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Title

Relationships between years of education, regional grey matter volumes, and working memory-related brain activity in healthy older adults

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Abstract

The aim of this study was to examine the relationships between educational attainment, regional grey matter volume, and functional working memory-related brain activation in older adults. The final sample included 32 healthy older adults with 8 to 22 years of education. Structural magnetic resonance imaging (MRI) was used to measure regional volume and functional MRI was used to measure activation associated with performing an n-back task. A positive correlation was found between years of education and cortical grey matter volume in the right medial and middle frontal gyri, in the middle and posterior cingulate gyri, and in the right inferior parietal lobule. The education by age interaction was significant for cortical grey matter volume in the left middle frontal gyrus and in the right medial cingulate gyrus. In this region, the volume loss related to age was larger in the low than high-education group. The education by age interaction was also significant for task-related activity in the left superior, middle and medial gyri due to the fact that activation increased with age in those with higher education. No correlation was found between regions that are structurally related with education and those that are functionally related with education and age. The data suggest a protective effect of education on cortical volume. Furthermore, the brain regions involved in the working memory network are getting more activated with age in those with higher educational attainment.

Keywords

Educational attainment; working memory; n-back task; functional magnetic resonance imaging; voxel-based morphometry; aging.

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Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent: Informed consent was obtained from all individual participants included in the study.

1. Introduction

Age is associated with a range of neural changes that concern both brain structure and brain function. Studies using voxel-based morphometry (VBM) for the analysis of grey matter (GM) volume have consistently reported age-related GM reduction in frontal, temporal, and parietal regions (Curiati et al. 2009; Good et al. 2001; Kalpouzos et al. 2009; Terribilli et al. 2011; for a review, see Matsuda 2013). A large number of studies have examined age-related changes in brain function and in task-related activation (see Li et al. 2015 for a meta-analysis). Many of them have focused on changes in working memory-related brain activation patterns, as age is associated with pronounced deficits in working memory (Dobbs and Rule 1989; Foos and Wright 1992; Salthouse et al. 1991; Wingfield et al. 1988). Functional neuroimaging studies using either positron emission tomography (PET) or functional magnetic resonance imaging (fMRI) reported a decreased activation of the dorsolateral prefrontal cortex (Reuter-Lorenz et al. 2000; Rypma & D'Esposito 2000) and an increase in the bilateral activation of frontal regions in older adults during working memory tasks (Reuter-Lorenz et al. 2000; Mattay et al. 2006).

However, although many studies have investigated the effects of age on brain structure and function, less is known about the impact education has on them in older adults. Educational attainment is considered to be a robust proxy of cognitive reserve. The concept of reserve was proposed to account for the lack of a systematic relationship between the amount of brain damage and the magnitude of symptoms. It was proposed that the inter-individual variability regarding the impact brain lesion has on clinical symptoms was related to differences in the amount of reserve. Reserve would thus reflect the individual's own brain ability to cope with disease-related or age-related neural changes in order to delay or minimize cognitive manifestations (Stern, 2002; 2012). Structural neuroimaging studies have reported contradictory results on the relationship between global brain volume measures and education in healthy older adults. Educational attainment was 1) negatively correlated with manual measures of peripheral cerebrospinal fluid volume (Coffey et al. 1999), 2) not correlated with cerebrospinal fluid volume (Kidron et al. 1997) or with total intracranial volume (TIV), according to a 4-year longitudinal cohort study of older adults aged 60-64 years at baseline (Christensen et al. 2009). Discordant results might be due to the fact that only some brain regions differ as a function of education. For instance, studies that have examined regional volume reported that higher educational attainment was associated with more GM volume in the right superior temporal gyrus, left insula, and bilateral anterior cingulate cortices (Arenaza-Urquijo et al. 2013), in the dorsomedial prefrontal and left anterior cingulate cortices (Rzezak et al., 2015), and more broadly in the temporo-parietal and frontal regions (Foubert-Samier et al. 2012; Steffener et al., 2016). Also, the effect may vary with age. There is a possibility that education has a maintenance role and contributes to protect against age-related decline. If this is the case, education's positive effect may only be visible later in life, i.e., in older samples of older adults. Interestingly, some behavioral results support that hypothesis. An interaction between age and education was found for tasks engaging working memory (Cordière, Cloutier & Belleville, 2016) and episodic memory (Adam, Bonsang, Grotz and Perelman, 2013) showing that the positive effect of education was only present in late old age. Considering that age-related regional GM atrophy may account for age-related cognitive decline, it may explain why Christensen et al. (2009) did not find this effect in an epidemiological sample in early old age. The use of a younger group of older individuals might preclude observation of such effect. A few functional neuroimaging studies have found evidence indicating that educational attainment is associated with differences in the brain's ability to recruit functional neural networks (Habeck et al. 2003; Scarmeas et al. 2003; Springer et al. 2005; Stern et al. 2005). Two of these studies reported larger recruitment of frontal and posterior regions in the older adults with higher levels of education compared to those with lower levels of education when performing a recognition memory task (Scarmeas et al. 2003; Springer et al. 2005). One study reported that working memory was associated with greater activation in parietal regions in older adults with higher education relative to those with lower education levels (Haut et al. 2005). In contrast, two other fMRI studies reported negative correlations between educational/occupational attainment (combined with IQ and social activities) and memory task-related brain activation in frontal regions (Bartrès-Faz et al. 2009; Solé-Padullès et al. 2009).

