Cortical amyloid accumulation is associated with alterations of structural integrity in older people with subjective memory complaints

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Abstract
We determined the effect of cortical amyloid load using 18F-florbetapir PET on cognitive performance and gray matter structural integrity derived from MRI in 318 cognitively normally performing older people with subjective memory impairment from the INSIGHT-preAD cohort using multivariate partial least squares regression. Amyloid uptake was associated with reduced gray matter structural integrity in hippocampus, entorhinal and cingulate cortex, middle temporal gyrus, prefrontal cortex, and lentiform nucleus (p < 0.01, permutation test). Higher amyloid load was associated with poorer global cognitive performance, delayed recall and attention (p < 0.05), independently of its effects on gray matter connectivity. These findings agree with the assumption of a two-stage effect of amyloid on cognition, (1) an early direct effect in the preclinical stages of Alzheimer’s disease and (2) a delayed effect mediated by downstream effects of amyloid accumulation, such as gray matter connectivity decline.

1. Introduction
Several studies have investigated the association between brain global amyloid load, as determined using amyloid PET or cerebrospinal fluid (CSF) Aβ42, and cortical gray matter volume changes, measured by MRI as a marker for neurodegeneration, in prodromal and clinical stages of Alzheimer’s disease (AD) as well as in cognitively intact individuals (Dore et al., 2013; Oh et al., 2011). Previous studies found associations of global amyloid load with basal forebrain volume in asymptomatic individuals at risk for AD, individuals with mild cognitive impairment (MCI) and in people with AD dementia (Grothe et al., 2014; Kerbler et al., 2015). These results are consistent with the early involvement of basal forebrain in amyloid pathology (Beach, 2008; Boncristiano et al., 2002). Furthermore, hippocampus volume was inversely correlated with global amyloid load in cognitively healthy subjects in some (Apostolova et al., 2010; Becker et al., 2011; Bourgeat et al., 2010), but not all studies (Chetelat et al., 2010a,b). During prodromal...
stages of AD with MCI, hippocampal atrophy was not associated with global or regional amyloid accumulation in most studies (Apostolova et al., 2010; Chetelat et al., 2010b). Thus, associations between cortical amyloid deposition and hippocampus atrophy were relatively weak in healthy controls and subjects with MCI (Teipel et al., 2014), consistent with postmortem evidence reporting that amyloid pathology occurs in the hippocampus only in the later stages of the disease (Royall et al., 2012).

The majority of previous studies have used univariate voxel— or region-of-interest—based analysis to assess the interaction between amyloid load and gray matter volume. One study in 52 healthy older individuals used multivariate analysis to identify the patterns of gray matter decreases and increases with global amyloid load (Oh et al., 2014).

Subjective memory complaints are considered an at risk factor for AD (Mitchell et al., 2014). So far, the association between higher amyloid load and gray matter volume was only studied in a sample of 49 people with subjective memory complaints (Chetelat et al., 2010a,b).

In this current study, we tested the association between amyloid load and gray matter atrophy in a large sample of 318 cognitively intact individuals with subjective memory complaints from the “INVeStIGation of AlzHeimer’s PredicTors in Subjective Memory Complainers” (INSIGHT-preAD) study. We used a multivariate assessment of structural integrity of cerebral gray matter volumes as a proxy of structural connectivity (Alexander-Bloch et al., 2013) based on multivariate partial least squares (PLS) regression (McIntosh and Lobaugh, 2004) to determine the association of global amyloid load with cerebral gray matter structure. Structural integrity as determined using PLS regression represents a spatially distributed covarying pattern of increase or decrease of gray matter volume with some external factor, here global amyloid load, across individuals. We expected a covarying network of reduced gray matter volume in limbic areas associated with larger brain amyloid load. We assessed whether the presence of an ApoE ε4 allele, as a genetic risk factor for sporadic AD (Zhong and Weisgraber, 2009), and education, as a proxy for cognitive reserve (Meng and D’Arcy, 2012), moderated the association between global amyloid load and gray matter integrity. Based on previous evidence in individuals with MCI (Chetelat et al., 2011; Mattsson et al., 2015), we expected that a network of gray matter decrease would mediate the effect of brain amyloid load on episodic memory and executive function.

