voxel-to-voxel multi-modal regressions between baseline grey matter maps and percent change $^{18}$FDG-PET maps (with total intracranial volume as a covariate) and between baseline $^{18}$FDG-PET maps and grey matter percent change maps using the biological parametric mapping toolbox (Casanova et al., 2007).

ROI-to-voxel distant intermodality analyses
We then assessed the relationship between baseline alterations and rates of change in distant brain areas (Fig. 1III and IV) to test the...
hypotheses listed in the ‘Introduction’. We used the main brain alterations observed in early Alzheimer’s disease, namely hippocampal atrophy, cingulate and uncinate fasciculi disruption, and posterior cingulate and medial prefrontal hypometabolism, as starting points in ROI-to-voxel analyses between baseline measurements and percent change maps. According to our hypotheses, these relationships were assessed in only the biologically expected (i.e. positive) direction.

**Correlation between baseline hippocampal atrophy and whole brain white matter MRI percent change maps**

First, the distant effects of baseline hippocampal atrophy on the evolution of white matter alterations were assessed (Fig. 1II). Baseline mean hippocampal grey matter values were extracted from the significantly atrophied hippocampal grey matter voxels (obtained by assessing the baseline pattern of grey matter atrophy), using a P uncorrected for multiple comparisons <0.001 threshold with k >50 mm^3. Anatomical delineation of the hippocampus, used for data extraction, was based on the Automated Anatomical Labelling software (Tzourio-Mazoyer et al., 2002). Since automated anatomical labelling was performed on the Montreal Neurological Institute single subject Colin27 T1-MRI, we segmented the raw T1-MRI of the Montreal Neurological Institute single-subject using VBM5.1, warped it onto the 17 amnestic MCI group template using the DARTEL pipeline and applied this transformation to the automatic anatomical labelling map using a nearest-neighbour interpolation. Positive regressions were then computed across the 17 patients with amnestic MCI between mean hippocampal baseline grey matter values and whole brain white matter percent change maps, with total intracranial volume as a covariate, using the ‘multiple regression’ SPM5 routine.

**Correlation between baseline ROI white matter values and whole brain 18FDG-PET and grey matter MRI percent change maps**

The effects of baseline white matter alterations in the two ROIs (cingulum bundle and uncinate fasciculus) on the progression of grey matter hypometabolism or atrophy over time were assessed (Fig. 1IV and VI). Mean baseline white matter values were extracted in these two regions for each patient from the significant clusters of the previous voxel-based regression analysis (between hippocampal baseline grey matter values and white matter percent change maps) thresholded at P uncorrected <0.001 with k >50 mm^3. For each cluster, individual baseline values of white matter were then entered as covariates in a regression analysis with whole brain 18FDG-PET percent change maps or grey matter percent change maps, using the total intracranial volume as a covariate and the ‘multiple regression’ SPM5 routine.

**Correlation between baseline ROI 18FDG-PET values and whole brain white matter percent change maps**

Finally, the relationships between baseline grey matter hypometabolism in the posterior cingulate cortex, the anterior cingulate or the subgenual areas and white matter atrophy percent change maps were assessed (Fig. 1V). Mean posterior cingulate baseline 18FDG-PET values were extracted from the significantly hypometabolic posterior cingulate voxels (obtained by assessing the baseline pattern of grey matter hypometabolism), using a P uncorrected for multiple comparisons <0.001 threshold with k >50 mm^3. Anatomical delineation of the posterior cingulate cortex, for data extraction, was based on the automated anatomical labelling software adapted to our template (see above). Positive regressions were then computed across the 17 patients with amnestic MCI between mean posterior cingulate baseline 18FDG-PET values and whole brain white matter percent change maps, with total intracranial volume as covariate, using the ‘multiple regression’ SPM5 routine.

Baseline mean anterior cingulate 18FDG-PET values were extracted from the significant cluster of the previous voxel-based regression analysis (between cingulum bundle baseline white matter values and 18FDG-PET percent change maps), using a P uncorrected for multiple comparisons <0.005 threshold with k >50 mm^3. Since this cluster did not encompass other anatomical regions, no anatomical labelling was necessary. Positive regressions were then computed across the 17 patients with amnestic MCI between baseline mean anterior cingulate 18FDG-PET values and whole brain white matter percent change maps, with total intracranial volume as a covariate, using the ‘Multiple Regression’ SPM5 routine.

