Distinct hippocampal subfields atrophy in older people with vascular brain injuries

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Cover title : Hippocampal subfields and vascular brain injuries

5997 words, 1 table, 3 figures

Key words: Hippocampus, hippocampal subfields, aging, neurovascular injury, small vessel disease

Abstract

Background and Purpose: Many neurological or psychiatric diseases affect the hippocampus during aging. The study of hippocampal regional vulnerability may provide important insights into the pathophysiological mechanisms underlying these processes; however, little is known about the specific impact of vascular brain damage on hippocampal subfields atrophy.

Methods*:* To analyse the effect of vascular injuries independently of other pathological conditions, we studied a population-based cohort of non-demented older adults, after the exclusion of people who were diagnosed with neurodegenerative diseases during the 14-year clinical follow-up period. Using an automated segmentation pipeline, 1.5T-MRI at inclusion and 4 years later were assessed to measure both white matter hyperintensities (WMH) and hippocampal subfields volume. Annualized rates of WMH progression and annualized rates of hippocampal subfields atrophy were then estimated in each participant.

Results*:* We included 249 participants in our analyses (58% women, mean age 71.8, median MMSE 29). The volume of the subiculum at baseline was the only hippocampal subfield volume associated with total, deep/subcortical and periventricular WMH volumes, independently of demographic variables and vascular risk factors (ß= -0.17, p=0.011; ß= -0.25, p=0.020 and ß= -0.14, p=0.029 respectively). In longitudinal measures, the annualized rate of subiculum atrophy was significantly higher in people with the highest rate of deep/subcortical WMH progression, independently of confounding factors (ß= -0.32, p=0.014).

Conclusions: These cross-sectional and longitudinal findings highlight the links between vascular brain injuries and a differential vulnerability of the subiculum within the hippocampal loop, unbiased of the effect of neurodegenerative diseases, and particularly when vascular injuries affect deep/subcortical structures.

Non standard abbreviations:

CA: Cornu-Ammonis, FCSRT: Free and Cued Selective Reminding Test, IST: Isaacs Set Test, MMSE: Mini Mental State Evaluation, TMT: Trail Making Test, WMH: White matter Hyperintensities

Introduction

White matter hyperintensities (WMH) measured using T2-weighted magnetic resonance imaging (MRI) are common in older people, and are thought to result from chronic hypoxia or ischemia and small infarcts associated with cerebral small vessel disease[1]. Although epidemiological studies have demonstrated an association between WMH and the risk of stroke or dementia[2], the precise link between WMH and neurological symptoms at the individual level remains poorly understood[3]. Indeed, WMH alone has been shown to contribute a modest degree of cross-sectional variation in cognition during aging[4]. In their initial longitudinal research on this topic, Schmidt and Fazekas found that associations between WMH progression and cognitive functioning were no longer significant after controlling for changes in brain volume, suggesting that cognitive decline in patients with vascular cognitive impairment was related to brain atrophy but not with the disconnection of white matter tracts or vascular pathology alone[5].

Many clinical and preclinical arguments suggest that the hippocampus is one of the brain regions most likely to be damaged by age-related chronic ischemia. Hippocampal hypometabolism and degeneration have been shown in different rodent models of chronic hypoperfusion or transient ischemia[6,7]. Furthermore, *post mortem* histological studies and *in vivo* imaging studies in older people have shown an association between WMH and medial temporal lobe atrophy[8,9,10]. However, imaging studies have also provided evidence for an additive effect of AD and WMH in hippocampal atrophy during aging[9]. Given that WMH often present as a comorbidity of AD, a recurring question is whether small vessel disease and AD pathology interact, making it difficult to determine to what extent hippocampal atrophy is the result of a neurodegenerative disease versus small vessel disease.

The study of hippocampal regional vulnerability has been proposed as a way to isolate pathogenic mechanisms affecting this archeocortical structure[11]. Indeed, the hippocampus is composed of numerous subfields with distinct morphological, cellular, molecular, functional, and connectivity profiles: the dentate gyrus, the cornu ammonis (CA, with subdivisions from CA1 to CA4), and the subiculum, which can be differentially affected by distinct neurological or psychiatric conditions[12,13,14]. If AD and small vessel disease affect hippocampal subfields differently, we hypothesized that the monitoring of regional hippocampal damage in older people could help clinicians to distinguish between these two pathophysiological processes. However, previous MRI studies investigating the link between WMH and specific hippocampal subfields atrophy in aging and vascular cognitive impairment have failed to clarify whether hippocampal atrophy is due to the accumulating burden of hypoxic/ischemic lesions or to the combination with frequent neurodegenerative disease in this population[15,16]. Furthermore, the quantitative relationship between the load of WMH and hippocampal

subfields volumes has never been investigated and there is a lack of longitudinal studies in this field of research.

The aim of this study was to assess properly the association between neurovascular damage and hippocampal subfields atrophy in older people, independently of the effect of neurodegenerative diseases. For that purpose, we measured hippocampal volume and the rate of hippocampal subfields atrophy, together with the volume and the rate of deep/subcortical and periventricular WMH progression using two MRI examinations at 4-year intervals in a population-based volunteer cohort of non-demented older adults. Thanks to the long follow-up of our cohort, we had the opportunity to investigate the association between small vessel disease and hippocampus atrophy avoiding bias due to other concomitant pathophysiological processes by excluding from analyses participants diagnosed with neurodegenerative disease within 14 years following the first MRI exam.

Methods

Data availability

Anonymized data will be shared by request with any qualified investigator for the sole purpose of replicating procedures and results and as long as data transfer is in agreement with EU legislation on the general data protection regulation.

