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ABSTRACT: More educated elders are less susceptible to age-related or pathological cognitive changes. We aimed at providing a comprehensive contribution to the neural mechanism underlying this effect thanks to a multimodal approach. Thirty-six healthy elders were selected based on neuropsychological assessments and cerebral amyloid imaging, i.e. as presenting normal cognition and a negative florbetapir-PET scan. All subjects underwent structural MRI, FDG-PET and resting-state functional MRI scans. We assessed the relationships between years of education and i) gray matter volume, ii) gray matter metabolism and iii) functional connectivity in the brain areas showing associations with both volume and metabolism. Higher years of education were related to greater volume in the superior temporal gyrus, insula and anterior cingulate cortex and to greater metabolism in the anterior cingulate cortex. The latter thus showed both volume and metabolism increases with education. Seed connectivity analyses based on this region showed that education was positively related to the functional connectivity between the anterior cingulate cortex and the hippocampus as well as the inferior frontal lobe, posterior cingulate cortex and angular gyrus. Increased connectivity was in turn related with improved cognitive performances. Reinforcement of the connectivity of the anterior cingulate cortex with distant cortical areas of the frontal, temporal and parietal lobes appears as one of the mechanisms underlying education-related reserve in healthy elders.

Keywords: education; cognitive aging; brain reserve; cognitive reserve; functional connectivity.

1. Introduction

The protective role of education on age-related or pathological changes in cognition is a long standing well documented concept supported by a large set of complementary data. Epidemiological studies have shown lower prevalence or incidence of dementia in elder population with high level of education (see Meng and D'Arcy, 2012 and Valenzuela et al., 2006 for a review and meta-analysis). Furthermore, neuropsychological studies have reported better cognitive test performance (Plassman et al., 1995; Wilson et al., 2009) and reduced rate of cognitive decline (Albert et al., 1995; Alvarado et al., 2002; Anstey et al., 2003; Christensen et al., 1997; Evans et al., 1993; Farmer et al., 1995; Lyketsos et al., 1999; White et al., 1994) in elders with higher levels of education. These investigations suggest that education affects the risk of late life dementia by its positive association with level of cognition or through its association with decreased rate of cognitive decline in aging. These findings are more generally interpreted under the reserve hypothesis (see Stern , 2002 for a review), with education being considered as one of the main proxy of reserve, although other factors (i.e. IQ, occupation, social and physical activities, complex mental activities) may also be considered (see Satz et al., 2011 for a review). The reserve concept, largely supported by neuroimaging studies, posits that brains with higher reserve could tolerate more aging or pathological effects, thereby minimizing the symptoms. That is, this hypothesis assumes that there are inter-individual differences in the tolerance to brain insult before clinical deficit emerges. At a theoretical level, two different (though not mutually exclusive) conceptualizations to account for reserve mechanisms have been proposed: the brain reserve and the cognitive reserve hypotheses. Under these hypotheses, the inter-individual differences in the tolerance to aging or pathological effects may be explained either by differences in anatomy such as greater brain volume,

head or intracranial size (cerebral or brain reserve, e.g., Katzman et al., 1988), or by differences in the ability to use brain networks in an effective way (cognitive reserve, Stern, 2002), namely efficiency, capacity or compensation (Stern 2009). Thus, brain reserve implies quantitative differences of anatomical substrate while cognitive reserve implies variability at the level of brain networks (Stern, 2009). In the field of Alzheimer's disease research, evidence suggests that reserve delays the clinical manifestations of dementia (Stern 2002). This has important implications including delayed diagnosis and consequently faster progression (Stern 2002) and shorter duration of diagnosed disease before death (Stern et al., 1995). Thus, the understanding of the mechanisms underlying the protective effect of education is a topic of major interest as it could be a key to delay the onset of dementia. In this line, several studies in Alzheimer's disease patients observed inverse relationships between indicators of reserve and brain measures (see Bartrés-Faz and Arenaza-Urquijo, 2011 for a review). This would be consistent with the idea that reserve allows to compensate or to better tolerate pathological effects, but do not provide evidence regarding the underlying neuroprotective mechanism. Hence, studies in healthy elderly are particularly interesting as they could provide information about education-related neuroprotective mechanisms associated with healthy brain aging, rather than about compensatory mechanisms when the pathology has already impacted the brain. The previous literacy on this topic however focused on pathologic populations while studies in healthy elders remain relatively rare (see Bartrés-Faz and Arenaza-Urquijo, 2011 for a review).