Baseline mean subgenual 18FDG-PET values were extracted from the significant cluster of the previous voxel-based regression analysis (between uncinate fasciculus baseline white matter values and 18FDG-PET percent change maps), using a P uncorrected for multiple comparisons <0.005 threshold with k >50 mm^3. Since this cluster did not encompass other anatomical regions, no anatomical labelling was necessary. Positive regressions were then computed across the 17 patients with amnestic MCI between baseline mean subgenual 18FDG-PET values and whole brain white matter percent change maps, with total intracranial volume as a covariate, using the ‘multiple regression’ SPM5 routine.

**Testing the unidirectionality of intermodality distant relationships**

As stated above, the unidirectionality of the correlations between baseline values of an alteration A and rate of change values of an alteration B was assessed by comparing the strength of this correlation with that of the correlation between baseline B and A rate of change, as well as that between baseline A and baseline B. Hence, for each significant relationship identified between two regions A and B in the voxel-to-voxel or ROI-to-voxel regression analyses above, we extracted the baseline and percent change mean values in the significant clusters and computed three multiple regression analyses using Statistica software (Statsoft, Tulsa, OK): (i) with baseline A values as the predictive variable and B percent change as the dependent variable; (ii) with A percent change as the predictive variable and baseline B as the dependent variable; and (iii) with baseline A as the predictive variable and baseline B as the dependent variable. The total intracranial volume was also introduced as a predictive variable in all multiple regression analyses. The three resulting partial correlation coefficients between A and B values were then statistically compared (Steiger, 1980).

**Evaluation of the relative contribution of grey and white matter atrophy to metabolism evolution through multiple regression analyses**

In a last set of analyses, we assessed whether white matter atrophy provides significant independent contribution to 18FDG-PET percent change, over and above local grey matter atrophy. We conducted three independent multiple regression analyses, one for each of the three metabolic ROIs (i.e. the posterior cingulate, anterior cingulate and subgenual areas). Each 18FDG-PET percent change ROI value was entered in a multiple regression including three predictive variables, i.e. the corresponding baseline white matter value, local baseline grey matter value and total intracranial volume.
Statistical threshold and display of results

Statistical Parametric Mapping-T maps of all previously described analyses were thresholded using a $P$ uncorrected $<0.001$ threshold with a $k > 50$ mm$^3$. For the sake of illustration, a lower threshold ($P < 0.005$) was used in some figures but this was then explicitly specified. Anatomical localization was based on the superimposition of the Statistical Parametric Mapping-T maps onto the group template and identification of the localization using the automated anatomical labeling software and anatomical atlases (Talairach and Tournoux, 1988; Tzourio-Mazoyer et al., 2002; Mori et al., 2005). The findings were rendered using the publicly available ‘Anatomist/BrainVISA’ and ‘MRICron’ software (http://www.brainvisa.info; http://www.sph.sc.edu/comd/rorden/mricron/).

Results

Profiles of grey matter atrophy, glucose hypometabolism and white matter atrophy

The profiles of baseline grey matter atrophy and hypometabolism have already been described elsewhere (Chételat et al., 2003a, 2005; Fouquet et al., 2009) and are presented here in a single integrated figure (Fig. 2) to provide a comprehensive view of brain alterations in this sample of patients with amnestic MCI. The profiles showed the prominent involvement of the hippocampus for grey matter atrophy and of the posterior cingulate cortex for hypometabolism in patients with amnestic MCI relative to controls. The largest changes over the 18-month follow-up period affected the medial and lateral temporal, parietal and frontal lobes for grey matter atrophy, and posterior cingulate, lateral parietal and prefrontal areas for metabolic changes (see Chételat et al., 2003a, 2005; Fouquet et al., 2009 for details). The profiles of cross-sectional and longitudinal white matter changes, not detailed elsewhere, are shown in Fig. 3. Baseline white matter atrophy mainly involved bilaterally the perforant path, the caudal part of the cingulum bundle and fornix as well as extensive left temporal white matter areas encompassing the superior and inferior longitudinal fasciculi, and right frontal white matter areas (Fig. 3, green). White matter atrophy evolution over the 18-month follow-up period concerned mainly a large bilateral frontoparietal cluster including the superior longitudinal fasciculus, rostral part of the cingulum bundle and superior fronto-occipital fasciculus, as well as the left uncinate fasciculus, bilateral perforant path and corticospinal tracts (Fig. 3, red).