The diverging functional results might be due to differences in the relationship between educational attainment and activation as a function of the level of task demand, or to differences in the relationship between educational attainment and age. Many models have proposed an inverted U-shaped function between brain activation and resource capacities (Callicott et al. 1999; Reuter-Lorenz 2002; Mattay et al. 2006). A similar pattern was proposed in the compensation related utilization of neural circuits' hypothesis (CRUNCH, Reuter-Lorenz & Cappell 2008; Schneider-Garces et al. 2010). The model proposes that older adults engage more neural circuits than young adults in order to meet task demands but that as task demand increases, older adults reach resource ceiling and additional neural circuits can no longer be recruited. Therefore, they are more likely than young adults to show over-activations at lower levels of task demand, and under-activations at higher levels (Cappell et al. 2010). A parallel effect may be anticipated between people with different levels of education: those with lower education might reach their brain responsivity limits sooner than the more educated ones. Interestingly, an fMRI study exploring the impact of individual differences on working memory-related brain patterns reported distinctive load-related brain patterns between low and high older performers. Low performers showed increased activation from low to intermediate levels of task demand, followed by decreased activation from intermediate to high levels, whereas high performers only showed increased activation from low to high levels of task demand (Nagel et al. 2009). It is thus critical to examine more systematically the relationship between task load and activation in older adults with high vs. low levels of education. Furthermore, little is known about the impact of age on brain activation in older age and how educational attainment interacts with age or modulates its effect. Aging covers a period that can span decades and marked cognitive changes occur from early to late old age (Borella, Carretti & De Beni, 2008; Hale et al., 2011). Education might reduce the detrimental effect of age on activation hence having a protective effect. One other possibility is that education is associated with compensatory activation differences. These compensatory effects are more prone to occur at an older age when biological aging is more likely to have its largest detrimental impact. The protective vs. compensatory effects can be assessed by examining whether the relationship between education and activation varies with age. However, the predictions are different: In the former case, one expects that the interaction would arise from the fact that individuals with less education would show a negative age-related activation effect whereas those with more education would show no age-difference on activation. If the interaction is due to compensatory processes occurring in those with higher education, one would expect that only individuals with high education would show age differences on activation.

There is also a need to better understand the relationship or lack of between education-related structural and functional differences. One important question is whether regions with education-related activation differences are also those showing differences in volume or in contrast, regions that are structurally unaffected by education. Another is to assess whether structural differences account for differences in activation. This was suggested in a study showing that a negative correlation between cognitive reserve and working memory-related brain activation disappeared after adjusting for regional brain volumes (Bartrès-Faz et al. 2009). If confirmed, this result would suggest that differences in brain volume might account for many of the education-related activation differences.