From a structural point of view, although negative relationships (Arenaza-Urquijo et al., 2011; Bastin et al., 2012; Coffey et al., 1999; Querbes et al., 2009) or null findings (Christensen et al., 2009; Seo et al, 2011) have also been reported, several neuroimaging studies in cognitively healthy elders highlighted positive relationships between brain

measures and education. Thus, greater gray matter volume and cortical thickness in temporoparietal areas (Foubert-Samier et al., 2012, Liu et al., 2012), larger cortical thickness in orbitofrontal lobe (Liu et al., 2012) as well as greater white matter volume in regions connecting these latter areas (Foubert-Samier et al., 2012) and decreased mean diffusivity in the bilateral hippocampus (Piras et al., 2011) have been found in elderly with greater education.

From a dynamic or functional point of view, several studies have investigated the effect of education by using measures of brain activity while cognitively healthy elderly individuals were performing a cognitive task ($H_2{}^{15}O$-PET and fMRI studies) or at rest (FDG-PET studies). Studies investigating cerebral metabolism at rest showed a negative association between a proxy of reserve combining education and intelligence and metabolic activity in temporoparietal areas (Bastin et al., 2012) or failed to show any influence of education (Perneczky et al., 2006, Scarmeas et al., 2003a). By contrast, functional studies during memory tasks showed functional reorganization of brain networks (compensation) in healthy elders with higher education compared to young individuals (Scarmeas et al., 2003b; Springer et al., 2005), and more efficient or optimal patterns of brain activation in elders with higher reserve proxies compared to elders with lower reserve proxies (Bosch et al., 2010; Solé-Padullés et al., 2009).

Finally, there has been no study to date assessing both structural and functional brain changes with education. This is important yet to highlight the relative contribution of education to cognitive and brain reserve as well as to provide a further understanding of the role of this factor in healthy brain aging. In a previous study, we failed to evidence positive relationships between a reserve proxy (combining verbal intelligence and education) and FDG-PET or resting state functional connectivity while inverse relationships were found (Bastin et al., 2012). We hypothesize that the selection of a

population of healthy elders based on strict criteria including amyloid imaging to exclude preclinical Alzheimer's disease cases would allow highlighting positive relationships. This hypothesis has found support in two recent works using either structural or functional (FDG-PET) imaging and highlighting inverse relationships in cognitively normal elders with normal *versus* pathological amyloid deposition (Arenaza-Urquijo et al., 2013, Ewers et al., 2013). With the aim to further understand the mechanisms underlying the positive effect of education on structural and functional brain measures, we thus selected a population of healthy elders based on both neuropsychological evaluation and cerebral amyloid imaging. We then assessed the relationships between education and gray matter volume, brain metabolism and resting state functional connectivity, expecting to find greater volume and/or metabolism or increased functional connectivity with increased education.

2. Material and methods

Thirty-nine right-handed healthy elders (mean age (±SD): 67.14 (±5.45), range 60-80; mean MMSE (±SD): 29.33 (±0.96), range 27-30; 24 women) were recruited after detailed clinical and neuropsychological examinations, part of which (25/39) were also included in Bastin et al. (2012). Neuropsychological testing included memory, language, executive functions, visuo-spatial functioning and praxis. All reported scores were in the normal range (i.e. within 1.65 standard deviation of the normal mean for age, gender and education). Subjects were screened for the lack of abnormalities based on stringent inclusion/exclusion criteria: (1) normal somatic examination, (2) body mass index in the normal range (i.e., 18.5-25), (3) no known vascular risk factor and smoking less than 10 cigarettes per day, (4) no alcohol or drug abuse, (5) blood pressure within normal limits (6) no history or clinical evidence of neurological disease, dementia or psychiatric disorder, (7) no current use of medication likely to interfere with cognitive

