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Abstract

While the hippocampus is constantly reported as the site of earliest and highest structural alteration in Alzheimer's disease (AD), findings regarding the metabolic status of this region are rather heterogeneous. It has been proposed that only a time-consuming individual region-of-interest (ROI) approach would allow the detection of hypometabolism in this complex and small area. Our main goal with this study is to assess whether more automatic and clinically useful methods would be sensitive enough when considering other methodological confounds. From a single PET dataset collected in 28 patients with amnestic Mild Cognitive Impairment (aMCI) and 19 controls, we assessed the effects of partial volume effect (PVE) correction, scaling (using vermis or global means), and analysis method (individual ROI *versus* more automatic template-based ROI or voxel-based approaches) on hippocampal hypometabolism detection in aMCI. PVE correction and scaling both showed a significant effect on group comparison, while the analysis method (individual *versus* template-based ROI) surprisingly did not. Hippocampal metabolic decrease was significant in all vermis-scaled conditions, and more so after PVE correction. Our findings highlight the crucial relevance of using reference-region-based (instead of global) scaling, and the higher sensitivity of PVE-corrected PET measures, to detect hippocampal hypometabolism in aMCI. They also show that hippocampal metabolic decline can be detected using template-based ROI as well as voxel-based methods. These findings have clinical relevance, since they support the validity of more automatic and time-saving approaches to assess hippocampal metabolism changes in aMCI and early AD.

Introduction

Brain structural and functional alterations have been consistently demonstrated with MRI and FDG-PET in Alzheimer's disease (AD). Early changes have been observed at the stage of amnestic Mild Cognitive Impairment (aMCI), which represents the clinical group that best reflects the prodromal stage of AD (Petersen *et al.*, 2005). In contrast to the growing evidence of early and marked structural alterations of the hippocampal region in AD (Nestor *et al.,* 2004), findings regarding the metabolic status of this key area at the early stage of the disease have been somewhat divergent. Hippocampal hypometabolism has been reported in aMCI and mild AD in several PET studies using various methods (De Leon *et al.,* 1997; Ouchi *et al.,* 1998; De Santi *et al.,* 2001; Nestor *et al.*, 2003; Anchisi *et al.*, 2005; Mosconi *et al.,* 2005; 2006) but not in others using either similar or different methodological approaches (Minoshima *et al.,* 1994; Desgranges *et al.,* 1998; Ibanez *et al.,* 1998; Ishii *et al.,* 1998; De Leon *et al.,* 2001; Alexander *et al.,* 2002; Herholz *et al.,* 2002; Nestor *et al.,* 2003; Mosconi *et al.,* 2005; 2006; Kawachi *et al.,* 2006), even though the finding of significant correlation between relative hippocampal metabolism and severity of episodic memory impairment suggested sensitivity was not the issue. The question of hippocampal metabolic preservation or alteration in early AD is crucial to support clinical diagnosis, to better understand the pathological mechanisms underlying this neurodegenerative disease and to guide pharmacological research.

The hippocampus is a small and complex structure particularly sensitive to methodological issues, which could thus have confounded previous PET results. Firstly, partial volume effect (PVE) corresponds to a limitation inherent to the camera and results in the contamination of metabolic values by those of neighbouring voxels. This effect, which is exacerbated by atrophic process, could lead to hypometabolism overestimation. However, the effect of PVE correction on PET findings is not clear since one previous study reported

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significant hippocampal hypometabolism in aMCI only before, but not after, correcting for PVE (De Leon *et al.,* 2001), while another study found a significant decrease in both conditions (Mosconi et al., 2005).

Secondly, in order to reduce inter-subject variability in global brain PET measures (which is known to affect also healthy subjects), PET data are scaled using either the global mean value of the whole brain, or the mean value of a known preserved cerebral region in AD (such as the pons or the cerebellar vermis). While the latter adequately reflects inter-individual variability, the former also reflects the pathological effect, i.e. a global decrease due to regional decreases, and would therefore lead to an underestimation of regional changes (Buchert *et al.*, 2005). However, both a presence and a lack of hippocampal hypometabolism were found after reference-region-based scaling (Herholz *et al.,* 2002; Mosconi *et al.,* 2006).