Voxel-to-voxel intermodality regressions reveal no relationship between baseline grey matter atrophy or hypometabolism and local changes over time

The voxel-to-voxel regression analyses between baseline grey matter maps and $^{18}$FDG-PET percent change maps, and between baseline $^{18}$FDG-PET maps and grey matter MRI percent change maps did not reveal any statistically significant findings (Fig. 1I and

![Figure 2](https://academic.oup.com/brain/article-abstract/133/11/3301/312337)
II). This suggests that baseline atrophy was not related to local metabolic decrease over the subsequent 18 months and conversely that baseline hypometabolism does not predict subsequent local grey matter atrophy.

Baseline alterations are found to be related to changes over time in distant brain regions

The same findings were obtained for all analyses described below when introducing age and conversion status (converters versus non-converters) as covariates (data not shown).

Hippocampal atrophy at baseline is related to subsequent disruption of both the cingulum bundle and the uncinate fasciculus

The results of the ROI-to-voxel regression between significantly atrophied hippocampal baseline grey matter values and white matter percent change maps are displayed in Fig. 4. Significant regressions were found with the caudal part of the right cingulum bundle, left temporal and orbitofrontal white matter clusters belonging to the uncinate fasciculus, left internal capsule and middle part of the corpus callosum.

Baseline alterations of both the cingulum bundle and the uncinate fasciculus are related to subsequent annual rates of cortical hypometabolism, but not of atrophy

The results of the ROI-to-voxel regressions between baseline cingulum bundle white matter values and $^{18}$FDG-PET percent change maps (Fig. 1IV) are displayed in Fig. 5. Significant regressions were found within the right posterior cingulate cortex (mostly its retrosplenial part; Brodmann areas 29/30), the right anterior cingulate cortex, the right parahippocampal/fusiform gyrus and the right caudate nucleus. The results of the ROI-to-voxel regressions between baseline uncinate fasciculus white matter values and $^{18}$FDG-PET percent change maps (Fig. 1IV) are displayed in Fig. 6. Significant regressions were found within extensive cortical areas, including the subgenual area, left dorsolateral prefrontal cortex, bilateral cingulate gyrus, right precuneus, right superior parietal lobule and left occipital lobe. The ROI-to-voxel regressions between either the cingulum bundle or the uncinate fasciculus baseline white matter values and grey matter MRI percent change maps (Fig. 1VI) did not reveal any significant findings.

Baseline metabolic alterations in either the posterior cingulate cortex or the medial prefrontal ROI are not related to subsequent white matter rates of atrophy

None of the three ROI-to-voxel regressions between baseline posterior cingulate, anterior cingulate or subgenual area $^{18}$FDG-PET...
Figure 4  Relationship between baseline hippocampal atrophy and white matter--MRI percent change maps. Results of the ROI-to-voxel regression analysis between baseline hippocampal grey matter volume and white matter percent change maps (thresholded at $P < 0.005$ uncorrected with $k > 50 \text{mm}^3$). Left side of the brain is on the left.

Figure 5  Relationship between baseline cingulum bundle atrophy and $^{18}$FDG-PET percent change maps. Results of the ROI-to-voxel regression analysis between baseline cingulum bundle (highlighted in Fig. 4) white matter volume and $^{18}$FDG-PET percent change maps (thresholded at $P < 0.005$ uncorrected with $k > 50 \text{mm}^3$). Left side of the brain is on the left.