or functional imaging measurements (except birth control pills, oestrogen replacement therapy and anti-hypertensive drugs), (8) normal standard T1 and T2-weighted MRI as assessed by a medical doctor. Moreover, individuals were also screened for the presence of amyloid deposition using florbetapir-PET imaging. They were classified as amyloid-negative or amyloid-positive using a neocortical standardized uptake value ratio cut off value of 1.1 (see La Joie et al., 2012 for the full description of the procedure). Of the initial 39 subjects involved in the study, three subjects with a positive florbetapir-PET scan (i.e., amyloid-positive) were excluded. All the analyses described below were conducted on the 36 remaining amyloid-negative healthy elderly.

The study was approved by the regional ethics committee and all subjects gave their informed consent**.**

2.3. Assessment of years of education

Years of education were assessed as years attending school (mean (± SD), 11.69 (± 3.45), range 7-20). Years of education and age were not correlated (p=.60).

2.4. Imaging protocol

All participants were scanned on the same MRI and PET scans at the CYCERON center (Caen, France). Ten out of the 36 participants only had the T1-weighted MRI scan, while the remaining 26 participants had a structural MRI scan, a FDG-PET scan and a resting-state fMRI scan. All assessments were obtained within 2 months from neuropsychological evaluation.

Structural MRI data. For each participant, a high-resolution T1-weighted anatomical image was acquired on a Philips (Eindhoven, The Netherlands) Achieva 3T scanner using a 3D fast field echo sequence (3D-T1-FFE sagittal, TR = 20 ms; TE = 4.6 ms; flip angle = 20°; 170 slices; slice thickness = 1 mm; FOV = 256x256 mm^2; matrix = 256x256).

PET data. Both FDG and florbetapir scans were acquired on a Discovery RX VCT 64 PET-CT device (General Electric Healthcare) with a resolution of 3.76x3.76x4.9 mm (FOV= 157mm). Forty-seven planes were obtained with a voxel size of 2.7x2.7x3.27 mm. A transmission scan was performed for attenuation correction before the PET acquisition.

FDG-PET. Participants were fasted for at least 6h before scanning. After a 30-min resting period in a quiet and dark environment, ≈200 MBq of FDG were intravenously injected as a bolus. A 10-min PET acquisition scan began 50-min post-injection.

Florbetapir-PET. Each participant underwent a 20 min PET scan, beginning 50 minutes after the intravenous injection of ≈ 4 MBq/kg of florbetapir (see La Joie et al., 2012).

Resting-state fMRI. Scans were acquired on a Philips (Eindhoven, The Netherlands) Achieva 3T scanner using an interleaved 2D T2 Star EPI sequence designed to reduce geometrical distortions by using parallel imaging, shorter echo time and smaller voxels (Villain et al., 2010), with a resolution of 2.8x2.8x2.8 mm, TR = 2.382 s; TE=30ms; Flip angle :80°, 44 slices, slice thickness= 2.8 mm ; no gap ; matrix = 80 x 80 ; FOV=224x224 mm^2 ; in plane resolution= 2.8x2.8 mm^2 ; 172 volumes per run. During this acquisition, subjects were asked to relax, lie still in the scanner and keep their eyes closed while not falling asleep.

2.5. Image preprocessing

All the images were pre-processed using Statistical Parametric Mapping 5 (SMP5) software (Wellcome Department of Imaging Neuroscience, Institute of Neurology, London, England) running on MATLAB 7.1. To allow the multimodal comparison of the results, the following procedure was carried out, similar to the one used in previous

studies from the laboratory (Chételat et al., 2008; Villain et al., 2010), including: (i) correction of PET data for partial volume effects (PVE) (ii) use of the same spatial normalization parameters for the MRI and PET data set, and (iii) use of a differential smoothing for each modality (see below) to equalize the resultant smoothness and obtain identical resolution for the three imaging modalities (Richardson et al., 1997, Van Laere and Dierckx, 2001).