Participants

Study participants were recruited as part of a longitudinal population-based cohort designed to evaluate the risk factors of dementia, the Bordeaux subset of the Three-City (3C) study[17]. During the 1999-2000 inclusion period, a personal letter including a brief description of the study protocol and an acceptance/refusal form were sent to non-institutionalized individuals aged 65 years and older randomly selected from electoral lists. Partner was also invited to participate in the study if meeting eligibility criteria. In case of no response, an attempt was made to contact subjects by telephone. After inclusion, people were then followed-up prospectively for up to 14 years. Data regarding demographic characteristics and vascular risk factors was collected at baseline. Of the initial cohort of participants with baseline MRI data (n=663), only non-demented participants with a MMSE >23, who agreed to have a second MRI 4 years later were included in the present hippocampal subfields analyses (n=364). Compared to the total Bordeaux-3C cohort (n=2104), subjects with at least one MRI (n=663) were younger (72.7±4.0 vs 75.5±5.3, $p<0.0001$), presented more frequently a high education level (44.0% vs 34.0%, $p<0.0001$), were more frequently male (42.8% vs 36.9%, $p=0.0097$) and had slightly higher mean MMSE score at baseline (27.7±1.9 vs 26.9±2.6, $p<0.0001$). There was no significant differences regarding APoE4 status. However, no significant differences in demographic data or neuropsychological tests were observed at baseline between the participants who completed one MRI exam and the subjects who completed two. Participants lost to follow-up after the second MRI were also excluded (Fig. 1A). All participants provided written informed consent consent prior to participation in the study. The study protocol was approved by the ethics committee of Kremlin-Bicêtre University Hospital (Paris, France).

Clinical and neuropsychological follow-up

In this cohort, clinical assessments were administered by trained psychologists at baseline and after 2, 4, 7, 10, 12 and 14 years. At each follow-up, a diagnosis of dementia was pre-specified at home by the neuropsychologist and a clinical validation of the diagnosis was made by a neurologist or a

geriatrician. The definitive diagnosis of dementia was ultimately made by a panel of independent neurologists based on the Diagnostic and Statistical Manual of Mental Disorders criteria (DSM-IV) and the etiology of dementia was made according to National Institute of Aging and international criteria at the time of diagnosis.

The initial neuropsychological battery consisted of the Mini Mental State Evaluation (MMSE: global cognitive functions), the Free and Cued Selective Reminding Test (FCSRT: verbal episodic memory - sum of the number of words retrieved during the three free or cued trials), the Isaacs Set Test (IST: semantic fluency), and the Trail Making Test part A and B (TMT-A and TMT-B: attention, information processing speed and executive functions ((number of correct moves/total time in seconds)x10)).

MRI acquisition and processing

Participants were scanned on a 1.5T Gyroscan Intera system (Philips Medical Systems) with a quadrature head coil. The protocol consisted of 3D high-resolution T1-weighted images acquired in transverse plane using magnetization prepared rapid gradient echo sequence (TR=8.5 ms, TE=3.9 ms, α=10°, FOV=240 mm, voxel size=0.94x0.94x1mm^3). T2-and proton density (PD)-weighted MRI were acquired using a 2D dual spin echo sequence (TR=4400ms, TE1=16ms, TE2=98ms, matrix size=256x256, voxel size=0.98x0.98x3.5mm^3). The same scanner and sequence were used for both the baseline and the 4-year follow-up MRI examinations.

For the volumetric analyses of total grey matter volume, intracranial volume and hippocampal subfields volumes, T1-weighted images were processed using the volBrain system (http://volbrain.upv.es)[18]. Next, the segmentation of hippocampal subfields was performed with the HIPS (HIPocampus subfield Segmentation) pipeline[19], based on a combination of non-linear registration and multi-atlas patch-based label fusions with systematic error correction. HIPS has been shown to significantly outperform other publicly available software such as FIRST or Freesurfer[20]. It uses a training library from a public repository (www.nitrc.org/projects/mni-hisub25) composed of manually labeled high resolution T1-weighted images[21] (Kulaga-Yoskovitz dataset). We used the Kulaga-Yoskovitz protocol instead of the Winterburn protocol (the other available segmentation protocol in the HIPS pipeline) because its segmentations were more reliable (0.88 vs 0.71) due to the use of a larger number of training cases (25 vs 5)[19] and a lower number of subfields (3 vs 5). To perform the segmentation, the images were up-sampled with a local adaptive super-resolution method to fit in the training image resolution[22]. The method provides automatic segmentation of hippocampal subfields gathered into three labels, based on morphology and intensity of densely myelinated molecular layers as follows: subiculum, CA1-3 and CA4/dentate gyrus (CA4-DG) (Fig. 1C). Quality

control of the image-processing pipeline for hippocampal subfields segmentation in this cohort was previously reported[23]. Briefly, two neurologists performed a visual assessment of sagittal, coronal, and axial slices of the 3D hippocampal volume: labels with segmentation errors were excluded or manually corrected using 3D-Slicer (www.slicer.org) in case of minor errors (inappropriate inclusion of choroidal plexus, para-hippocampal T1-hypointensities, CSF "pockets": n=44/327 subjects with hippocampal subfields segmentation, Fig 1A). Baseline grey matter and hippocampal volumes were normalized with intracranial cavity volume (ICV), and annualized rates of atrophy for each participant was calculated as follows: ((volume after 4 years – volume at baseline)/volume at baseline)/4.

We used an automatic WMH detection algorithm that has been previously described, validated and applied to the 3C cohort[24]. Briefly, it consisted of a pre-processing step including registration (alignment of the T1 and T2/PD volumes), non-brain tissue removal and bias field correction; a second step of WMH detection in T2 images, including removal of false positives (using the CSF volume of the subject provided by SMP99) and a third post-processing step including the generation of WMH probability maps at the individual and sample levels (in stereotactic space), descriptive volumetry, localization and classification of WMH. When their distance to the ventricular system was less than 10 mm, WMH were labeled as periventricular, otherwise they were labeled as deep/subcortical. Annualized rates of WMH progression were calculated as follows: ((volume after 4 years – volume at baseline)/volume at baseline)/4.