2. Material and methods

2.1. Participants

Participants were recruited in the INSIGHT-preAD study, a monocentric academic university based cohort derived from the Institute for Memory and Alzheimer’s Disease (IMZA) at the Pitié-Salpêtrière University Hospital in Paris, France, with the objective to investigate the earliest preclinical stages of AD and its development including influencing factors and markers of progression (Dubois et al., in preparation).

The INSIGHT-preAD study includes 318 cognitively normal Caucasian individuals from in and around Paris, between 70 and 85 year old, with subjective memory complaints and with defined brain amyloid status. The study aims at 7-years of follow-up, and in February 2017, the first wave of follow-up after 1 year was still ongoing. Demographic, cognitive, functional, nutritional, biological, genetic, genomic, imaging, electrophysiological, and other assessments were performed at baseline. Subjective memory complaints were confirmed by an affirmative answer to both of the following questions: (1) “Are you complaining about your memory?” and (2) “Is it a regular complaint which lasts more than 6 months?”

Detailed inclusion and exclusion criteria can be found in the Supplementary Panel 1.

Demographic characteristics, including cognitive performance and ApoE genotype, are shown in Table 1. Each participant had a total recall at the Free and Cued Selective Reminding Test (FCSRT) in the normal range (mean 46.1 ± 2.0).

Written informed consent was provided by all participants. The study was approved by the local Institutional Review Board, and has been conducted in accordance with the Helsinki Declaration of 1975.

2.2. Cognitive tests

A comprehensive neuropsychological battery was administered to all participants of the INSIGHT-preAD cohort including the Mini-Mental State Examination (MMSE; Folstein et al., 1975) to assess global cognition, the Digit span (forward and backward; Wechsler, 1997), and the FCSRT (Arniva et al., 2007; Buschke, 1984) for short- and long-term memory; Letter and Category Verbal Fluency test (Benton, 1968; Cardebat et al., 1990; Shao et al., 2014) for verbal fluency, the Rey-Osterrieth Complex Figure Copy (Fastenau et al., 1999) for visuospatial abilities; the Trail Making Test (TMT; Tombaugh, 2004) and the Frontal Assessment Battery (Dubois et al., 2000) for attention and executive function assessment. To reduce the number of comparisons, we a priori decided to explore the associations of amyloid load and structural integrity only with the measures of global cognition, episodic memory, and executive function, respectively. Therefore, we selected performance in the MMSE, the delayed recall of the FCSRT, and the difference between TMT-B and TMT-A performance (time TMT-B minus time TMT-A; Drane et al., 2002) as measures of executive function (Arbuthnott and Frank, 2000).

2.3. ApoE genotype

DNA was prepared from frozen blood samples using the 5 Prime ArchivePure DNA purification system according to the manufacturer’s instructions. ApoE genotypes were determined for each individual using PCR-based Sanger sequencing. Exon 4 from ApoE gene containing the SNP corresponding to the ε3/ε4 alleles was amplified using PCR with the following primers: APOE sense, 5′-TAAGCTTGGACCGCGTCTCCAGGA-3′; APOE antisense, 5′-ACAGAATTGCCGCCGCTGTACAC-3′. For each sample, the reaction mixture (50 µL) contained 200 ng of genomic DNA, 10-µL PCR Flexi buffer (5X), 3-µL MgCl2 (25 mM), 1-µL dNTPs (10 mM), 1-µL of each forward and reverse primers (10 µM), and 0.25-µL GO Taq DNA polymerase (Promega). The cycling program was carried out after a preheating step at 95°C for 2 minutes and 35 cycles of denaturation.
at 95 °C for 1 minute, annealing at 68 °C for 1 minute, and extension at 72 °C for 1 minute. The amplified fragments were then purified and sequenced using the same primers.

2.4. MRI acquisition

MRI acquisitions of the brain were conducted using a 3-Tesla scanner with parallel imaging capabilities (Siemens MAGNETOM Verio, Siemens Medical Solutions, Erlangen, Germany). The scanner used a quadrature detection head coil with 12 channels (transmit-receive circularly polarized CP-head coil).