In the present study, we investigated the relationship between educational attainment and 1) regional GM volume and 2) working memory-related brain activity in healthy older adults. We used a working memory task for several reasons. Working memory is a critical component of cognition and was suggested to determine inter-individual differences on complex cognitive abilities including reasoning, language and problem solving in both younger and older adults. Many models have suggested that changes in working memory capacity accounts for much of the age-related cognitive decline. Working memory is thus a compelling cognitive target for studies interested in identifying the brain correlates of inter-individual differences in cognitive reserve. Among the different working memory tasks, the n-back task was considered to be particularly interesting. Considerable research has been done with this task and its neuroanatomical substrates are well known. The n-back task typically involves activation of specific working memory-related cortical regions such as the lateral premotor cortex, the dorsal cingulate and medial premotor cortex, the prefrontal cortex, the frontal poles and the posterior parietal cortex (for a meta-analysis, see Owen et al. 2005). Also, as the level of load increases, greater activation is found in the frontal (e.g. Braver et al., 1997; Jonides et al., 1997) and parietal regions (e.g., Jonides et al., 1997). Hence, by allowing a parametric manipulation of its load, the task is particularly well suited to assess whether the effect of education on activation varies as a function of task demand.

We also assessed whether educational attainment buffers the negative effect of age on brain structure and examined whether the relationship between education and activation varies with age by entering it as an interaction term in the analyses. Using structural MRI for regional brain volume and fMRI with an n-back task and two levels of load, we performed correlational analyses between years of education and 1) regional GM volume and 2) n-back-related brain activation. Similar analyses were done using education-by-age as a term of interaction. Combining structural and functional neuroimaging techniques in the same design allowed us to examine the relationship between structure and function. Thus, we first looked at the overlap between the education-related differences associated with regional volume and those associated with working memory-related brain activation. Second, we examined if education-related regional GM volume differences accounted for the impact of education on the working memory-related brain activation pattern.

It was hypothesized that educational attainment would be positively related to GM volume in frontal, temporal, and parietal regions. At the functional level, working memory brain activation patterns were expected to differ as a function of educational attainment and to be modulated by task load. Age was expected to modify the effect of education on brain structure and function. Finally, we anticipated that some correlation would be found between education-related differences in structure and function, although we did not expect that controlling for brain structure would entirely remove the impact of education on working memory-related brain activity.

2. Methods

2.1. Participants

Forty healthy older adults were recruited from the “banque de participants” of the research center of the Institut universitaire de gériatrie de Montréal and via advertisements in senior centers. All participants were French-speaking and community-dwelling individuals living in the Montreal area, were right handed, and had normal or corrected-to-normal vision. Participants were tested on a range of clinical tests including the digit span subtest of the Wechsler Adult Intelligence Scale - III (Wechsler, 1997) and the Trail Making Test A and B (Adjutant General's Office, 1944). Their mood and cognition were briefly measured with the Geriatric Depression Scale (GDS, Yesavage et al. 1985) and the Montreal Cognitive Assessment (MoCA, Nasreddine et al. 2005) respectively. Exclusion criteria were alcoholism or substance abuse; presence or history of a neurological disorder or stroke; presence or history of a severe psychiatric disorder; general anesthesia in the past six months and presence of any medication that could affect cognitive or cerebral functioning. Participants were excluded if their MoCA score was below the cut-off stratified by age and educational attainment for the North American population (Rossetti, Lacritz, Cullum & Weimer, 2011) to exclude participants with mild cognitive impairment (MCI) or dementia. Furthermore, participants with a GDS score above 5/15 were excluded. Eight participants were excluded because reaction times (RT) and accuracy were not recorded due to a technical issue that occurred with the response box. The demographic and clinical characteristics of the 32 remaining participants (25 women) are presented in Table 1. Years of education ranged from 8 to 22 years and age ranged from 60 to 84 years old. The local ethics committee approved of this experiment, and each participant provided informed written consent.

Please insert Table 1 about here

2.2. N-back paradigm

A verbal n-back task was used for the fMRI assessment. The stimulus material consisted of letters that were presented in white on a black background in the center of a screen. Participants were shown a sequence of letters and were asked to decide for each one whether it matched the letter that preceded it by n places in the sequence, i.e., the last letter for the 1-back condition or the letter presented two letters back for the 2-back condition.