A total of 249 participants were finally included in our analyses after exclusions based on the quality of MRI post-processing at both timepoints, as well as 49 participants who developed neurodegenerative disease during the 14-year follow-up period (36 Alzheimer's diseases, 12 Parkinson's disease or dementia with Lewy bodies, and one frontotemporal lobar degeneration) (Fig. 1A).

Statistical analyses

Statistical analyses were performed with Prism software 8 (Graphpad) and XLstats 19.4 (Addinsoft). First, patients were classified into three subgroups based on their WMH volume (cross-sectional measures at baseline, Fig. 2) or according to the progression of WMH volume between the two MRI (Fig. 3). We defined a group with a low level of WMH volume or WMH progression ($\leq$ 25th percentile), a moderate level (25th-75th percentile), and high level ($\geq$75th percentile). In univariate analyses, the χ^2 test was used to compare categorical variables and Mann-Whithney or analyses of variance (ANOVAs) were performed to compare quantitative variables among groups, followed by Sidak multiple comparisons tests. Then, hippocampal subfields volumes and annualized rate of

atrophy found to be significantly associated ($p<0.05$, before Sidak correction) with WMH volumes or rate of WMH progression were predicted with multivariate linear regression models. For each hippocampal subfield, the first model included WMH volumes (or rate of progression) and demographic variables (age, gender and educational level) known as nuisance variables in MRI volumetric studies. The second model included the variables of model 1 and additionally vascular risk factors including high blood pressure, body mass index, diabetes, smoking and alcohol consumption. Finally, we performed a sensitivity analysis on the longitudinal MRI data by running the same regression models without excluding the 49 patients who developed neurodegenerative diseases (n=298 participants).

Results

Demographic, clinical characteristics at baseline and follow-up rates

The baseline characteristics of the whole analytic sample and according to total WMH load at baseline is reported in table 1. The mean duration of follow-up was 12.2±2.2 years according to our exclusion criteria (participants lost to follow-up the visit after the second MRI were excluded to allow a confident exclusion of all neurodegenerative cases). There was no association between baseline WMH load and duration of follow-up (p=0.98). Among the 249 participants included in the analyses, four developed vascular dementia over time (one after 12 years follow-up and three after 14 years).

Association between WMH and hippocampal subfields volumes at baseline

We split the population into three groups based on the presence of WMH, defined as low (<25th percentile), moderate (25th to 75th percentile), and high levels of WMH (>75th percentile), with measurements calculated for total, deep/subcortical, and periventricular WMH (Fig. 2A). In univariate analyses, CA4-DG and subiculum volumes were significantly lower in people with the highest WMH load at baseline, relative to the total volume of WMH ($p<0.001$ for both CA4-DG and subiculum, Fig. 2B), the volume of deep/subcortical WMH (both p=0.01, Fig. 2C), and the volume of periventricular WMH (p=0.002 and $p<0.001$, Fig. 2D). In comparison, no significant differences between WMH groups were observed for the total volume of grey matter, with only a tendency toward smaller grey matter volumes when total WMH loads were moderate or high (44.5% of ICV vs 42.9% vs 42.2%, respectively in the low, moderate and high level of total WMH groups, p=0.07).

In multivariate analyses, regression models using hippocampal subfield as dependent variables showed that the volume of CA4-DG was no longer associated with total, deep/subcortical, or periventricular WMH volumes when demographic variables (or demographic variables and vascular risk factors) were added into the models, whereas older age was still a predictor of CA4-DG volumes ($p<0.0001$ in all models). In contrast, the volume of the subiculum was still associated with the volume of total WMH, independently of demographic variables (model 1: age, gender and educational level; ß= -0.20, p=0.002) and vascular risk factors (model 2: age, gender, educational level, high blood pressure, body mass index, diabetes, smoking and alcohol consumption; ß= -0.17, p=0.01), with the volume of deep/subcortical WMH (ß= -0.16, p=0.02 in model 1 and ß= -0.25, p=0.02 in model 2), and with the volume of periventricular WMH (ß= -0.14, p=0.02 in model 1 and ß= -0.14, p=0.03 in model 2). In all these statistical models, age was also shown to be an independent predictor of smaller subiculum volumes (ß from -0.26 to -0.23, $p<0.001$). Diabetes was associated with smaller CA4-DG volume in univariate analyses (p=0.04); smoking (p=0.001 and p=0.02) and alcohol consumption (p=0.01 and

p=0.007) were associated with smaller CA4-DG and subiculum volumes but none of the vascular risk factors were found to be predictors of smaller hippocampal subfields volumes independently of WMH and demographic variables.

Association between the progression of WMH volumes and the annualized rates of hippocampal subfields atrophy over 4 years

To study the longitudinal dynamics of hippocampal subfields atrophy and its link with WMH progression, we calculated the annualized rate of hippocampal subfields atrophy and the annualized rate of WMH progression during the initial 4-year follow-up period. The mean annualized increases in WMH volume were 11.6% (±15.6) for total WMH volume, -0.5% (±8.9) for deep/subcortical WMH volume, and 39.3% (±157.0) for periventricular WMH volume. The progression of total WMH was highly correlated with periventricular WMH ($r=0.97$, $p<0.0001$) but less with deep/subcortical WMH ($r=0.32$, $p<0.0001$). The progression of deep/subcortical WMH was not correlated with the progression of periventricular WMH ($r=0.07$, $p=0.22$).