For the anatomic study, 3D TurboFLASH sequences were performed (orientation sagittal; repetition time 2300 ms; echo time 2.98 ms; inversion time 900 ms; flip angle 9°; 176 slices; slice thickness 1 mm; field of view 256 × 256 mm; matrix 256 × 256; bandwidth 240 Hz/Px).

2.5. PET acquisition

All flurbetapir-PET scans were acquired in a single session on a Philips Gemini GXL CT-PET scanner 50 (+5) minutes after injection of approximately 370 MBq (333–407 MBq) of flurbetapir. PET acquisition consisted of 3 × 5 minutes frames, a 128 × 128 acquisition matrix and a voxel size of 2 × 2 × 2 mm³. Images were then reconstructed using iterative LOR-RLMA algorithm (10 iterations), with a smooth post-reconstruction filter. All corrections (attenuation, scatter, and random coincidence) were integrated in the reconstruction. Lastly, frames were realigned, averaged, and quality-checked by the CATI team (http://cati-neuroimaging.com).

2.6. MRI data processing

The processing of structural MRI scans was implemented through statistical parametric mapping, SPM8 (Wellcome Dept. of Imaging Neuroscience, London) and the VBM8-toolbox (http://dbm.neuro.uni-jena.de/vbm/) implemented in MATLAB, Version 7.1 (Mathworks, Natick, MA, USA). First, images were segmented into gray matter (GM), white matter, and CSF partitions using the tissue before the free segmentation routine of the VBM8-toolbox. The GM and white matter partitions of each subject were then high-dimensionally registered to a crisp template of average anatomy in Montreal Neurological Institute (MNI) space (IXI-template) using the Diffeomorphic Anatomic Registration using Exponentiated Lie algebra (DARTEL) algorithm (Ashburner, 2007). The IXI-template is part of the VBM8-toolbox and was derived by DARTEL intersubject alignment of 550 healthy control subjects of the publicly available IXI-Database (http://www.brain-development.org).

Flow-fields resulting from the DARTEL registration to the IXI-template were used to warp the GM segments and voxel-values were modulated for the affine and the nonlinear effects of the high-dimensional normalization. This preserves the total amount of GM volume. Finally, modulated warped GM segments were resliced in a high-dimensional normalization. This preserves the total amount of template were used to warp the GM segments and voxel-values in the IXI-Database (http://www.brain-development.org).

2.7. PET data processing

Reconstructed PET images were analyzed with a pipeline developed by the CATI, a neuroimaging platform funded by the French Plan Alzheimer (http://cati-neuroimaging.com). Structural MRI images were coregistered to flurbetapir-PET images using SPM8 with visual inspection to detect any coregistration errors. Using inverse deformation fields and matrix transformation from MRI data processing, composite cortical ROIs (left and right precuneus, posterior and anterior cingulate, parietal, temporal, and orbitofrontal cortex) derived from (Clark et al., 2012) and a reference region (in pons and whole cerebellum) were placed in the individual native PET space. After correcting for partial volume effect with the RBV-sGTM method (Thomas et al., 2011), parametric PET images were created for each individual, by dividing each voxel with the mean activity extracted from the reference region. Finally, standard uptake value ratios (SUVR) were calculated by averaging the mean activity of all cortical ROIs in the individual PET native space. The processing steps are described in Supplementary Fig. 1.

The SUVR threshold to determine amyloid positivity was extracted performing a linear correlation between the above method and the one used by Joshi et al. (2015), using PET images of normal controls, MCI and AD patients from the IMAP cohort (Besson et al., 2015; Supplementary Fig. 2). Indeed, several previous studies have shown that positivity cutoffs could be reliably converted between tracers and processing methods, using the linear association across subjects (Landau et al., 2013; Villeimagne et al., 2012). The positivity threshold of 1.10 associated with Joshi’s method (Joshi et al., 2015) was defined as the confidence limit for the upper 5% of the SUVR distribution based on 2 groups of respectively, 10 and 11 healthy young controls (age interval 38–52 years) and corresponded to a value of 0.88 with our method. Thus, all INSIGHT subjects with a SUVR above 0.88 were considered as amyloid positive.