Finally, previous PET studies either used a region of interest (ROI) approach, which consists in manually delimiting the outline of *a priori* determined cerebral regions, or a whole-brain voxel-based Statistical Parametric Mapping (SPM) method. Hippocampal hypometabolism has mostly been reported using the ROI approach but not with SPM. In addition, several studies using both methods on the same data reported a lower sensitivity of SPM compared to the ROI approach (De Leon *et al.,* 2001; Nestor et al., 2003; Mosconi *et al.,* 2005), which was thought to arise from the spatial resolution degradation required in the SPM procedure. However, the other above-mentioned methodological confounds may also be involved. Moreover, previous studies used the SPM approach without *a priori* hypothesis upon the hippocampus, so that statistical thresholds were much more stringent than those used in ROI studies due to correction for multiple comparisons. For both methods to be compared, a similar hypothesis-driven approach (and thus comparable thresholds) should be used. Since the ROI method is particularly time-consuming and observer-dependant, it seems of particular

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clinical relevance to assess whether a more automatic method could prove to be sensitive enough and just as accurate to detect hippocampal dysfunction even in patients with aMCI.

Our main goal with this study is thus to determine the least demanding methodological approach allowing the accurate measurement of hippocampal metabolic changes in aMCI, as a more conservative pre-AD situation, by systematically assessing the potential confounding effects of PVE correction, scaling procedure and method of analysis.

Materials and Methods

Participants

Subjects' characteristics are summarized in Table 1. A total of 47 subjects were studied, including 28 patients with aMCI and 19 healthy elderly. aMCI patients were prospectively recruited through a memory clinic, which they all attended for a complaint of memory impairment. Following medical, neurological, neuropsychological, and neuroradiological investigations, they were selected according to previously described stringent criteria for aMCI (Chételat *et al.,* 2005), i.e. isolated episodic memory deficits with preservation of other cognitive domains, daily-living activities and global cognitive capacities.

The 19 unmedicated healthy right-handed elderly had no cerebrovascular risk factors, mental disorder, substance abuse, history of head trauma, and standard MRI or biological abnormality. Two years later, none of the healthy controls had converted to either aMCI or dementia, while 11 aMCI patients had converted to AD during the follow-up period (from 18 to 36 months), 15 were still classified as aMCI, and 2 were unclassified because of incomplete follow-up clinical examination.

All subjects included in this study gave informed consent for the protocol, which was approved by the Regional Ethics Committee. Within an interval of two months at most from

inclusion for the controls and a few days for aMCI patients, MRI and PET examinations were performed.

Neuroimaging procedure

All subjects underwent a high-resolution T1-weighted volume MRI scan, which consisted of a set of 128 adjacent axial cuts parallel to the anterior-posterior commissure (AC-PC) line and with slice thickness 1.5 mm and pixel size 1x1 mm, using the SPGR (spin gradient recalled) gradient echo sequence (repetition time, TR=10.3 ms; echo time, TE=2.1 ms; field of view, FOV=24x18 cm; matrix=256x192). Standard correction for field inhomogeneities was applied.

PET data were collected as previously described (Chételat *et al.,* 2003), using the high-resolution PET device ECAT Exact HR+ with isotropic resolution of 4.6x4.2x4.2 mm (FOV=158 mm). In brief, [18]FDG uptake was measured in the resting condition, with eyes closed, in a quiet and dark environment. 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 (volume acquisition), using a voxel size of 2.2x2 .2x2.43 mm.

The overall experimental design is displayed in Figure 1. All PET data were either PVE-corrected or not, and scaled by either the vermis or the global mean. Hippocampal metabolic values were then extracted from these four PET data sets, using either an individual ROI (from hippocampal ROI drawn on individual MRI scans; see below), or a template-based ROI (from a single hippocampal ROI drawn on the mean MRI template of the whole sample) approach, or analyzed through the voxel-based SPM approach. Hippocampal anatomic boundaries were drawn blinded to diagnostic group using MRIcro (http://www.sph.sc.edu/comd/rorden/mricro.html), by the same observer (KM) according to

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previously published anatomical guidelines (Nestor *et al.,* 2003; Mosconi *et al.,* 2005) already used in our laboratory (Viard *et al.,* In Press). All tracings were checked by an independent skilled observer (GC), any changes being made by joint agreement.