Next, we split the population into three groups based on the progression of WMH, defined as low (<25th percentile), moderate (25th to 75th percentile), and high levels of WMH (>75th percentile), with measurements calculated for total, deep/subcortical, and periventricular WMH (Fig. 3A). In univariate analyses, the annualized rate of subiculum atrophy was significantly higher only in people with the highest rate of deep/subcortical WMH progression ($p=0.002$, Fig. 3C). This association was independent of demographic variables (model 1: ß= -0.25, $p=0.04$) and vascular risk factors (model 2: ß= -0.32, $p=0.01$). Age (model 1: ß= -0.08, $p<0.001$ and model 2: ß= -0.07, $p<0.001$) and alcohol consumption (ß= -0.02, $p=0.01$) were also identified as independent predictors of the annualized rate of subiculum atrophy.

As a sensitivity analysis, we performed the same linear regression analyses on longitudinal MRI data without excluding the 49 patients who went on to develop neurodegenerative diseases (n=298). The annualized rate of subiculum atrophy was still significantly associated with the rate of deep/subcortical WMH progression in these analyses (model 1: ß= -0.33, $p=0.01$; model 2: ß= -0.39, $p=0.004$).

When baseline total, periventricular or deep/subcortical WMH volumes were added into the regression models, they were not associated with the rate of subiculum atrophy (all p-values >0.6) and they did not change other significant associations.

Discussion

Thanks to the long clinical follow-up of this cohort, we were able to assess accurately the impact of neurovascular injuries on hippocampal subfields, unbiased of the effect of these neurodegenerative diseases. We found that the volume of the subiculum was the only hippocampal subfield volume associated with total, deep/subcortical, and periventricular WMH lesions, independently of age, gender, educational level, and vascular risk factors. Furthermore, using longitudinal MRI measures, we showed that people with higher deep/subcortical WMH progression rates also presented with higher subiculum atrophy rates, independently of demographic variables or vascular risk factors. These results suggest a differential vulnerability within the hippocampus for vascular brain damage, with the subiculum presenting the highest vulnerability to deep/subcortical WMH lesions.

Our results corroborate two previous small cross-sectional studies showing significant shape or volume modifications of the subiculum in patients with subcortical vascular mild cognitive impairment[15,16]. This differential vulnerability of the subiculum to vascular injuries has also been observed in animal studies using anoxia-ischemic models[7] and potentially involves the glucocorticoid pathway. Indeed, both the human and the rodent subiculum are enriched in glucocorticoid receptors, which have been shown to potentiate ischemic injury in neurons[25]. While the volume of the subiculum was associated with total, deep/subcortical, and periventricular WMH volumes at baseline, the annualized rate of atrophy was only associated with the progression of deep/subcortical WMH. It highlights the relevance to consider deep/subcortical and periventricular WMHs separately because these measures were not correlated and they could correspond to patients with distinct neuropathology[1] and different rates of hippocampal atrophy. For instance, some authors reported elevated levels of activated microglia in periventricular white-matter lesions but not in deep/subcortical lesions[26], which were associated with oxidative stress markers related to hypertension[27]. Accordingly, a recent genetic study concluded that periventricular WMH was more associated with ischemic stroke while loci associated with deep/subcortical WMH were implicated in vascular, astrocyte, and neuronal dysfunction[28]. Finally, regarding the biological correlates of our findings, we found that high alcohol consumption was also an independent predictor of the annualized rate of subiculum atrophy, as previously suggested in a small cross-sectional study of patients with alcohol dependence[29].

Interestingly, univariate analyses revealed significant associations between WMH and CA4/DG volumes; however, in contrast to the subiculum, these results were no longer significant after controlling for age. This statistical link between CA4/DG volume and age is consistent with our previous study on the same cohort showing that the dentate gyrus is the most vulnerable subfield to the

effects of aging[23]. We have also shown in this previous work that the annualized rate of CA1-3 atrophy was associated with an increased risk of developing Alzheimer's clinical syndrome. Taken together, our results suggest that monitoring of regional hippocampal vulnerability can provide crucial insights into the phenotypic variability and pathophysiological mechanisms underlying neurological disorders associated with aging: the dentate gyrus is the most vulnerable subfield to the effects of aging, CA1-3 is the primary target of AD, and the subiculum is differentially affected by neurovascular injuries. Since many older patients with cognitive decline and hippocampal atrophy exhibit both vascular and concomitant AD pathology[30], our work suggests that studying hippocampal subfields volumes could help clinicians to identify the pathology that most affects the hippocampus on these patients.

Several factors support the external validity of the present work. Vascular risk factors, including smoking, body mass index, and diabetes, were significantly associated with greater total WMH volumes at baseline, consistent with previous studies[31,32]. Interestingly, while smoking and alcohol consumption were associated with smaller subiculum at baseline in univariate analyses, vascular risk factors were not associated with the volume of the subiculum or its annualized rate of atrophy in our regression models when WMH are taken into account. It suggests that they are not associated with subiculum damage independently of WMH, or that the vascular risk factors analyzed here do not measure the overall vascular risk (for instance hypertension was analyzed without distinction between treated and untreated patients). In the present work, we found a mean annualized rate of total WMH progression of +11.6%/year in our population, which is consistent with previous longitudinal studies in older adults (ranging from 4.4% to 37.2%)[3]. Interestingly, the mean progression of periventricular WMH was rather high (39.3%/year) whereas the mean progression of deep/subcortical WMH was negligible (-0.5%/year)[33]. The volume of deep/subcortical WMH can even decrease in some participants, with the same small effect size in both the 3C cohort and other cohorts[33]. However, a quarter of the population (>75e percentile, Fig. 3A) had a progression of deep/subcortical WMH between 5% and 40%/year, driving our conclusions about subiculum atrophy. As previously discussed, these findings highlight that the classification of WMH into deep/subcortical and periventricular is clinically meaningful because their causes and consequences are likely to be different.