2.8. Statistical analysis

To determine the covariance of gray matter integrity with the global amyloid load, we used PLS regression (McIntosh and Lobaugh, 2004) using the PLS software (Rotman Research Institute, https://www.rotman-baycrest.on.ca/index.php?section=84) implemented in MATLAB R2013a. This approach operates on the covariance between brain voxels and allows assessment of an integrated network of brain regions that together covaries with some external measure (Krishnan et al., 2011), here the global amyloid load. PLS yields a new set of variables, the so-called latent variables (LVs), where each LV identifies a pattern of brain regions that conjointly covaries with global amyloid load. Each voxel of the brain has a weight on each LV, called the salience of this voxel on the LV. The significance of each LV has been assessed using permutation tests with 100 permutations at a p < 0.05 threshold. In addition, we used bootstrap estimation (with 100 bootstrap iterations) to determine the reliability of the saliences for the brain voxels determining each LV. The bootstrap ratios of salience follow a standard z-score distribution, where a ratio of >1.96 corresponds to a p value of <0.05 and a ratio of >2.58 to a p value of <0.01. In contrast to univariate analysis, the permutation tests and the saliences were determined in a single analytical step so that there is no need for multiple comparison correction.

We determined a model for the main effect of global flurbetapir uptake on gray matter integrity. To control for age, sex, MMSE score, and total intracranial volume (TIV), these variables were regressed out of both the flurbetapir SUVR values (except TIV) and the gray matter segments using multiple regression and keeping the respective residuals that then were entered into the PLS regression, as previously described (Ziegler et al., 2013).

In a second model, we assessed the association of age with gray matter integrity, controlling for sex, TIV, and amyloid load.

In a third model, we determined the main effect of ApoE4 genotype (binarized into no ApoE4 allele vs. at least one ApoE4 allele) and the interaction effect of ApoE4 genotype by global amyloid load on gray matter integrity, controlling for age, sex, MMSE score, and TIV.

In a fourth model, we determined the effect of education on gray matter integrity, again after partialling out the effects of age, sex, MMSE score, and TIV. Due to the highly variable frequency...
distribution of educational achievements, we decided to binarize education into people with primary education or lower (less than 11 years of education) and secondary education or higher (at least 11 years of education).

A moderating effect of binarized education on the association between amyloid and gray matter integrity was tested using an ANCOVA model, with the main effects of education, SUVR, and the interaction of education by SUVR on the brain scores of the LV that had been found associated with global florbetapir SUVR.

Finally, we performed mediation analysis to determine the direct and indirect effects of florbetapir SUVR on cognitive performance, mediated by brain structural integrity scores (Fig. 1), following Baron's and Kenny's (Baron and Kenny, 1986) steps that assess the presence of:

1. A direct effect: regression of cognitive score (Y) on amyloid (X)
2. An effect a: regression of volume (M) on amyloid (X)
3. An effect b: multiple regression of amyloid (X) and gray matter covariance (M) on cognitive scores (Y)

When these 3 steps were fulfilled, we determined the mediation analysis with Sobel's test (Sobel, 1986) using the macro PROCESS for SPSS (available at: http://processmacro.org/index.html) with a bias-corrected bootstrapped 95% confidence interval for assessing the indirect effect with 5000 bootstrap samples. For mediation analysis, we used unstandardized residuals of predictors and outcomes, controlling for age, sex, and education.

3. Results

Of the 318 participants, 63 had a global amyloid load above the positivity threshold. Amyloid load was significantly associated with age ($r = 0.15, p < 0.006$) indicating higher amyloid load with higher age, but not with sex ($r = 0.025, p = 0.65$). In structural PLS regression, controlling for age, sex, TIV, and MMSE score, global florbetapir SUVR values were associated with gray matter volume reductions in a network encompassing bilateral hippocampus, right predominant entorhinal cortex, anterior cingulate and cingulate body, medial temporal gyrus, bilateral prefrontal cortex, and bilateral lentiform nucleus ($p < 0.01$, permutation test). In addition, higher amyloid load was associated with the expansion of ventricular spaces, but also with increased gray matter volume in superior prefrontal and occipital cortical areas ($p < 0.01$, permutation test) (Figs. 2 and 3).