Figure 6  Relationship between baseline uncinate fasciculus atrophy and $^{18}$FDG-PET percent change maps. Results of the ROI-to-voxel regression analysis between baseline white matter volume in the uncinate fasciculus (highlighted in Fig. 4) and $^{18}$FDG-PET percent change maps (thresholded at $P < 0.005$ uncorrected with $k > 50 \text{mm}^3$). Left side of the brain is on the left.
values and white matter percent change maps (Fig. 1V) revealed any significant findings.

The same findings were obtained for all analyses when introducing age and status conversion (converters versus non-converters) as covariates (data not shown).

**Intermodality distant relationships show a specific direction from hippocampal atrophy to white matter alterations and from white matter alterations to grey matter hypometabolism**

The results of the comparison analyses between the partial correlation coefficients of the regressions of baseline to percent change, percent change to baseline, and baseline to baseline are reported in Table 2. They show that the partial correlations between baseline hippocampal atrophy and white matter percent change values in the cingulate and uncinate fasciculi were higher than the partial correlations between baseline white matter values and baseline or percent change hippocampal atrophy, and that the partial correlations between baseline white matter alterations and 18FDG-PET percent change values were higher than the corresponding partial correlations between baseline 18FDG-PET values and white matter baseline or percent change values. The statistical comparison among these partial correlation coefficients (Steiger, 1980) revealed that almost all of these differences were statistically significant (see Table 2 for details).

**The relationships between baseline white matter alterations and metabolic changes in the ROI are independent of local baseline grey matter atrophy**

The results of the ROI-based multiple regression analyses between 18FDG-PET percent change ROI values and the corresponding baseline white matter values and local baseline grey matter values are reported in Fig. 7. All multiple regression analyses were significant and revealed that only the baseline white matter alteration, but not the baseline local grey matter atrophy, provided a significant independent contribution to the model.

### Table 2 Test of the unidirectionality of intermodality distant relationships

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<th>Alteration B</th>
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<td></td>
<td></td>
<td>Baseline A–B percent change</td>
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<td>Hippocampus grey matter</td>
<td>Cingulum bundle white matter</td>
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<tr>
<td>Hippocampus grey matter</td>
<td>Uncinate fasciculus white matter</td>
<td>0.75 (7 × 10⁻⁵)</td>
</tr>
<tr>
<td>Cingulum bundle white matter</td>
<td>Posterior cingulate cortex ¹⁸FDG-PET</td>
<td>0.72 (2 × 10⁻⁵)</td>
</tr>
<tr>
<td>Cingulum bundle white matter</td>
<td>Anterior cingulate cortex ¹⁸FDG-PET</td>
<td>0.74 (1 × 10⁻⁵)</td>
</tr>
<tr>
<td>Uncinate fasciculus white matter</td>
<td>Subgenual area ¹⁸FDG-PET</td>
<td>0.65 (6 × 10⁻⁵)</td>
</tr>
</tbody>
</table>

Multiple regressions between baseline-to-percent change, percent change-to-baseline, and baseline-to-baseline values of the ROIs highlighted in the ROI-to-voxel analyses (with total intercranial volume as a covariate), and statistical comparisons among the partial correlation coefficients highlighted in the regressions. Asterisk indicates that the r value is significantly lower than the corresponding partial correlation coefficient for baseline A–B percent change (*P < 0.05; **P < 0.01).

### Discussion

This study investigated the sequential relationships between the main grey and white matter structural and metabolic alterations observed in amnestic MCI. Our findings indicate that, in amnestic MCI, hippocampal atrophy is related to subsequent disruption of both the uncinate fasciculus and the cingulum bundle. Fibre loss in these white matter tracts is in turn related to the metabolic decrease observed in the posterior cingulate and medial orbitofrontal cortices. This interpretation is based on three sets of results from independent successive analyses: (i) we found baseline hippocampal grey matter atrophy to be related to white matter changes over time and baseline white matter atrophy to metabolic changes over time, but neither baseline white matter nor baseline 18FDG-PET alterations were significantly related to subsequent grey or white matter changes; (ii) these specific relationships of baseline to percent change were significantly stronger than the corresponding correlations of percent change to baseline or baseline to baseline, suggesting a unidirectional sequential relationship; and (iii) the relationship between the baseline white matter alterations and the grey matter metabolism decrease over time remained statistically significant even after removing the effect of baseline local grey matter atrophy, indicating that disruption of white matter tracts provides independent contribution to progressive metabolic changes, over and above local grey matter atrophy.