Regarding the limitations of the study, we acknowledge that our findings are based on up-sampled 1.5T MRI and that there is currently a lack of protocol harmonization regarding the definition of hippocampal subfields[34]. However, we have previously demonstrated that our postprocessing pipeline significantly improves the segmentation results compared with classical interpolation methods[19]. Regarding technical limitations, we also acknowledge that our quantitative measures of neurovascular damage rely only on WMH measured on T1 and T2/PD-weighted images, and do not take into account other markers of small vessel diseases such as microbleeds or dilated peri-vascular spaces[2]. The

present study also lacks an assessment of amyloid and tau pathology in order to study the isolated impact of vascular damage on hippocampal subfields volumes, as neither PiB-PET nor tau-PET were available at the time of inclusion (1999-2000). Although clinical criteria for AD and vascular dementia may overlap and correlate moderately with neuropathological data, the strength of our study is the long clinical follow-up of 14 years allowing the probable exclusion of participants who would later develop all types of neurodegenerative diseases: this distinction marks a clear advantage over a previous cross-sectional study in which subcortical vascular dementia was defined on the basis of the negativity of PiB-PET (excluding only patients with AD)[16]. Finally, we did not report associations between the longitudinal evolution of neuropsychological performances of participants, and either WMH or hippocampal subfields volumes. Indeed, due to our selection criteria of healthy older people (median MMSE at baseline 29) and the exclusion of all future cases of neurodegenerative (or mixed) dementia, only four patients went to develop vascular dementia during follow-up. While our population was selected to study the unbiased pathological and anatomical associations between vascular damage and hippocampal subfield volumes, future studies should be designed to investigate correlations between hippocampal subfields atrophy, vascular risk factors and cognitive performance. It will be of great interest to study the different memory processes in this context because there is functional evidence that the subiculum is particularly involved in episodic retrieval, while other hippocampal subfields rather support the encoding of novel information[35].

Funding

The 3C Study is conducted under a partnership agreement among the *Institut National de la Santé et de la Recherche Médicale* (INSERM), Bordeaux University, and Sanofi. The *Fondation pour la Recherche Médicale* funded the preparation and initiation of the study. The 3C Study is also supported by *Caisse Nationale Maladie des Travailleurs Salariés*, *Direction Générale de la Santé*, *Mutuelle Générale de l'Education Nationale*, *Institut de la Longévité, Conseils Régionaux d'Aquitaine et Bourgogne, Fondation de France,* and the Ministry of Research-INSERM Programme *"Cohortes et collections de données biologiques"*. The follow-ups have also been funded by ANR 2007LVIE 003, the "*Fondation Plan Alzheimer*" and the Caisse Nationale de Solidarité pour l'Autonomie (CNSA). This work benefited from the support of the project DeepvolBrain of the French National Research Agency (ANR-18-CE45-0013) and by the Spanish DPI2017-87743-R grant from the Ministerio de Economia, Industria y Competitividad of Spain. In addition, this study was achieved within the context of the Laboratory of Excellence TRAIL ANR-10-LABX-57 for the BigDataBrain project. Finally, we thank the Investments for the future Program IdEx Bordeaux (ANR-10- IDEX- 03- 02, HL-MRI Project), Cluster of excellence CPU and the CNRS. VP also received grants from Fondation Bettencourt Schueller (CCA-Inserm-Bettencourt). The sponsors did not participate in any aspect of the design or performance of the study, including data collection, management, analysis, and the interpretation or preparation, review, and approval of the manuscript.

Disclosures

None

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Figure legends

Figure 1. Datasets. (A) Flowchart of the study. (B) Examples of T2-weighted images of two subjects presenting WMH volume at baseline in the highest range of the cohort (both in periventricular and in deep/subcortical localizations). (C) Examples of hippocampal segmentations with the HIPS software of two extreme cases, one with very low total hippocampal volume (top panel, normalized volume=0.36% of ICV) and one with high hippocampal volume (bottom panel, normalized volume=0.63% of ICV). The method provides automatic segmentation of hippocampal subfields gathered into 3 labels: subiculum, CA1-3 and CA4/dentate gyrus (CA4-DG).

Figure 2. Association between hippocampal subfields and WMH volumes at baseline. (A) Dot plots showing the distribution of total, deep/subcortical and periventricular WMH volumes at baseline. Participants were further classified into subgroups with low level of WMH (<25th percentile), moderate level of WMH (25^{th} to 75^{th} percentile) and high level of WMH (>75^{th} percentile) for total, deep/subcortical or periventricular WMH. (B – D) Normalized hippocampal subfields volumes were compared between subgroups: asterisks above the histograms refer to Sidak's multiple comparisons test after ANOVA (* $p<0.05$, **$p<0.01$, ***$p<0.001$). Hashtag after the subfield name refers to a significant association after adjustment on demographic variables and vascular risk factors (# $p<0.05$).

Figure 3. Associations between annualized rates of hippocampal subfields atrophy and the progression of WMH volumes over 4 years. (A) Dot plots showing the distribution of total, deep/subcortical and periventricular annualized rate of WMH progression during 4-year follow-up. Participants were further classified into subgroups with low WMH progression rate (<25th percentile), moderate WMH progression rate (25^{th} to 75^{th} percentile) and high WMH progression rate (>75^{th} percentile) for total, deep/subcortical or periventricular WMH. (B – D) Annualized rate of hippocampal subfields atrophy were compared between subgroups: asterisks above the histograms refer to Sidak's multiple comparisons test after ANOVA (* $p<0.05$, **$p<0.01$). Hashtag after the subfield name refers to a significant association after adjustment on demographic variables and vascular risk factors (# $p<0.05$).