Structural MRI data. T1-weighted images were pre-processed using the VMB5.18 toolbox (Structural Brain Mapping Group, Christian Gaser, Department of Psychiatry, University of Jean, Germany) and the optimized VBM protocol described in detail elsewhere (Good et al., 2001). MRI data were iteratively segmented and normalized with the bias correction option and the application of Hidden Markov Field weighting to enhance the accuracy of segmentation. Then the spatially normalized gray matter segments were modulated correcting for the effects of non-linear warping (but not affine transformation) so that brain size variation is already taken into account and intracranial total volume is not needed to be controlled for. These images were smoothed at 10 mm full width at half maximum (FWHM), and finally entered into the multiple regression model.

PET data. FDG-PET data were first corrected for PVE using the voxel-by-voxel method proposed by Müller-Gärtner et al. (1992) and modified by Rousset et al., (1998) as implemented in the PMOD software (PMOD Technologies Ltd., Adliswil, Switzerland). PVE-corrected PET images were then coregistered onto corresponding MRI, normalized using the normalization parameters defined from the corresponding MRI scan and scaled using the mean PET value of the cerebellar gray matter (in order to control for individual variations in global PET measures). Resultant maps were then smoothed using a Gaussian kernel of 9.3x9.3x8.7 (resulting in a smoothness equivalent

to that of the MRI images smoothed at 10mm) and masked to exclude non-gray matter voxels.

Resting-state fMRI. First, datasets were checked for the lack of artefact due to head movements (> 3 mm translation or 1.5 degree rotation) or of abnormal variance distribution through the application of the TSDiffana routine (http://imaging.mrccbu.cam.ac.uk/imaging/DataDiagnostics). EPI volumes were then corrected for slice timing and realigned on the first volume. Data were then spatially normalized using a technique designed to reduce geometrical distortions effects (Villain et al., 2010). Briefly, this procedure includes for each individual (1) a coregistration of the mean EPI volume, non-EPI T2*, T2 and T1 volumes, (2) a warping of the mean EPI volume to match the non-EPI T2* volume, (3) a segmentation of the T1 volume using the VBM 5.1 'Segment' procedure with the International Consortium for Brain Mapping (ICBM)/Montreal Neurological Institute (MNI) priors, (4) a normalization of the coregistered T1, EPI, and non-EPI T2* volumes using the parameters obtained from the T1 segmentation, (5) a 4mm FWHM smooth of the EPI volumes. Finally, a binary mask was created from the group segmented mean gray matter T1 volume in conjunction with the mean non EPI-T2* volume in the MNI space (including only voxels with values above .25 in both mean images). This mask was used in further analyses to include only the voxels of interest (i.e. located in the gray matter and showing a signal on the EPI-T2* scan).

For the purpose of functional connectivity analyses, the regions showing a significant correlation with years of education from both the structural MRI and the functional FDG-PET analyses (see below) were selected using a conjunction mask and used as seeds. The individual mean time-courses were extracted for each of these regions/seeds, and individual functional connectivity maps were computed for each seed

according to the following steps: i) a bandpass filter (.01-.08 Hz) was applied to the time series of each seed to remove low and high-frequency drift components of resting fMRI data (Chételat et al., 2013; Mevel et al., 2013); ii) regression of the six parameters generated from realignment of head motion as well as the white matter and cerebrospinal fluid time courses was performed ; iii) the positive correlation coefficients between the averaged time course in each seed region and the time course of each voxel across the whole gray matter using the T1-non EPI T2* mask was computed. A Fisher's z transform, as well as an 8 FWHM smooth, were then applied to the resulting individual connectivity maps and the resulting images were entered into multiple regression analyses in SPM5.

2.6. Statistical analyses

Statistical Package for Social Science (SPSS) for Windows (V.17.0) was used for the demographical data. Results were considered significant at $p<.05$ and when necessary correction for multiple comparisons was carried out.