To assess the specificity of the amyloid effect, we determined the association of age with gray matter structural integrity, controlling for age, sex, TIV, and amyloid load. As shown in Fig. 4, concordant effects with lower gray matter volume associated with higher amyloid load and higher age were predominantly located in bilateral putamen and in parts of bilateral hippocampus.

Presence of at least one ApoE ε4 allele was associated with higher florbetapir SUVR ($r = 0.26, p < 0.001$), controlling for age, sex, and MMSE score, but not with gray matter integrity ($r = 0.84$, permutation test), and there was no interaction effect of amyloid load by ApoE on gray matter structural integrity ($p = 0.76$, permutation test).

When assessing the association of structural integrity with education, we found no significant effect of education on gray matter integrity ($p = 0.76$, permutation test). In addition, as shown in Fig. 5, there was no significant difference in florbetapir SUVR values between the control groups ($t = 1.13, 316 df, p = 0.26$). Consequently, education had no moderating effect on the association between florbetapir SUVR values and gray matter structural integrity ($F[1,314] = 0.27, p = 0.60$). Likewise, education was not moderating the effect of amyloid on MMSE, FCSRT delayed recall, and TMT-B minus TMT-A, respectively ($p$ for interaction effect of education by amyloid $= 0.13$ for all comparisons).

Following Baron’s and Kenny’s (Baron and Kenny, 1986) steps of mediation analysis, the direct effects were significant for the association of amyloid load with MMSE score at trend level ($r = 0.30; 95\% \text{ CI } [0.14, 0.47]$), controlled for age, sex, and education, indicating smaller MMSE score with higher amyloid load. In addition, amyloid load was significantly associated with FCSRT delayed recall performance, controlled for age, sex, and education ($r = 0.122, 312 df, p = 0.031$), and there was a positive effect of amyloid load on TMT-B minus TMT-A performance, controlling for age, sex, and education ($r = 0.15, p = 0.007$), indicating that higher amyloid load associated with more time needed to perform the task. In addition, step 2, regression of gray matter integrity on amyloid load was significant in controlling for age, sex, and education ($r = -0.44, p < 0.001$).

Finally, however, for step 3, the effect of gray matter integrity in a multiple regression model together with amyloid load was not significant for any of the cognitive outcomes, that is, MMSE, FCSRT delayed recall, and TMT-B minus TMT-A ($p > 0.51$) for all comparisons, indicating that gray matter integrity is not functioning as a mediator for the effect of amyloid on the cognitive outcomes.

To account for rare instances where the absence of a direct effect (a or b) is not associated with the absence of an effect a-by-b, as pointed out by Andrew F. Hayes (Hayes, 2013), page 166 ff., we carried out additional mediation analyses using Sobel's test. Consistent with the negative outcome of Baron’s and Kenny's steps, the indirect effects of amyloid load on MMSE (path coefficient $= 0.14, p = 0.30$; 95% confidence interval $[0.10, 0.40]$), on FCSRT delayed recall (path coefficient $= 0.10, p = 0.75$; 95% CI $[0.48, 0.74]$), and on TMT-B minus TMT-A (path coefficient $= -0.46, p = 0.44$; 95% CI $[-17.1, 6.2]$) through GM integrity were not significant.

When we assessed Baron’s and Kenny’s steps (Baron and Kenny, 1986) for a subsample of 144 cases enriched for some degree of minimal cognitive impairment (MMSE 27 and 28), we only found a significant correlation between amyloid and TMT-B minus TMT-A, after controlling for age, sex, and education ($r = 0.20, p < 0.02$). However, the effect of gray matter integrity in a multiple regression model together with amyloid load was not significant for TMT-B minus TMT-A in this subgroup as well ($\beta = 0.10, p = 0.26$), consistent with the lack of an indirect effect in Sobel's test ($p = 0.14$).