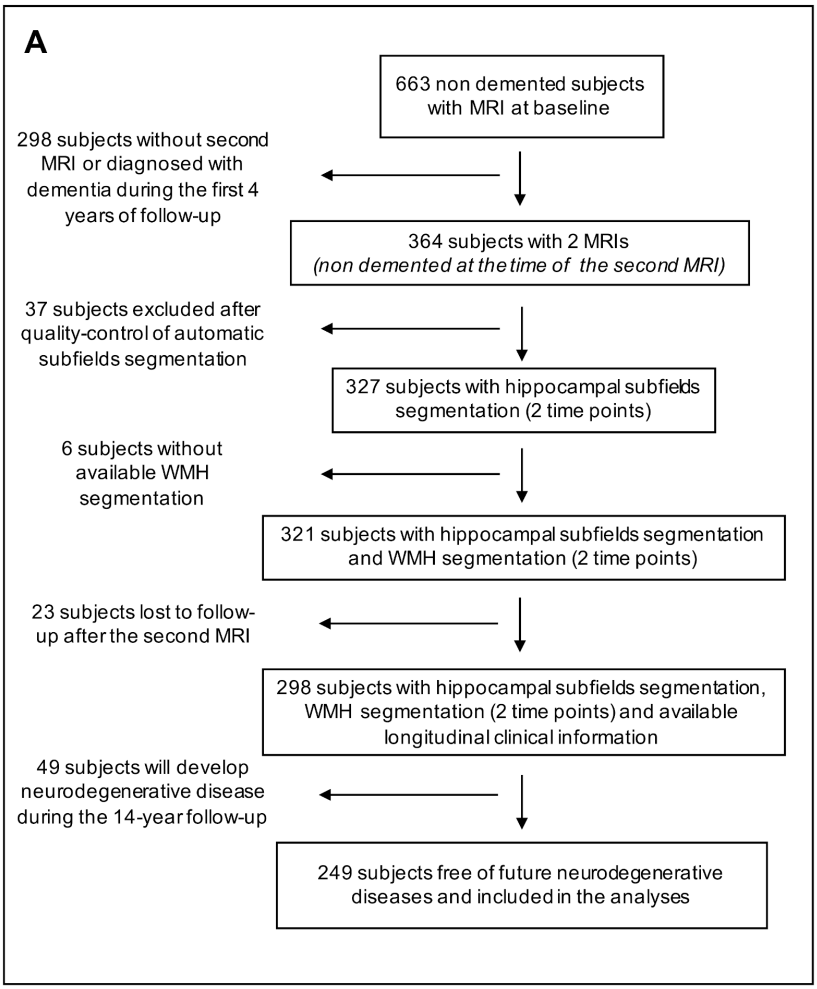
Table

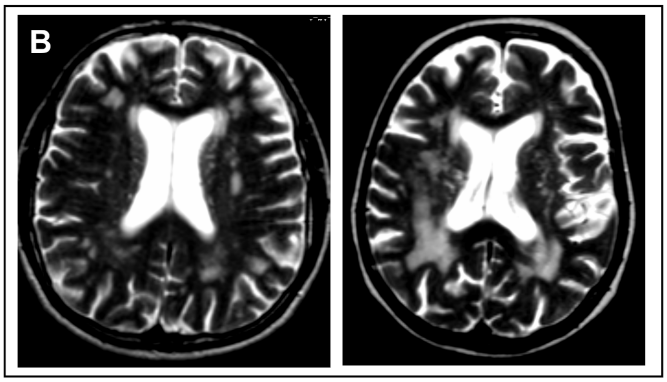
	Whole study sample (n=249)	Total WMH < 25th percentile	Total WMH 25th – 75th percentile	Total WMH > 75th percentile	*p*-value
Demographical variables at baseline					
Age, mean (SD)	71.8 (3.7)	71.3 (3.5)	71.8 (3.7)	72.1 (3.9)	0.437
Gender, women %	58.0%	80.7%	56.5%	39.7%	<0.0001
Education level, high %	53.0%	48.4%	53.2%	57.1%	0.632
Neuropsychological tests at baseline					
MMSE, median [range]	29 [24-30]	28 [24-30]	29 [24-30]	29 [24-30]	0.354
FCSRT free recall, mean (SD)	25.3 (5.7)	26.4 (6.0)	25.5 (5.7)	24.9 (5.6)	0.326
FCSRT total recall, median [range]	46 [21-48]	47 [36-48]	46 [21-48]	47 [30-48]	0.287
Isaacs set test 60s, mean (SD)	70.8 (14.6)	69.0 (13.2)	72.3 (14.5)	72.5 (14.1)	0.267
TMT-A, mean (SD)	5.0 (1.5)	4.7 (1.3)	5.2 (1.5)	5.2 (1.4)	0.033
TMT-B, mean (SD)	2.4 (1.1)	2.3 (1.1)	2.4 (1.1)	2.4 (1.1)	0.746
Vascular risk factors					
High blood pressure[1], %	68.7%	61,3%	69.4%	74.6%	0.27
Body mass index, mean (SD)	25.9 (3.9)	24.8 (3.7)	26.0 (3.8)	27.0 (4.2)	0.008
Diabetes mellitus[2] %	6,8%	3,2%	3,3%	17.5%	0.002
Smoking (pack-year), mean (SD)	10.4 (19.5)	5.3 (13.6)	10.0 (18.4)	16.2 (24.5)	0.008
Alcohol consumption (g/day), mean (SD)	12 (12.6)	8.5 (11.2)	12.2 (12.0)	14.8 (14.4)	0.021
History of stroke, %	4.4%	0%	4.8%	7.9%	0.09
History myocardial infarction, %	4.8%	3.2%	4.8%	6.3%	0.72