For VBM analyses, correlations were computed between years of education and the smoothed modulated normalized gray matter segments, using the multiple regression voxelwise analysis of SPM5. In line with the main objectives of the present study, we focused on the positive correlations (for example, higher gray matter volume being related to higher years of education). However, the results for the reverse contrast (negative correlations) were also reported for the sake of completeness. The same analysis was performed for PET images. As explained before, seed-connectivity analyses (Biswal et al., 1995; Fox et al., 2005) were carried out based on the overlapping regions of both former analyses (gray matter volume and PET). These connectivity maps were then regressed against years of education. All the analyses were controlled for age and gender. A p (uncorrected) <.005 threshold was used for all voxel-

based analyses. In order to consider only the larger clusters a cluster size of k>1500 was used for all the analyses.

3. Results

Correlations between gray matter volume and years of education

Significant correlations were found between years of education and the volume of the right superior temporal gyrus, left insular cortex and bilateral anterior cingulate gyrus (see Figure 1, Table 1). Two clusters located in the bilateral lingual gyrus were found in the reverse contrast (negative correlation).

Correlations between brain metabolism and years of education

Years of education positively and significantly correlated with the metabolism of the anterior cingulate cortex (see Figure 2, Table 1). Nothing significant was found in the reverse contrast (negative correlation).

Areas where both gray matter and metabolism were related to years of education

The overlapping region in the two previous analyses included a single cluster located in the anterior cingulate gyrus (see Figure 3, Table 1). This region was used as a seed for the connectivity analyses as explained above (see methods).

Seed-connectivity analyses: correlation with years of education

The anterior cingulate functional connectivity maps were thus obtained and correlated with years of education. Significant positive correlations were found between years of education and the connectivity of the anterior cingulate cortex with the right hippocampus, right posterior cingulate, left inferior frontal lobe and left angular gyrus (Figure 4). Nothing significant was found in the reverse contrast (negative correlation).

Complementary analyses: Relationships with cognitive performances

These results led to conduct complementary analyses so as to assess whether education-related increase in anterior cingulate functional connectivity would be associated with an increase in cognitive performance. For this purpose, two cognitive domains particularly relevant in aging and reserve studies were selected: memory (Buckner, 2004) and executive functions (Buckner, 2004; Siedlecki et al., 2009; Stern et al., 2008). Verbal episodic memory was assessed using the 16-word delayed recall from Grober & Buschke (Grober and Buschke, 1987; Van der Linden et al., 2004). Semantic memory capacity and access to lexical memory and retrieval were evaluated using the phonological ("P" letter) and semantic ("animals" category) word fluency task (Cardebat et al., 1990). Finally, shifting and inhibition capacities were assessed using the Trail Making Test B (TMT, Army Individual Test Battery, 1944) and the Stroop test (Stroop, 1935), respectively. Firstly, partial correlations between education and cognitive scores introducing age and sex as covariates were performed using two-tailed Pearson's correlation in SPSS. Secondly, two-tailed Pearson's correlation was computed between the cognitive scores and the connectivity values extracted from the regions showing a significant effect of education in the seed-based connectivity analyses (i.e. between the anterior cingulate cortex and the righ hippocampus, right posterior cingulate, left inferior frontal lobe and left angular gyrus). Results were considered as significant when $p<.01$ (.05/5, after correcting for multiple comparisons, i.e., five cognitive tests).

Higher years of education were related to better performances in almost all tests, with correlations with semantic and phonological fluencies and Stroop test surviving correction for multiple comparisons. Moreover, the anterior cingulate cortex connectivity correlated positively with phonological fluency performance for all regions (as a trend for the inferior frontal gyrus), with semantic fluency for the hippocampus

and as a trend for the inferior frontal gyrus, and trends as observed for a positive correlation with episodic memory performance for the hippocampus and the right posterior cingulate cortex (see Table 2 for details).

4. Discussion

This is the first study assessing the association between education and both structural and functional brain imaging measurements in healthy elders selected using both neuropsyschological evaluation and cerebral amyloid assessment (i.e., presenting normal cognition and a negative florbetapir-PET scan). It highlights a significant positive relationship between education and gray matter volume and metabolism in similar brain areas, as well as between education and functional connectivity in turns related with improved cognitive performances. More specifically, more educated elderly showed both greater gray matter volume and greater brain metabolism in the anterior cingulate cortex, as well as greater gray matter in the right superior temporal gyrus and left insular cortex. Interestingly, the results of the functional connectivity analyses showed that higher education was also associated with an increase in the functional connectivity of the anterior cingulate area with the right hippocampus, right posterior cingulate cortex, left inferior frontal lobe and left angular gyrus, which was itself associated with improved cognitive performance (mainly in phonological fluencies).