Participants performed the task in a blocked design with five runs of three blocks (baseline, 1-back and 2-back conditions). Each n-back block was composed of an instruction (6 s) and the 1-back or 2-back conditions (72 s). The third block was composed of an instruction (3 s) and a baseline condition (cross fixation, 36 s). Each n-back condition consisted of 24 trials (8 of which were targets) and each trial (3 s) was composed of a stimulus (2.5 s) followed by a crosshair during inter-stimulus intervals (0.5s). Each run started with the baseline condition and continued with both n-back conditions. No feedback was given. The tasks were implemented using E-prime software (Psychology Software Tools, Inc.). Stimuli were presented using a projection system (Epson, EMP-8300) and were visible to participants in a mirror attached to the head coil. Responses were given on fiber-optic response pads (Brain Logics, BLBRS-FO-A). Before we started scanning, a short practice sequence for each n-back condition was carried out outside of the scanner to ensure that the instructions were understood.

2.3. MRI and fMRI parameters

Participants underwent structural and functional magnetic resonance imaging (MRI) examinations on a Siemens TIM Trio 3T MRI system (Siemens Medical Solutions, Erlangen, Germany), using the Siemens 12-channel receive-only head coil at the neuroimaging unit of the research center of the Institut universitaire de gériatrie de Montréal (http://www.unf-montreal.ca/siteweb/Home_en.html). The structural MRI images were acquired using a high-resolution T1-weighted MPRAGE sequence (TR: 2300 ms /TE: 2.98 ms, flip angle: 9°, FOV: 256 x 256 mm, 176 slices, voxel size 1 mm^3 , matrix size: 256x 256 pixels). Blood oxygen level-dependent (BOLD) signal was acquired using a standard T2*-weighted gradient echo EPI sequence (TR: 3000 ms, TE: 30 ms, flip angle: 90°, FOV: 192 x 192 mm , 38 slices, voxel size 3 mm^3 with a gap of 0.6 mm-distance factor [20%], matrix size: 64 x 64 pixels). Acquisition was in axial orientation co-planar with AC-PC, whole-brain coverage. Order of acquisition was ascending. The functional images were acquired in one run, and the first three volumes were automatically discarded by the fMRI scanner.

2.4. MRI image preprocessing

A voxel-based morphometry (VBM, Ashburner & Friston 2000) analysis was performed on structural imaging data in MATLAB 7.1.2 (http://www.mathworks.com), using the statistical parametric mapping (SPM12) software (http://www.fil.ion.ucl.ac.uk/spm). Before preprocessing, the T1-weighted images were manually reoriented along the anterior–posterior commissure line, and the anterior commissure was set as the origin of spatial coordinates. Then, the standard segmentation option was used to segment images into GM, white matter, and cerebrospinal fluid (Ashburner and Friston 2005). Next, Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL, Ashburner 2007) was applied on segmented images to generate a study-specific template, which improves the inter-subject alignment of smaller inner structures during normalization (Klein et al. 2009). The template was registered to standard Montreal Neurological Institute (MNI) space and used to generate Jacobian scaled modulated grey and white matter images from each subject, which were spatially normalized to MNI space and smoothed with an isotropic Gaussian kernel of 8-mm full width at half maximum (FWHM).

2.5. MRI statistical analysis

To assess the effect of education and how educational attainment modulates the effect of age on GM volume, two separate whole-brain multiple regression analyses were conducted. First, the effect of education was assessed with the years of education as the covariate of interest. Second, considering that education may modulate the effect of age on GM volume, the analysis was run with the term resulting from the interaction of years of education and age as the covariate of interest. Then, to verify the effect of age on GM volume, the same analysis was run using years as the covariate of interest. Gender was entered as nuisance variable to adjust for its effect on regional brain tissue volumes. GM volume analyses were corrected for total intracranial volume (TIV) by using the global values to proportionally scale the original voxel values. TIV was calculated for each subject by summing the voxel values of the GM, white matter, and cerebrospinal fluid segmentations of each image. GM volume analyses were masked to optimize sensitivity and exclude false positives outside GM tissue, with an explicit threshold GM mask created using the Masking toolbox (Ridgway et al. 2009). The statistical significance threshold was set to $p < 0.001$ uncorrected. Only clusters that survived an extent threshold of $k = 100$ voxels were considered. Results are visualized using xjView toolbox (http://www.alivelearn.net/xjview). Analyses of variance (ANOVA) were used to further explore the source of the interaction when present. To create dichotomous independent variables, participants were divided based on a median split for age (age < 67 and ≥ 67 years), and years of education (education < 16 and ≥ 16 years) and the ANOVA was run using the regional volume found from the interaction as the dependent variables.