Data handling and transformation steps are detailed in Figure 2. PET data were first coregistered onto their corresponding MRI data set using SPM2 and corrected for PVE using a modified Müller-Gartner voxel-based method fully implemented in the "PVE-lab" software (Quarantelli *et al.,* 2004). Next, both PVE-corrected and uncorrected coregistered PET datasets were spatially normalized for the template-based ROI and voxel-based SPM approaches.

The four resulting PET data sets (PVE-corrected or uncorrected, and spatially normalized or not) were then scaled, both by the vermis and by the global means of the corresponding condition. These means were automatically extracted from the coregistered and spatially normalized (PVE-corrected and uncorrected) PET scans, using the fMRI-ROI analysis toolbox of SPM and the Automated Anatomical Labeling (AAL) template (Tzourio-Mazoyer *et al.,* 2002) commonly used in our laboratory (see Figure 2). This procedure allows the automatic extraction of the mean PET value of 116 anatomically labelled brain ROIs. These 116 means were averaged to obtain the global mean, while the value of the 'vermis 1-2' ROI label was used as the vermis mean, which was the best metabolically preserved region in preliminary analysis comparing aMCI to controls (data not shown), consistent with the well-known metabolic preservation of this structure in AD (Desgranges *et al.,* 2002). Finally, i) for the SPM voxel-based and template-based ROI approaches, the four normalized PET datasets were smoothed (FWHM=14), masked (to exclude white-matter, CSF and non-brain voxels) and then either entered into the voxel-based SPM analysis, or used to extract hippocampal PET values from the template-based ROI; and ii) for the individual ROI approach,

hippocampal PET values were extracted from the PVE-corrected and uncorrected, and vermis or global-scaled, PET datasets.

<u>Statistical analyses:</u>

Firstly, to assess the effect of each methodological factor and how they interact with groups, i.e. in hippocampal hypometabolism detection, a general ANCOVA with three factors (PVE correction, scaling and ROI method) and two levels each (PVE-corrected versus not corrected; vermis versus global scaling; individual ROI versus template-based ROI) and two groups (aMCI and controls) was performed, introducing age as nuisance variable. Note that the same analysis was first performed adding the hippocampal laterality (right versus left) as another factor. However, since no significant effect of, or interaction with, this factor was observed, only the results of the ANCOVA on the (left plus right) averaged hippocampal mean will be shown. LSD tests, correcting for multiple comparisons, were done post-hoc on significant interactions. All the results were considered as significant when $p < 0.05$.

Secondly, aMCI and control hippocampal values were compared for each ROI condition separately, using eight independent ANCOVAs introducing age as a nuisance variable. These eight comparisons between groups were performed to assess, in each condition separately, whether hippocampal metabolism is significantly decreased in aMCI compared to controls and at which degree and level of significance, in order to compare the relative accuracy of each method. For a comparison with previous positive and negative reports, we used the $p < 0.05$ (uncorrected for multiple comparisons) statistical threshold.

Finally, four independent voxel-based SPM analyses comparing aMCI versus controls in each condition (PVE-corrected and uncorrected, vermis or global scaled PET data) were also performed, using the "Compare-populations: 1 scan/subject (Ancova)" routine of SPM 2 and introducing age as nuisance variable. We first adopted a Small Volume Correction (SVC)

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approach for hippocampal hypometabolism detection, as required for *a priori* hypothesis-driven analyses. This approach allows statistical inferences about the significance of regional effects in statistical parametric maps to be made when the approximate location of the effect is specified in advance (Friston, 1997), and the coordinates and level of significance of hippocampal peaks to be extracted. The hippocampal template-based ROI binary image was entered as *a priori,* and a p<0.05 statistical threshold (FWE corrected for multiple comparisons onto the hippocampal ROI) was used. For the sake of comparison with previous SPM studies, we also reported the results of the whole-brain SPM analysis, using a statistical threshold of p<0.05 (FWE–corrected for multiple comparisons in the whole brain).