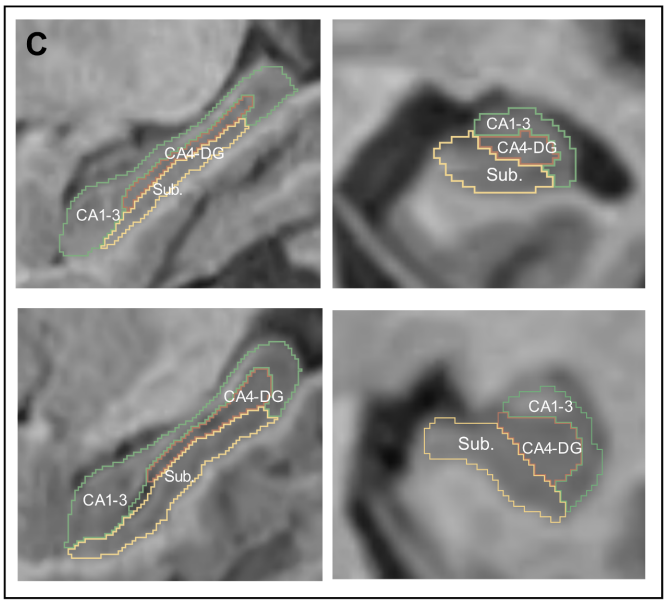
Table 1. Baseline characteristics of participants and according to total WMH volume quartiles at baseline. FCSRT: Free and Cued Selective Reminding Test; MMSE: Mini Mental State Examination; TMT: Trail-Making Test. *p*-values refer to χ^2 test and ANOVA, to compare variables among the three groups.

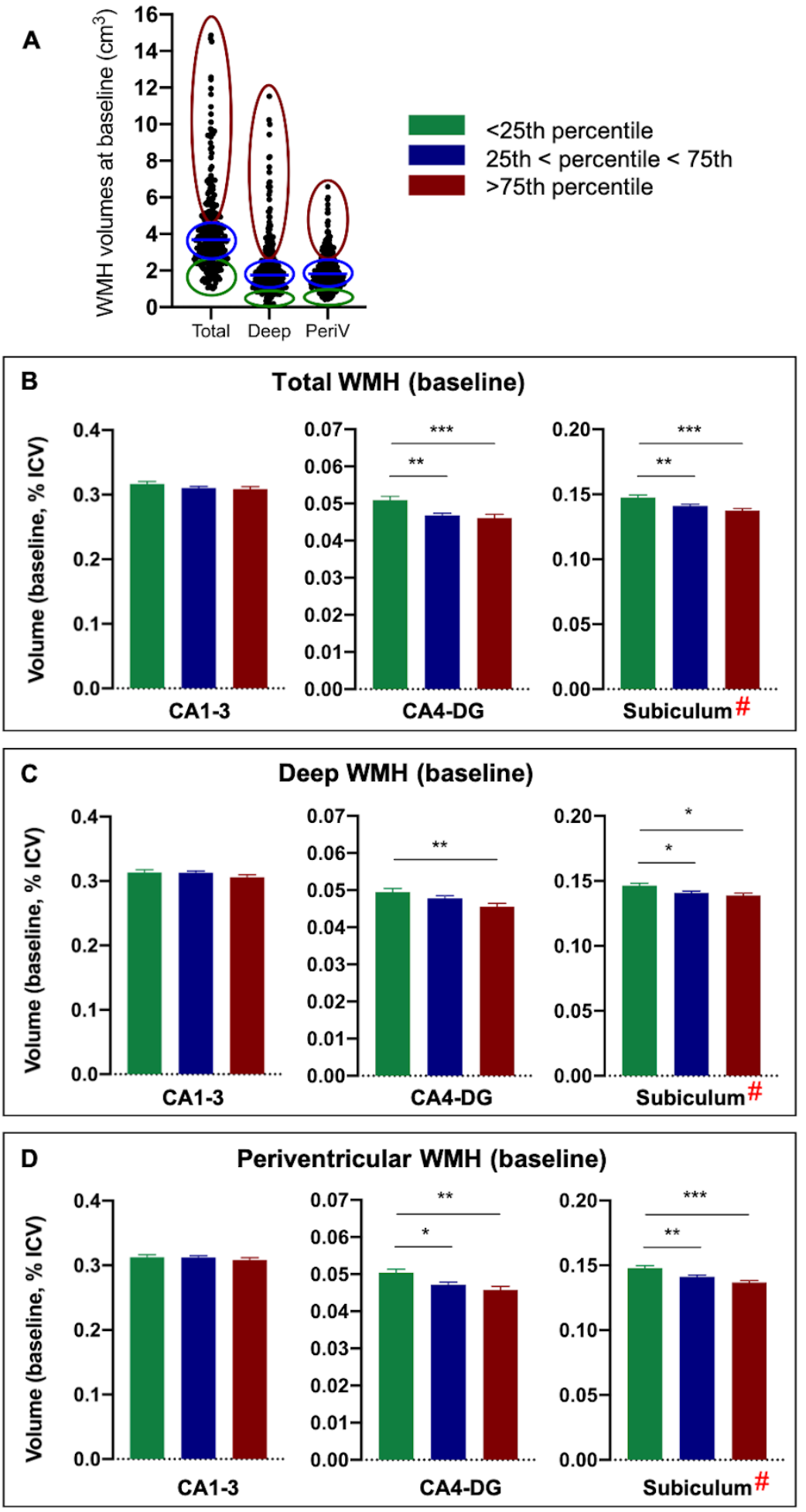
1. Hypertension was defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg or by antihypertensive drug use