2.6. *fMRI image preprocessing*

Functional imaging data were analyzed in MATLAB 7.1.2 (http://www.mathworks.com), using the statistical parametric mapping (SPM8) software (http://www.fil.ion.ucl.ac.uk/spm). First, images were motion corrected, the temporal processed volumes of each subject were realigned to mean volume to remove the head motion, and subjects with more than 3 mm of translation in x, y, or z axis and 1u of rotation in each axis were removed. Second, images were slice-time corrected to the middle slice using SPM8's Fourier phase shift interpolation. Third, images were co-registered with each subject's anatomical MRI image. Fourth, images were spatially normalized to the echo-planar imaging (EPI) template via their corresponding mean image, resliced by 3 mm^3 voxels and smoothed with a Gaussian kernel of 9-mm FWHM.

2.7. fMRI statistical analysis

The first level of statistical analysis was fixed-effects analysis based on the general linear model (GLM) with a box-car response. GLM analysis was performed using regressors, which were generated by convolving the time course of the condition's onsets and duration with canonical hemodynamic response function (HRF). For both n-back conditions (1-back and 2-back), activation during each condition was contrasted with baseline (cross-fixation). The specific contrasts of interest were [1-back > baseline] for 1-back-induced activation, [2-back > baseline] for 2-back-induced activation, and [2-back > 1-back] for load-induced activation. The resulting set of images was used for a second level of analysis where subjects were treated as a random variable. The significance level for each single contrast was set to $p < 0.05$ after whole-brain correction (family-wise error, or FWE). The clusters of activation were displayed only when they contained more than 50 contiguous voxels.

To assess the effect of education and how it interacts with or modulates the effect of age on task-related activation, two sets of whole-brain multiple regression analyses were conducted. First, separate analyses studying positive and negative correlations between fMRI images and educational attainment were performed with SPM8, adjusting for gender, age, and behavioral performances for the three contrasts of interest. Second, to assess the relation between age and education on task-related activation, regression analyses were conducted with the education-by-age interaction term as the covariate of interest. To perform these analyses, we used the 'multiple regression' model implemented in SPM8. Education was entered as an independent variable and activation as a dependent variable. Results were interpreted if they reached both a voxel-wise threshold of $p < 0.001$ (uncorrected) and a threshold of $p < 0.05$ (corrected) at the cluster level. Analyses of variance (ANOVA) were used to further explore the source of the interaction when present. Dichotomous variables were created for age and education with median split as described above and the ANOVA was run using peak activation on the region(s) of interaction as the dependent variables.

2.8. Combined VBM/fMRI statistical analysis

To control for the effect of regional GM volume, the relationships between education and fMRI brain activity were adjusted for GM volumes in a whole-brain voxel-based manner. A statistical toolbox known as Biological Parametric Mapping (BPM) was used to assess the direct comparison across VBM and fMRI modalities (Casanova et al. 2007). In this model, age, gender, and behavioral performance were also included as control variables. To reduce sensitivity to outliers and heteroscedasticity, we used the software package robust BPM (rBPM) that allows for the inclusion of voxel-wise regressors and implements a robust regression model (Yang et al. 2011).

To assess the relationship between volume and activation difference without the confounding impact of education, – as each is defined by its relationship to education, partial correlation analysis was performed. Proportional regional volume within each region of interest (ROI) that correlates with years of education or education*age interaction was extracted per participant using the MarsBar region of interest toolbox for SPM (Brett et al. 2002). The same procedure was used to extract beta values within each ROI that correlates with years of education or education*age interaction. Correlations between the extracted values from each ROI of VBM and fMRI data were then evaluated and adjusted for years of education or education*age interaction.

3. Results

3.1. *Behavioral n-back data*

Behavioral performance on both n-back conditions was assessed in terms of RT (hits only) and accuracy (proportion of hits minus false alarms; Snodgrass & Corwin, 1988) and is reported in Table 2. Paired *t*-test analyses on behavioral performances between the two n-back conditions revealed significant differences on accuracy and RT. Accuracy was higher and RT were lower in the 1-back condition compared to the 2-back condition, respectively $t(31) = 2.64$, $p < 0.01$ and $t(31) = -5.04$, $p < 0.0001$. No correlation was found between behavioral performance and years of education and there was no effect of the education by age interaction term.