Results

The general ANCOVA (Table 2 and Figure 3) showed, firstly, a significant main effect of Group only (aMCI<controls; Figure 3a). Secondly, both PVE and scaling factors interacted with Group. Post-hoc LSD tests revealed that PVE-corrected hippocampal metabolic values were higher than non-corrected values in controls (p=0.007) but not in aMCI (p=0.42; Figure 3b), and that global scaling led to significantly lower hippocampal values than vermis scaling in both controls ($p=10^{-12}$) and aMCI ($p=10^{-8}$), the difference being more marked for the former (Figure 3c). Finally, a significant interaction between the three factors was also demonstrated, indicating a differential effect of PVE correction in aMCI compared to controls in the vermis-scaled condition, but not in the global-scaled condition (Figure 3d), such that inter-group differences were significant only in the former.

The eight independent group comparisons revealed a significant hippocampal hypometabolism in aMCI compared to controls in the four vermis-scaled conditions (with and without PVE correction, using individual or template-based ROI approach; Table 3). No significant difference was found in any corresponding global-scaled condition.

Similarly, using the SVC approach, significant hippocampal hypometabolism was found in vermis-scaled (PVE corrected and uncorrected; Table 4), but not in global-scaled, conditions. Note that without *a priori* hypothesis regarding involvement of the hippocampus, i.e. in the whole-brain SPM analyses, we did not observe significant hippocampal hypometabolism in aMCI in any of the four conditions. Significant decreases only concerned the bilateral posterior cingulate, right precuneus, and bilateral caudate and thalamus (Figure 4). Lowering the threshold, there was no significant decrease in the global-scaled conditions even using a very liberal p(uncorrected)< 0.05 threshold. However, in the vermis-scaled conditions, significant hippocampal hypometabolism was detected from a p(FDR–corrected)< 0.05 threshold.

Discussion

First, this study provides insights into previous literature discrepancies regarding hippocampal hypometabolism in prodromal AD, by clearly describing the influence of the main methodological confounds and highlighting their respective relevance when assessing this issue. Our data show that both PVE correction and scaling method should be considered, as suggested by their significant interaction with the Group factor. In contrast, the analysis methods (i.e. individual versus template-based ROI) did not significantly influence the detection of hippocampal hypometabolism, which seems surprising (see Introduction). Second, our findings confirm the finding of early hippocampal metabolic reduction reported in several previous studies that used an individual ROI method, and they show for the first time that more automatic template-based ROI or *a priori* voxel-based approaches such as SVC are sensitive enough to detect this significant, albeit subtle, reduction.

The scaling method and PVE correction both influenced the assessment of relative hippocampal metabolism in aMCI as compared to controls, between-group differences being

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increased in the vermis-scaled and PVE-corrected conditions. The effect of scaling was largely expected since, as already highlighted in previous studies (see Introduction), global scaling induces an underestimation of regional supra-tentorial hypometabolism. Failure to detect significant hippocampal hypometabolism when using global scaling here is thus consistent with previous studies using the same scaling procedure in patients with aMCI or even with mild AD compared to controls (Minoshima *et al.,* 1994; Ibanez *et al.,* 1998; Ishii *et al.,* 1998; Herholz *et al.,* 2002; Chételat *et al.,* 2003; Kawachi *et al.,* 2006). Although already highlighted previously (Buchert *et al.,* 2005),,the crucial relevance of using reference-region-based scaling deserves further emphasis, especially as global scaling is still commonly used in voxel-based PET studies (and probably accounts for negative findings concerning hippocampal hypometabolism). This also likely applies to SPECT, where global normalization is endemic, although this issue would merit to be specifically addressed to ensure that the present findings also apply to SPECT.