A comprehensive overview of grey matter atrophy, hypometabolism and white matter alterations in amnestic MCI at baseline and their evolution over the 18-month follow-up period were presented. Apart from white matter atrophy, these findings have already been published and discussed (Chételat et al., 2003a, 2005; Fouquet et al., 2009). Regarding white matter atrophy, volume loss was found at baseline in patients with amnestic MCI compared with controls in the cingulum bundle (caudal part), fornix, perforant path and frontal and temporal white matter. These findings, obtained using voxel-based morphometry, are consistent with previous studies using diffusion tensor imaging that reported diffusivity increase or anisotropy decrease in the same set of white matter areas (Fellgiebel et al., 2004; Medina et al., 2006; Huang et al., 2007; Zhang et al., 2007; Ukmar et al., 2008; Chua et al., 2009; Kiuchi et al., 2009; Stricker et al., 2009; Wang et al., 2009), while atrophy of the perforant path has been previously reported...
in a voxel-based morphometry study on amnestic MCI (Stoub et al., 2006). The assessment of the progression of white matter atrophy over the 18-month follow-up period demonstrated a significant tissue loss within the rostral cingulum bundle, superior longitudinal fasciculus, superior fronto-occipital fasciculus, uncinate fasciculus, perforant path and corticospinal tract. This is the first longitudinal study describing white matter change over time throughout the whole brain either in amnestic MCI or in Alzheimer’s disease. Fractional anisotropy changes over a 3-month follow-up period have been previously assessed in amnestic MCI using diffusion tensor imaging in a selected set of ROI (including the fornix, cingulum bundle, corpus callosum splenium and cerebral peduncles), showing significant changes within the rostral part of the cingulum bundle only (Mielke et al., 2009). Our findings are consistent with this report, although we assessed the whole brain over a much longer follow-up period, and the involvement of additional tracts was revealed. Our findings of significant white matter loss in the superior longitudinal, superior fronto-occipital and uncinate fasciculi suggest the disruption of frontal connections with the parietal, occipital and temporal lobes, respectively. Moreover, the perforant path was also found to be progressively atrophied over the follow-up period.

**Figure 7** Relative contribution of distant baseline white matter atrophy and local baseline grey matter atrophy onto glucose hypometabolism evolution, using multiple regression analyses. (A) Multiple regression using posterior cingulate 18FDG-PET percent change values as a dependent variable and baseline measures of cingulum bundle white matter volume and posterior cingulate grey matter volume (and total intracranial volume) as predictive variables. The multiple regression is statistically significant ($R^2 = 0.61$, $P = 6.10^{-5}$), and only the cingulum bundle baseline white matter provides a significant independent contribution to the model ($P = 0.03$, partial $r = 0.55$; black slope), while baseline posterior cingulate atrophy shows no significant independent contribution ($P = 0.14$, partial $r = 0.40$; grey slope). (B) Multiple regression using anterior cingulate 18FDG-PET percent change values as a dependent variable and baseline measures of cingulum bundle white matter volume and anterior cingulate grey matter volume (and total intracranial volume) as predictive variables. The multiple regression is statistically significant ($R^2 = 0.68$, $P = 2 \times 10^{-5}$), and only the cingulum bundle baseline white matter provides a significant independent contribution to the model ($P = 2 \times 10^{-5}$, partial $r = 0.72$; black slope), while baseline anterior cingulate atrophy shows no significant independent contribution ($P = 0.45$, partial $r = 0.21$; grey slope). (C) Multiple regression with subgenual 18FDG-PET percent change values as a dependent variable and baseline measures of uncinate fasciculus white matter volume and subgenual grey matter volume (and total intracranial volume) as predictive variables. The multiple regression is statistically significant ($R^2 = 0.44$, $P = 0.049$), and only the cingulum bundle baseline white matter provides significant independent contribution to the model ($P = 8 \times 10^{-3}$, partial $r = 0.65$; black slope), while baseline anterior cingulate atrophy shows no significant independent contribution ($P = 0.87$, partial $r = -0.05$; grey slope).
suggestions that this early process still progresses while atrophy spreads to several neocortical areas.