4. Discussion

Higher amyloid load was associated with reduced structural integrity, indicating a consistent pattern of brain volume increase or decrease, in hippocampus, cingulate gyrus, and associated brain regions in 318 cognitively normally performing older individuals with subjective memory complaints. These effects are remarkable since already in the absence of manifest cognitive decline, amyloid
accumulation is associated with structural brain changes affecting areas that are typically involved in early AD. Education and ApoE ε4 genotype did not moderate the association between amyloid load and brain structural integrity. Contrary to our expectation, effects of amyloid load on episodic memory and executive function were independent from brain structural integrity.

Our findings of decreased structural integrity of hippocampus and related brain areas in cognitively normally performing individuals with higher amyloid load agree with a previous study using multivariate analysis in 52 cognitively healthy individuals. This study showed reduced gray matter volume with higher amyloid load in several brain areas that overlapped with our current findings, including the hippocampus, lateral and basal temporal cortex, and bilateral prefrontal cortex (Oh et al., 2014). Other studies have determined structural integrity and covarying morphometric changes in AD and aging using seed-based analyses (Montembeault et al., 2016), graph analysis (Carmeli et al., 2014), or multivariate methods such as joint independent component analysis (Quyang et al., 2015; Willette et al., 2014) or scaled sub-profile modeling (Guo et al., 2014), but these studies have not considered the association of such changes with amyloid load. Our findings also agree with several univariate studies assessing associations between amyloid load and gray matter volume (Apostolova et al., 2010; Becker et al., 2011; Bourgeat et al., 2010). Pointing in a similar direction, in a study on 72 cognitively intact individuals, subjects without increased amyloid load were numerically less likely to present with reduced cortical thickness or hippocampus volume, albeit this effect was not statistically significant (Wirth et al., 2013). In a previous voxel-based analysis, 44 healthy controls exhibited higher hippocampus volume with higher temporal lobe amyloid load (Chetelat et al., 2010a); global amyloid load, however, was not associated with gray matter volume (Chetelat et al., 2010b). In contrast, in the same sample 49 subjective memory complainers exhibited reductions of gray matter volume in hippocampus, anterior and posterior cingulate cortex, and temporoparietal regions with higher global or temporal lobe amyloid load (Chetelat et al., 2010a,b). Our findings agree with this previous report.

In addition, it is interesting to compare our findings with the typical pattern of cortical atrophy found in cases with prodromal AD and AD dementia relative to controls. Similar to our findings, previous studies reported atrophy in prodromal AD and AD dementia cases in lateral and medial temporal, and prefrontal cortex (Bakour et al., 2009; Balthazar et al., 2009; Teipel et al., 2012);
however, extending beyond the effects of amyloid on cortical GM integrity in our sample, precuneus and posterior cingulate gyrus (Baron et al., 2001; Teipel et al., 2016) as well as lateral inferior and superior parietal cortices (Harper et al., 2017; Su et al., 2016) were involved in the atrophic pattern of prodromal and manifest AD. The more restricted effects in our sample seem not too surprising since our cohort includes cases without evidence of AD pathology (with subthreshold amyloid load) as well as preclinical AD cases (with suprathreshold amyloid load) so that amyloid effects are expected to be less pronounced than in manifest stages of AD. In addition, the effects of atrophy in AD likely result not only from amyloid, but also from tau pathology changes (Xia et al., 2017); in our current analysis, however, we had no estimate of tau pathology.