PVE correction was also expected to increase metabolic mean values, compensating for the loss of GM activity (in the hippocampus) due to spill-out onto non-GM tissues (such as the surrounding CSF). Previous studies reported either similar (Ibanez *et al.,* 1998; De Leon *et al.,* 2001; Mosconi *et al.,* 2005) or greater (Meltzer *et al.,* 1996) increase of hippocampal values in aMCI relative to controls after PVE correction, while we found a smaller increase in aMCI (more so in the vermis-scaled condition, see Figure 3d). This apparent divergence likely reflects the fact that in the present study the vermis mean value used for scaling was extracted from PVE-corrected PET data, while previous studies used PVE-uncorrected PET values (Meltzer *et al.,* 1996; De Leon *et al.,* 2001). Thus, the vermis mean value slightly decreased following PVE correction (data not shown), more so in controls than in aMCI (-6% and -1.5%, respectively), resulting in the hippocampus/vermis ratio to

increase more in controls than aMCI after PVE correction, despite the fact that the non-scaled hippocampal mean increased in both groups (7% and 5%, respectively).

Overall, our findings therefore emphasize the need to use both PVE-corrected and vermis-scaled PET data to detect early hippocampal metabolic decrease in early AD. In contrast, we did not find the analysis method (individual versus template-based ROI approach) to significantly influence hippocampal metabolism measurement, and even automatic voxel-based approaches appeared to be sensitive to detect such a decrease based on *a priori* hypothesis. This was an unexpected finding since previous negative reports regarding hippocampal hypometabolism in early AD were mainly assigned to the use of insufficient anatomical precision when assessing this small and complex structure. More specifically, the negative reports using whole-brain SPM approach (Desgranges *et al.,* 1998; Alexander *et al.,* 2002; Herholz *et al.,* 2002; Chételat *et al.,* 2003; Mosconi *et al.,* 2005) contrasting with studies using the individual ROI approach (De Leon *et al.,* 1997; Ouchi *et al.,* 1998; De Santi *et al.,* 2001; Nestor *et al.,* 2003), were assumed to arise from the spatial resolution degradation induced by normalization and smoothing steps required in the SPM procedure (De Leon *et al.,* 2001; Chételat *et al.,* 2003; Nestor *et al.,* 2003; Mosconi *et al.,* 2005). However, *a priori* hypotheses, and thus more liberal statistical thresholds, were used with the ROI approach but not with SPM (see Introduction). Consistent with previous findings, we did not find significant hippocampal hypometabolism with SPM when assessing the whole brain without *a priori* hypothesis and using a very stringent threshold. However, when extracting SPM hippocampal mean values based on *a priori* hypothesis (and corresponding more liberal cut-off) or when using SVC based on the same *a priori* hypotheses, significant hypometabolism emerged. Our results therefore suggest that previous negative findings using SPM were likely due to the use of too stringent thresholds but not to methodological limitations as previously assumed. This finding is potentially relevant for future investigations

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and clinical applications considering clear advantages of fully-automatic voxel-based methods over laborious individual ROI approach. Over and above time-saving and objectivity, which are of particular clinical relevance, only SPM allows to obtain information upon the specificity of the functional deficit, i.e. the degree of hippocampal hypometabolism relative to other brain structures, and about the localization of this effect within the hippocampus.

However, our findings were obtained in patients with MCI and thus with minimal atrophy compared to more severely affected cohorts, and the reliability of voxel-based approach in this study might have been due, at least in part, to the population of patients studied. Mosconi *et al.* (2005) have also developed a hippocampal mask (HipMask) that proved to be sensitive when assessing hippocampal hypometabolism in both MCI and AD, and suggested that the precision of their method may be inversely related to the severity of hippocampal atrophy. Indeed, the HipMask obtained from one patient cohort is then applied to other, independent, cohorts. While only HipMask has thus far been statistically tested for anatomical accuracy and overlap, the present template-based ROI approach is expected to be less dependent on population differences since the ROI is outlined on the customized template, itself specific to the studied population. Consequently, the ROI is specific to the particular population, and thus would take into account severe atrophy if it was present. However, both automated methods depend on the quality of spatial normalization which itself may decrease as atrophy increases. The validity of our method to population with severe hippocampal atrophy would therefore require specific validation.

Overall, this study provides further support to the presence of hippocampal hypometabolism in prodromal AD. This finding is consistent with several previous studies in aMCI and AD that used the rigorous individual ROI approach on regionally-scaled PVE-corrected PET data (De Santi *et al.,* 2001; Nestor *et al.,* 2003; Mosconi *et al.,* 2005).