In a previous cross-sectional study in mild Alzheimer’s disease, baseline measures of hippocampal grey matter atrophy were found to correlate to cingulum bundle atrophy, itself related to posterior cingulate glucose hypometabolism (Villain et al., 2008), consistent with the known strong anatomical connection via the cingulum bundle between the hippocampus and posterior cingulate cortex (Kobayashi and Amaral, 2003). In the present study, we also found significant relationships between these three alterations in an independent sample of patients with amnestic MCI (Fig. 8). Importantly, the present study shows that this relationship was not present at baseline but was found between baseline hippocampal grey matter atrophy and subsequent cingulum bundle atrophy, as well as between baseline cingulum bundle atrophy and subsequent posterior cingulate hypometabolism. These findings therefore support the hypothesis of a sequential relationship between these pathological events. Although other processes may also independently contribute to each of the events, our results strongly suggest that hippocampal atrophy induces a progressive breakdown of cingulum fibres, which itself progressively leads to posterior cingulate hypometabolism. This proposed sequence of events would be in agreement with animal lesion studies where experimentally induced neuronal death and inherent axonal Wallerian degeneration were shown to lead to long-term neuronal alterations in projection sites of damaged neurons, such as decreases in glucose consumption and in the expression of metabolic-relevant genes and alteration of synaptic plasticity (Meguro et al., 1999; Albasser et al., 2007; Machado et al., 2008; Poirier et al., 2008; Garden et al., 2009). The baseline-to-baseline relationships observed in the Alzheimer’s disease stage (Villain et al., 2008) but not in the present study may reflect the long-term consequence of this process.

Our findings also highlight a second pathway of related sequential events, starting from hippocampal atrophy and eventually leading to hypometabolism in two medial prefrontal regions, namely the anterior cingulate and subgenual areas (Fig. 8). In particular, specific relationships were found between hippocampal atrophy and progression of (i) uncinate fasciculus disruption, in turn predicting subgenual hypometabolism, and (ii) caudal cingulum bundle disruption, in turn related to anterior cingulate hypometabolism. The present findings are consistent with those obtained in our previous 18FDG-PET study (Fouquet et al., 2009), but they emphasize the temporality, thereby further suggesting a causality between these different events. Our interpretation is consistent with strong neuroanatomical evidence for a direct connection between medial temporal lobe structure and medio-orbital frontal cortex via the uncinate fasciculus (Barbas and Blatt, 1995; Carmichael and Price, 1995; Kondo et al., 2005; Zhong et al., 2006; Saleem et al., 2008). Taken together, our data also support the idea that the posterior cingulate cortex is an important crossroads on the indirect hippocampofrontal route passing through the cingulum bundle (Mufson and Pandya, 1984; Morris et al., 1999; Kobayashi and Amaral, 2003; Kobayashi and Amaral, 2007; Shibata and Naito, 2008).

White matter atrophy was found to influence remote metabolism over and above local grey matter structural alterations, which is consistent with our previous finding in Alzheimer’s disease (Chételat et al., 2009). Nonetheless, the multiple regression analyses showed that distant and local atrophy together accounted for only 45–70% of total metabolic change variance in these regions. Although the remaining unexplained 30–55% may be due to methodological limitations (see below), they might also reflect additional local mechanisms contributing to grey matter hypometabolism and white matter disruption, such as soluble and insoluble β-amyloid, inflammation, oxidative stress, amyloid angiopathy, myelin loss and astrocyte degradation or gliosis (Buckner et al., 2008; Chételat et al., 2008; Gouw et al., 2008; Mosconi et al., 2008; Bai et al., 2009a). It is possible that some of these alterations, such as myelin disruption, in turn induce remote grey matter loss through retrograde or Wallerian degeneration (Bai et al., 2009a; Bartzokis, 2009), although the present study does not support this hypothesis.