Increase of structural integrity, representing consistent increase of gray matter volume with global amyloid load (outside the border zone of the inner ventricular spaces) involved superior prefrontal and occipital lobe areas; these regions overlapped with areas of increased GM covariance with higher amyloid load in a previous multivariate study (Oh et al., 2014) that had also checked for positive associations; these previous findings included prefrontal and occipital lobe regions as well, albeit spreading more to parietal lobe regions than the positive findings in our study. In analogy to the concept of cognitive reserve, we tested a moderating effect of education, a proxy of cognitive reserve (Meng and D’Arcy, 2012), on this association. According to the cognitive reserve paradigm, education would be expected to moderate the effect of amyloid on
downstream events such as cognitive performance or on some substrate of cognitive performance, such as brain structure or function. However, we did not find any moderating effects of education on these associations. This lack of an effect suggests that the covarying increase of gray matter volume in superior prefrontal and parietal areas does not reflect a trait marker of higher cerebral reserve that allows people to remain cognitively stable despite cortical amyloid accumulation. Instead, the increase in cortical gray matter with higher amyloid load independently of education may point to a state marker of reactive increase of amyloid accumulation. Previous work has shown increase of local and remote cortical metabolisms with increased amyloid levels in healthy older people (Ossenkoppele et al., 2014; Teipel et al., 2016), possibly related to reactive metabolic increase due to Aβ accumulation (Shankar et al., 2007). Together with previous findings (Oh et al., 2014) our findings may suggest that in preclinical stages of AD not only hypermetabolism, but also cortical gray matter increase may occur as reaction to global or local Aβ accumulation. This interpretation needs further longitudinal evaluation. Our interpretation would predict that people with higher amyloid-related increases of cortical gray matter remain cognitive stable longer during clinical follow-up than people without reactive gray matter increase. With the forthcoming waves of the INSIGHT-preAD cohort, we will be able to test this hypothesis.

We did not find a direct effect of ApoE ε4 carrier status on gray matter integrity or a moderating effect of ApoE ε4 on the association between amyloid and structural integrity. This adds yet another negative finding to the large body of studies showing no consistent effect of ApoE ε4 on gray matter integrity in non-demented people, as reviewed by Fouquet et al. (2014). It further agrees with an earlier study that had found a strong association of ApoE ε4 genotype with amyloid accumulation, but not with gray matter volume in healthy older people (Drzezga et al., 2009). In summary, these data suggest that ApoE ε4 effects on gray matter integrity, if present at all, are subtle in preclinical stages of AD.

We studied an independent effect of aging on brain structural integrity, controlling for amyloid load. We found that concordant effects of lower gray matter volume associated with higher amyloid load and higher age were predominantly located in bilateral putamen and in parts of bilateral hippocampus. The convergence of effects in the hippocampus for aging as well as amyloid effects is consistent with the age effect on hippocampus volume reported in a range of previous study (for a recent review see Pini et al. (2016)); thus, recently we reported a significant association between age and hippocampus volume reported in a range of previous study (for a recent review see Pini et al. (2016)); thus, recently we reported a significant association between age and hippocampus volume in a population based sample of 3184 people covering the age range from 21 to 90 years of age (Teipel et al., 2015). So far, the association of age with gray matter volume, however, has mostly been studied without considering the effect of amyloid. Our data suggest that there may be a joint effect of amyloid and aging on gray matter volume in some networks, where each of these factors acts at least partly independent from the other.

Higher amyloid load was associated with poorer global cognitive, episodic memory and executive performance, independently of gray matter integrity. While effect sizes were relatively low ($r = 0.11–0.15$), these findings agree with previous results in healthy individuals in studies on amyloid PET and cognition (Chetelat et al., 2011; Mattsson et al., 2015; Petersen et al., 2016). In a meta-analysis across 33 studies, the majority of studies reported that higher amyloid load as determined by different amyloid measures (CSF, blood, PET) was weakly but significantly associated with poorer episodic memory and executive function in cognitively healthy older individuals (Hedden et al., 2013). We found no mediating effect of gray matter integrity on the association between amyloid load and cognitive performance. This agrees with previous studies where gray matter volume did not mediate the association of global amyloid load with episodic...
memory in cognitively healthy controls (Chetelat et al., 2011; Mattsson et al., 2015). In contrast, one previous study found a stronger effect of a composite marker of neurodegeneration (including hypometabolism and atrophy) than of amyloid on cognition in cognitively healthy older people (Wirth et al., 2013a). Clinicopathological studies reported that markers of neurodegeneration (which in turn are closely associated with atrophy) rather than amyloid load are correlated with cognition in people with more advanced stages of AD (Nelson et al., 2012). In conclusion, in more advanced stages of AD, neurodegeneration is the key determinant of cognition. In cognitively healthy people spanning the range from normal aging to preclinical AD, the overall effect of amyloid load on cognitive performance is small, and may be independent from gray matter atrophy, in agreement with the assumption that amyloid accumulation precedes brain atrophy in the pathogenesis of AD (Hardy and Selkoe, 2002). As a note of caution, it has rightly been stated that "[... ] cross-sectional studies investigating associations between cognition and amyloid-β in cognitively normal older people were conflicting. Because such studies define the sample a priori as cognitively normal, any evidence of cognitive dysfunction must be subtle and difficult to detect." (Augustin, 2016, page 26).