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Nevertheless, results from the whole-brain SPM analysis emphasize that, although definitely present and significant, hippocampal hypometabolism was less marked than that found in other regions such as the posterior cingulate (see Figure 4). Since we did not use exactly the same method to assess hippocampal and posterior cingulate metabolism (i.e. a ROI drawn on the customized template and a ROI automatically generated from the AAL template respectively), our results should be viewed with some caution. However, Nestor *et al.* (2003) reported the same finding of a predominant hypometabolism in the posterior cingulate as compared to the hippocampus, using strictly the same MRI-based ROI method for both regions. The hippocampus being the site of highest and earliest structural alteration, the discrepancy between the degree of atrophy and hypometabolism in this structure will require further investigation.

It should be noted that our aMCI sample was heterogeneous in that it included both patients that will rapidly convert to AD and patients that will not, or may do so but later. We did however use stringent criteria to select a population as homogeneous as possible, so that it best represents the prodromal stage of AD. The high conversion rate (39%) of our aMCI sample supports this view. Moreover, to specifically address the issue of early AD, we repeated the analysis on the population of aMCI patients who subsequently converted (data not shown). The results were in line with those from the whole aMCI sample regarding the effects of the methodological factors, the presence of hippocampal hypometabolism in early AD and the accuracy of automatic voxel-based approaches to detect this hippocampal effect, suggesting that our findings were not confounded by sample heterogeneity and apply to early AD at large.

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In conclusion, our findings indicate that PET data should be PVE-corrected and scaled using a preserved reference region to accurately assess hippocampal metabolic change in early AD. More importantly, we showed that hippocampal hypometabolism can be detected not only through the time-consuming individual ROI method, but also using more automatic methods such as template-based ROI or SPM voxel-based approaches. These findings are potentially of high clinical relevance, since they provide evidence towards the validity of these more time-saving and accessible methods in assessing hippocampal hypometabolism in early AD.

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Table 1: Characteristics of the control and aMCI samples

	Controls	*aMCI*
n	19	28
Age: mean (SD)	63.5 (8.6) years *	73 (8) years
Range	(51-84)	(55-87)
% Female	53	57
MMSE : mean (SD)	-	27.2 (1.3)
Range	-	(24-29)

* Significantly different from aMCI (p = 0.0003)

Table2: Results of the general ANCOVA with three factors (PVE correction, scaling and ROI method), two groups (aMCI and controls), and age as nuisance variable, on the averaged (left plus right) metabolic hippocampal values.

	F values (1,44)	p values
Principal effects		
Group	7.69	0.008 (a)
PVE	0.48	0.49
Scaling	0.33	0.57
ROI method	0.68	0.41
Significant Interactions with Group		
Group x PVE	7.75	0.008 (b)
Group x Scaling	9.60	0.003 (c)
PVE x Scaling x Group	7.81	0.008 (d)

a, b, c, d: significant interactions are respectively illustrated in Figure 3a, b, c, and d.

Table 3: Averaged (left plus right) metabolic hippocampal means (standard deviation) by group and by condition, p and F values of the between-group comparisons and corresponding percent changes.

	Template-based ROI		Individual ROI	
	PVEc	**no PVEc**	**PVEc**	**no PVEc**
Vermis Scaling				
Controls	1.10 (0.24)	1.00 (0.14)	1.29 (0.29)	1.08 (0.15)
aMCI	0.91 (0.13)	0.91 (0.08)	1.12 (0.14)	0.99 (0.10)
F value	6.33	4.26	4.06	3.21
P value	0.004*	0.02*	0.02*	0.05*
Percent change	*- 17 %*	*- 9 %*	*-13 %*	*-8 %*
Global scaling				
Controls	0.84 (0.03)	0.82 (0.03)	0.61 (0.03)	0.79 (0.03)
aMCI	0.84 (0.05)	0.82 (0.04)	0.63 (0.04)	0.81 (0.06)
F value	0.08	0.08	0.97	0.70
P value	0.92	0.92	0.39	0.5
Percent change	*0 %*	*0 %*	*+3 %*	*+ 2%*

*PVEc= PVE correction, *= significant between-group difference (p< 0.05).*

Table 4: Results of the SVC approach in each vermis-scaled condition: Talairach coordinates (cluster size in number of voxels), and T and p values of hippocampal peaks of significant hypometabolism in aMCI compared to controls.