There are several limitations to the current study. Our sample of patients with amnestic MCI is relatively small, which may limit our statistical power. Therefore negative findings should be considered with caution. However, the sample was extremely homogeneous clinically. Moreover, all the findings that have been published from this cohort are fully consistent with the literature and/or consistently replicated with sometimes much larger samples (e.g. Risacher et al., 2009). Although limited in size, our sample therefore seems to well represent prodromal/early Alzheimer’s disease. Other aetiologies for amnestic MCI are possible (Petersen and Negash, 2008), but the amnestic MCI criteria used here, namely, single-domain amnestic MCI (Petersen and Negash, 2008), defines the most specific clinical entity for the early stage of Alzheimer’s disease (Mitchell and Shiri-Feshki, 2009). Still, the results depicted here are essentially a snapshot of the dynamics of structure and

**Figure 8** Overview of the results of the intermodality analyses highlighting the chronology of events in amnestic MCI. (1) Baseline hippocampal atrophy (red) is related to both cingulum bundle (blue) and uncinate fasciculus (yellow) white matter atrophy rates of change (orange arrows). (2) Baseline cingulum bundle white matter atrophy in turn relates to posterior cingulate (green) and anterior cingulate (pink) metabolic rates of change (dark red arrows), and baseline uncinate fasciculus white matter atrophy to subgenual (cyan) metabolic rate of change (dark red arrow).
metabolism over a short observation period and do not represent the whole variability spectrum or the entire course of Alzheimer’s disease. Note also that the use of voxel-based morphometry to assess white matter alteration could be disputed. Diffusion tensor imaging is probably more specific than voxel-based morphometry to axonal damage (Gouw et al., 2008) and allows better identification and isolation of white matter tracts. However, our findings are highly consistent with those obtained using diffusion tensor imaging (see above and Villain et al., 2008), and both methods are known to produce highly correlated assessment of white matter damage (Gouw et al., 2008). As white matter atrophy measured with voxel-based morphometry can result from white matter–CSF boundary changes rather than proper white matter alteration, we conducted supplementary white matter analyses where this boundary shift was controlled for by measuring ‘white matter density’ modifications. We were thus able to demonstrate that our findings were not a mere reflection of white matter–CSF boundary shift (Supplementary Figs 1 and 2). Note also that findings obtained using voxel-based morphometry within the deep grey matter could be biased due to segmentation issues in these regions (Ashburner and Friston, 2005), therefore we decided not to consider such findings in the present study. Also, one could argue that the sequential analysis approach used in this study is biased, as the ROI definition for data extraction between the imaging modalities is not symmetric and therefore not independent. There was no unique method allowing the individualization of all the ROIs of the study, therefore we used a sequential approach that could be applied to most ROIs. Note however that we also used alternative methods for ROI definitions and the findings were almost unchanged (Supplementary Tables 1 and 2). Besides, the estimation of longitudinal changes is based on measurements at two time points only, which is more sensitive to test-retest noise than multiple assessments and assumes a linear change between the two measures, which is not necessarily true (Jack et al., 2008; Schuff et al., 2009). Finally, even if the sequential relationships highlighted here provide strong arguments for causal links and diaschisis in Alzheimer’s disease, they do not provide direct evidence for these mechanisms, since other factors not assessed here such as amyloid deposition may mediate these relationships (Delatour et al., 2004; Chételat et al., 2010).

Conclusion

In summary, this study shows a temporal sequence of events supporting the causality between the different brain alterations observed in Alzheimer’s disease using in vivo neuroimaging. Hippocampal atrophy appears to cause progressive disruption in at least two brain pathways, namely the cingulum bundle, itself responsible for posterior and anterior cingulate hypometabolism, and the uncinate fasciculus leading to metabolism decrease in the subgenual cortex (Fig. 8). Our findings further reinforce the relevance of the disconnection process as a pathophysiological mechanism underlying brain alteration progression in the course of Alzheimer’s disease. As we are entering the era of large multicentre multi-modal imaging longitudinal studies, our approach can be used to integrate other modalities such as amyloid deposition into the above causal model in order to further our understanding of the pathological mechanisms underpinning the cascade of events eventually leading to Alzheimer’s disease-related dementia.

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Supplementary material

Supplementary material is available at Brain online.

References


Sequence of events in early Alzheimer’s disease

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