Compared with previous works (Apostolova et al., 2010; Becker et al., 2011; Bourgeat et al., 2010; Chetelat et al., 2010a,b; Oh et al., 2014; Wirth et al., 2013c), one strength of our study is the use of partial volume effect correction for amyloid signal quantification. Partial volume effect correction has been found to improve diagnostic accuracy, consistency of longitudinal changes, and associations of amyloid PET with postmortem histological Aβ plaque load (Rullmann et al., 2016). Partial volume effects are related to the degree of gray matter atrophy and thus can confound the association between amyloid PET signal and gray matter volumes.

Several limitations need to be considered with our study as well. First, the INSIGHT-preAD cohort represents an urban, highly educated, and cognitively high performing sample from the older population. In addition, the use of subjective memory complaints as inclusion criteria intended to enrich the risk for preclinical AD in this cognitively very high performing sample. This is an advantage as it allows studying effects of amyloid on brain structure and function in preclinical stages of AD, but it also limits the generalizability of findings to the aging population at large. Second, with this cross-sectional analysis, we cannot determine the relevance of amyloid accumulation and structural integrity for decline of cognitive performance. However, the number of cases is high and follow-up is ongoing in the INSIGHT-preAD cohort so that in future we will be able to study if the association of amyloid with brain morphology is predictive for cognitive decline, as suggested by previous findings (Wirth et al., 2013b).

In summary, we found predominant decreases of structural integrity of gray matter volumes as a proxy of structural connectivity (Alexander-Bloch et al., 2013) in a network encompassing the hippocampus, cingulate gyrus, and associated brain regions in a large cohort of cognitively normally performing older individuals with subjective memory complaints. Against our initial expectation, education was not a relevant moderator of this association. Amyloid load had a relatively weak but significant effect on global cognitive and episodic memory performance that was not mediated by gray matter integrity. Specifically, based on our cross-sectional findings, we hypothesize that in later stages of AD the direct effect of amyloid on cognition will be complemented by an indirect effect mediated by the downstream consequences of amyloid accumulation, such as decline of gray matter structural connectivity. The longitudinal follow-up data of the INSIGHT-preAD cohort over the next years will aid the testing of this hypothesis.

Disclosure statement

Drs Teipel, Cavedo, Weschke, Grothe, Rokkova, Fontaine, Dauphinot, Gonzalez-Escamilla, Bertin, Potier report no biomedical financial interests or potential conflicts of interest. Dr Dubois has received consultant fees from Lilly, Boehringer Ingelheim and has received grants from Roche for his institution. Dr Hampel reports having received lecture fees from Biogen, Roche, research grants from Pfizer and Aid (paid to the institution), travel funding from Axovant, Eli Lilly and company, Takeda and Zinfandel, GE Healthcare, Oryzon Genomics, consultancy fees from Jung Diagnostics, Cytox Ltd, Axovant, Anavex, Takeda and Zinfandel, GE Healthcare, Oryzon Genomics, and participated in scientific advisory boards of Axovant, Eli Lilly and company, Cytox Ltd, GE Healthcare, Takeda Zinfandel, Oryzon Genomics, Roche Diagnostics. Dr Habert reports having received honoraria from Lilly, Piramal, and GE as a speaker.

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SJT, EC, BD, and HH have contributed to the conception and design of the study, acquisition and analysis of data, and drafting a significant portion of the manuscript. SW, MJG, and GG-E have contributed to the analysis of data and drafting a significant portion of the manuscript. KR, GF, LD, M-CP, HB, and MOH have contributed to the acquisition of data and drafting a significant portion of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2017.05.016.

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