Our findings showing greater education-related volume in the superior temporal gyrus and anterior cingulate cortex are consistent with a recent report including 331 non-demented elderly (Foubert-Samier et al., 2012). This study, using voxel-based morphometry analyses, showed an association between education and gray matter volume in large and distributed brain regions mainly including the temporo-parietal and frontal cortices, together with greater white matter volume in areas connecting these regions. Moreover, larger regional cortical thickness in the temporal and insular cortex

has been recently reported in an investigation including 113 healthy controls (Liu et al., 2012). These results are also consistent with those assessing the effect of education together with other potential factors thought to impact reserve (i.e, IQ, physical and social activities) showing higher total gray matter volume (Solé-Padullés et al., 2009) and fronto-parietal volume (Bartrés-Faz et al., 2009) in elderly with higher indexes of reserve. The present study also shows a positive correlation between education and brain metabolism in the anterior cingulate gyrus. As mentioned in introduction, only a few studies to date have assessed the relationship between years of education and brain metabolism in healthy elders. These previous studies did not find any association (Perneckzy et al., 2006; Scarmeas et al., 2003a) or reported only negative associations (Bastin et al., 2012). Similarly, reverse relationships between education and brain structure (Arenaza-Urquijo et al., 2011; Coffey et al., 1999; Querbes et al., 2009; Teipel et al., 2009) have also been reported. The reasons for these differences are unclear. In the present study where individuals were screened for the lack of amyloid deposition in addition to normal cognition, the relationships were almost all positive. It is likely that positive and negative relationships co-exist in different parts of the brain as found for example in subjective memory impairment (Scheef et al., 2012) or recently in high educated prodomal AD subjects (Morbelli et al., 2013) that would be more or less detectable according to the sample and/or the method. Nevertheless, the present study suggests that a more strict definition of healthy aging from both neuropsychological and neuroimaging biomarker perspectives, may facilitate studies investigating mechanisms related to healthy brain aging. Thus, previous differences may reflect the heterogeneity of the samples between studies (for example, the inclusion of amyloid-positive subjects) as well as methodological differences. Still, the existence of positive relationships in specific brain regions as evidenced here is reinforced by the fact that these relationships

were found with both structure and function in the same brain regions, and in regions consistent with previous report also showing a positive association.

The anterior cingulate cortex showed greater gray matter volume and greater brain metabolism in more educated subjects. Using a seed-based functional connectivity approach, we showed that the effects of education on the volume and functioning were paralleled by changes in the functional connectivity. Higher years of education were related with increased connectivity between the anterior cingulate cortex and the right hippocampus, left posterior cingulate, left angular gyrus and left inferior frontal gyrus. Interestingly, the right hippocampus metabolism also positively correlated with years of education when using a lower cluster size threshold (even at $p<.001$, see supplementary figure 1B), and the sensitivity of this structure to education and mental activity has been suggested in previous studies (for example, Valenzuela et al., 2008, Piras et al, 2011, Draganski, 2006).

The association we found between education, cognitive performance and connectivity values provides a support to the interpretation of these findings, as they suggest a link between increased connectivity and improved cognitive function. More specifically, better performances in memory and fluencies were related to increased connectivity of the anterior cingulate cortex, although only fluencies survived multiple comparison correction. This suggests that greater connectivity of this region might represent one of the neural bases of more preserved cognitive performance in more educated elders, and thus, might have an important role in healthy brain aging. This idea is reinforced by a recent study in 258 healthy elders (Rosano et al., 2012) where the anterior cingulate cortex and medial temporal lobe were found to be the neuroimaging correlates of maintaining cognitive function over a period of 10 years. Also, no aging effects (Fjell et al., 2009) or positive aging effects (Salat et al., 2004) have been

reported in the anterior cingulate. These analyses were only mean to show that changes with education in functional connectivity were at least partly related to cognitive changes with education. We do not state that these relationships are specific, neither to the modality nor to the regions studied here (and further analyses rather suggest they are not).