	Peaks: x y z	*k*	*T (Z)*	*Voxel-level*		
				p$_{FWE}$	p$_{FDR}$	p$_{uncorrected}$
PVE correction						
Left hippocampus	*-28 -37 0*	*1124*	3.90 (3.59)	0.003	0.002	<0.0001
Right hippocampus	*14 -9 -15*	*1075*	3.61 (3.36)	0.006	0.002	<0.0001
No PVE correction						
Left hippocampus	*-26 -37 -5*	*1124*	3.72 (3.45)	0.006	0.007	<0.0001
Right hippocampus	*32 -37 -5*	*1045*	2.96 (2.81)	0.040	0.008	0.002

Figure 1*:* Schematic representation of the experimental design with 12 conditions corresponding to three methodological factors (PVE correction: yes/no, scaling: global/vermis, and analysis method: individual ROI/template-based ROI/voxel-based SPM).

Figure 2: Schematic representation of data handling and transformation steps in the different experimental conditions. (**a) PVE correction:** PET data were corrected for PVE due to both CSF and WM using the voxel-based method initially proposed by Müller-Gartner *et al.*(1992) and slightly modified by Rousset *et al.* (1998). This so-called 'modified Müller-Gartner' method is described in detail elsewhere and fully implemented in the "PVE-lab" software (Quarantelli *et al.,* 2004)**, (b) Spatial normalization:** coregistered PET data were normalized using the parameters of their corresponding MRI obtained from the optimal VBM procedure (Good *et al.,* 2001) already used in our laboratory (Chételat *et al.*, 2003). In brief, whole brain, GM, WM and CSF customized priors were created from the MRI data of all subjects. Then, optimal spatial normalization parameters were calculated from the normalization of the GM datasets onto the customized GM template, and applied to the coregistered (PVE-corrected and uncorrected) PET data sets, **(c) Scaling:** The resulting PET data sets were scaled by the global and by the vermis means extracted from the normalized PET of the corresponding condition using the fMRI-ROI analysis toolbox of SPM and the AAL template. (**d) Smoothing and masking procedures**: the resultant PET data sets were smoothed with a 14 mm isotropic Gaussian filter to blur individual variations in gyral anatomy and to increase the signal-to-noise ratio. Then, as classically performed in SPM analyses of PET data to restrict statistical analyses to GM voxels, the resultant PET images were masked. The mask was obtained by thresholding the GM customized template above a 0.25 value corresponding to a higher than 25 percent chance for the voxel to belong to GM. This value has been

selected a posteriori as the best compromise to avoid inclusion of non-GM voxels without clipping the GM fraction, **(e) ROI:** Hippocampal anatomic boundaries were traced both on each individual MRI scans and on the whole-brain customized template on coronal sections, from anterior to posterior, with simultaneous sagittal monitoring (Viard *et al.,* In Press).

Figure 3: Hippocampal means of aMCI (green) and controls (red) illustrating the principal effect of the group factor (a), and the significant group x PVE (b), group x scaling (c), and group x PVE x scaling (d) interactions. PVEc= PVE correction, No PVEc= No PVE correction

Figure 4: (a) Results of the SPM analysis on the vermis-scaled PVE-corrected PET data, using a p(FWE-corrected)< 0.05, k> 500 threshold. Significant hypometabolism was found in aMCI compared to controls in the bilateral posterior cingulate, right precuneus, bilateral caudate and thalamic nuclei. The other conditions led to similar results, with only slight differences in cluster size and level of significance, vermis scaling leading to more significant and more extended clusters than proportional scaling. **(b)** Mean (standard deviation) of bilateral metabolism in the posterior cingulate and hippocampus in controls and aMCI. These data are extracted from the PVE-corrected, vermis-scaled, SPM pre-processed PET data using the posterior cingulate ROI of the AAL labelled template (Tzourio-Mazoyer *et al.,* 2002) **(c)** and the hippocampal template-based ROI **(d)**.