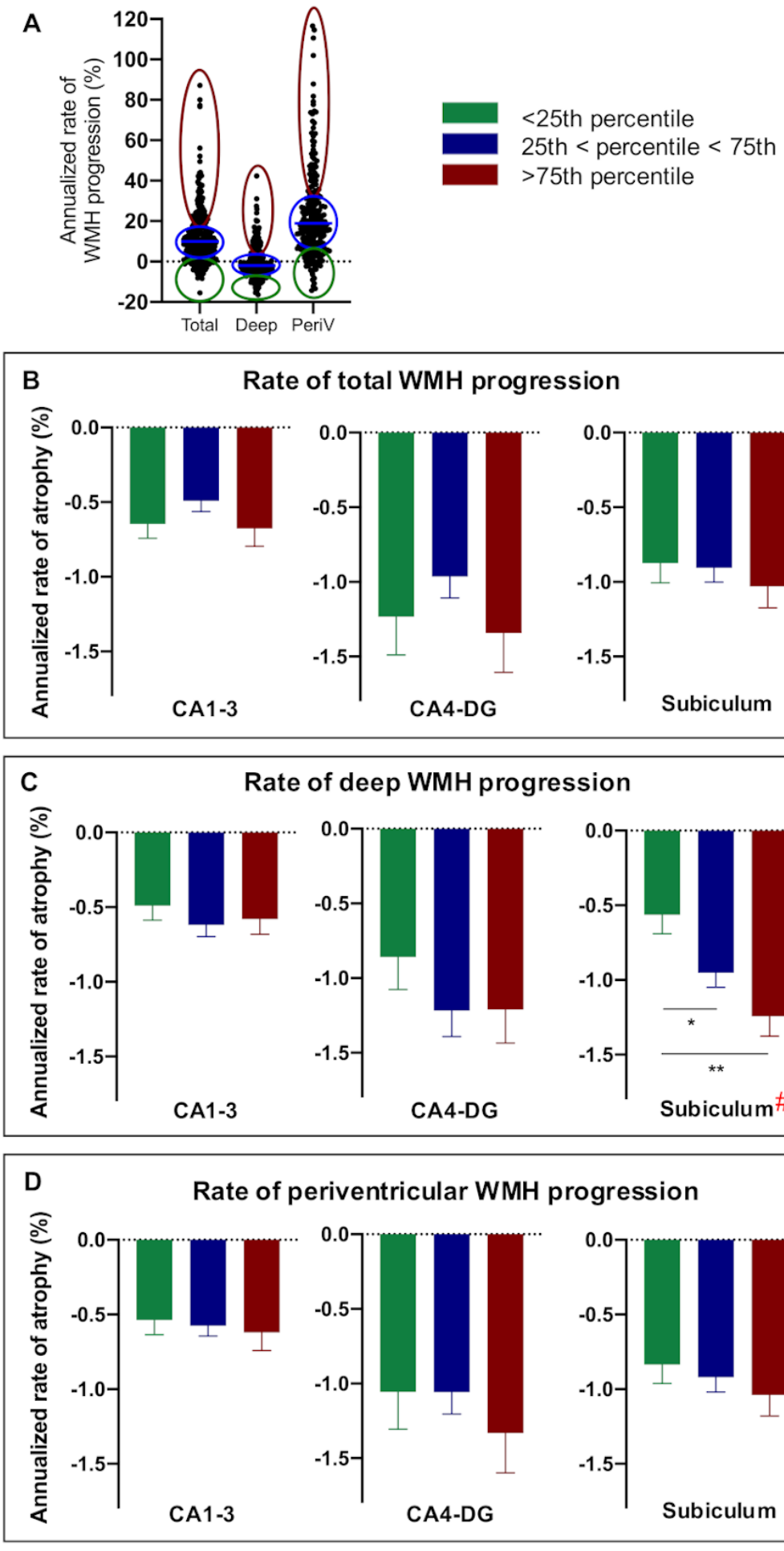
2. Diabetes mellitus was defined as glycemia >7 mmol/L or by antidiabetic treatment use


A
663 non demented subjects with MRI at baseline
298 subjects without second MRI or diagnosed with dementia during the first 4 years of follow-up
364 subjects with 2 MRIs (non demented at the time of the second MRI)
37 subjects excluded after quality-control of automatic subfields segmentation
327 subjects with hippocampal subfields segmentation (2 time points)
6 subjects without available WMH segmentation
321 subjects with hippocampal subfields segmentation and WMH segmentation (2 time points)
23 subjects lost to follow-up after the second MRI
298 subjects with hippocampal subfields segmentation, WMH segmentation (2 time points) and available longitudinal clinical information
49 subjects will develop neurodegenerative disease during the 14-year follow-up
249 subjects free of future neurodegenerative diseases and included in the analyses


B


C
CA4-DG
Sub.
CA1-3
CA1-3
CA4-DG
Sub.
CA4-DG
Sub.
CA1-3
CA1-3
Sub.
CA4-DG


A
WMH volumes at baseline (cm3)
16
14
12
10
8
6
4
2
0
Total
Deep
PeriV
<25th percentile
25th < percentile < 75th
>75th percentile
B
Total WMH (baseline)
Volume (baseline, % ICV)
0.4
0.3
0.2
0.1
0.0
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0.20
0.15
0.10
0.05
0.00
***
**
CA1-3
CA4-DG
Subiculum#
C
Deep WMH (baseline)
Volume (baseline, % ICV)
*
**
CA1-3
CA4-DG
Subiculum#
D
Periventricular WMH (baseline)
Volume (baseline, % ICV)
*
**
***
CA1-3
CA4-DG
Subiculum#


A
Annualized rate of WMH progression (%)
120
100
80
60
40
20
0
-20
Total
Deep
PeriV
<25th percentile
25th < percentile < 75th
>75th percentile
B
Rate of total WMH progression
Annualized rate of atrophy (%)
0.0
-0.5
-1.0
-1.5
CA1-3
CA4-DG
Subiculum
C
Rate of deep WMH progression
Annualized rate of atrophy (%)
0.0
-0.5
-1.0
-1.5
*
**
CA1-3
CA4-DG
Subiculum#
D
Rate of periventricular WMH progression
Annualized rate of atrophy (%)
0.0
-0.5
-1.0
-1.5
CA1-3
CA4-DG
Subiculum