Finally, in the field of cognitive reserve, it has been suggested that control processes may be a relevant component of reserve (see Stern et al., 2008). This is reliable with the idea that the anterior cingulate cortex integrates input from various sources and contributes to the regulation of processing in other brain regions (see Bush et al., 2000; Shackman et al., 2011).

Some limitations of this study need to be addressed. First, our sample size was relatively small. However, the sample was selected with stringent criteria and all individuals were assessed in the same centre. Second, the use of a relatively permissive threshold may have led to false positives. We are however confident in our findings as i) the results survived a p cluster-level corrected threshold and would also survive a more stringent $p<.001$ threshold (see supplementary data) ii) the results are consistent among modalities and iii) the results are consistent with those of previous studies that reported positive correlation with education. Third, the cross sectional design of the study is a further limitation as innate differences and birth years (cohort effect) are not controlled (although there was no association between years of education and age). Furthermore, we did not record information about hormone replacement therapy, especially in women, which is thought to affect a variety of cognitive functions (see Pompili et al., 2012). Also, we are aware that multiple regression models do not assess causation, i.e. this study does not allow to conclude on the causality between education, functional connectivity and cognition. Finally, different findings may have been

obtained with a different proxy of reserve. We decided to use only education (instead of a composite measure) as the proxy of reserve because different proxies may have different effects in terms of brain regions and in the direction of the relation (positive versus negative), so this seems as the most appropriate choice in the context of the present study. For example, a recent study showed that among other variables (i.e. occupation and leisure activities) education was the only measure associated with brain structure (Foubert-Samier et al., 2010), and another study (Pillai et al., 2012) showed that education was not related to brain regions associated to intelligence.

In summary, the main finding of this cross-sectional study in healthy elders is that education relates to both gray matter structure and metabolism, with increases related and paralleled by greater functional connectivity, itself associated with improved cognitive performances. Over and above structural and functional changes in anterior cingulate and parietal and temporal areas, reinforcement of the connectivity between these structures appears as one of the mechanisms underlying education-related reserve in healthy elders. Further studies are needed to explore whether and how these findings could be exploited in brain disease prevention programs.

4. Acknowledgments

This study was funded by Agence Nationale de la Recherche (ANR LONGVIE 2007), Programme Hospitalier de Recherche Clinique (PHRC National 2008), Région Basse Normandie, and Institut National de la Santé et de la Recherche Médicale (Inserm). E.M.A.U was funded by a predoctoral grant from the Generalitat de Catalunya. DB-F was supported by a Spanish Ministerio de Ciencia e Innovación research grant (SAF2009-07489). The authors gratefully acknowledge M. Fouquet, N. Villain, Vincent de La Sayette, S. Egret, A. Pélerin, C. Schupp, J. Dayan, A. Quillard for their role in this study.

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Fig 1. Brain areas and scatter plots showing the results of the voxel-wise multiple regression between years of education and gray matter volume (p uncorrected< .005, k> 1500). Details of the peaks are given in Table 1. R: right, L: left.

Fig 2. Brain areas and scatter plots showing the results of the voxel-wise multiple regression between years of education and brain metabolism (p uncorrected< .005, k> 1500). Details of the peaks are given in Table 1. R: right, L:left.

Fig 3. Sagittal views showing the overlap between gray matter volume and metabolism results: areas showing positive association with education on gray matter volume (red), metabolism (blue) and both volume and metabolism (purple).

Fig. 4. Sagittal and coronal views and scatter plots of the voxel-wise multiple regression between education and anterior cingulate functional connectivity (p uncorrected< .005, k> 1500). Details of the peaks are given in table 1. R: right, L: left.

Supplementary, Figure 1. Sagittal views showing the results of the voxel-wise multiple regression models (corrected for age and sex) for gray matter volume (A, in red), metabolism (B, in blue) and functional connectivity (C, in green). P (uncorrected) <.001 and K>300.