Please insert Table 2 about here

3.2. *Structural MRI data*

Whole-brain voxel-based analyses revealed significant positive correlations between years of education and GM volume in the right medial and middle frontal gyri, the right cingulate gyrus (BA 6, 32), the right inferior parietal lobule (BA 40), and in the right posterior cingulate gyrus and anterior cerebellum (see Figure 1 and Table 3). More education was associated with more volume in those regions. No negative correlations were observed. A second set of whole-brain analyses indicated a significant education by age interaction for the left middle frontal gyrus (BA 46) and the right medial cingulate gyrus (BA 24) (see Figure 2 and Table 4). Significant negative correlations between years and GM volume were also found in the frontal, temporal, parietal and occipital regions (see Table 5). Older age was associated with less volume in those regions. No positive correlations were observed. For both sets of analyses, only the effect of age remained significant in the parietal regions when applying a cluster-wise threshold of $p < 0.05$ (FWE corrected).

Please insert Figure 1 and 2 and Tables 3, 4 and 5 about here

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A 2 (Age) x 2 (Education) factorial ANOVA was performed within each ROI to further explore the source of interaction (see figure 2). In the left middle frontal ROI, the ANOVA confirmed the Age*Education interaction, $F(1, 28) = 8.78$, $p < 0.01$, but there were neither Age, $F(1, 28) = 6.64$, ns, nor Education, $F(1, 28) = 1.38$, ns, main effects. Post-hoc analyses revealed that in the low education group, GM volume was significantly smaller in the old-old than on the young-old group but there was no age effect in participants with high education. In the right middle cingulate ROI, the ANOVA also confirmed the Age*Education interaction, $F(1, 28) = 7.41$, $p < 0.02$, but there were neither Age, $F(1, 28) = 2.19$, ns, nor Education, $F(1, 28) < 1$, main effects. Here again, post-hoc analyses revealed that in the low education group, GM volume was significantly smaller in the old-old than in the young-old group but there was no age effect in participants with high education (see Figure 2).

3.3. fMRI data

Task analysis. Cortical activation associated with both n-back conditions ([1-back > baseline] and [2-back > baseline]) were found in several cortical regions: 1) bilateral temporal regions including the inferior, middle, and superior gyri, and the parahippocampal gyri; 2) bilateral frontal regions including the inferior, medial, middle, and superior frontal gyri, and the precentral gyri; 3) bilateral parietal regions including the inferior and superior parietal lobules, the supramarginal and angular gyri, the precunei and postcentral gyri; and 4) regions of the limbic system including the hippocampi, the amygdalae, and the cinglulate gyrus (see Figure 3 and Table 6). The load effect ([2-back > 1-back]) revealed increased cortical activation from the 1-back to 2-back conditions in bilateral fronto-parietal regions.

Please insert Figure 3 and Table 6 about here

Taking into account education, age, gender, and behavioral performances, we found a significant effect of the education by age interaction for the left superior, middle and medial gyri (BA 9) in the 1-back condition ([x: -39, y: 26, z: 32] *t*: 5.87, $p < 0.001$, k = 192 voxels; see Figure 4). The larger the age, the larger the difference due to education was. No education or interaction effect was found in the 2-back condition. No negative correlations were observed in either n-back condition. No negative or positive correlations were found between years of education and load-induced activation (2-back vs. 1-back) and no significant effect of the education by age interaction on load-induced activation was found

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Please insert Figure 4 about here

An ANOVA was carried out to better understand the education*age interaction. In the 1-back condition, the results confirmed the age*education interaction, *F*(1, 28) = 10.50, $p < 0,005$. None of the main effects were significant (Age: *F*(1, 28) <1; Education: *F*(1, 28) = 1.35, ns). Post-hoc analyses indicated that the age effect was only present in participants with higher education. In those participants, this ROI was significantly more activated in the old-old compared to the young-old group. Furthermore, the education effect was present only in the old-old group (see figure 4).. Participants with more education showed more brain activity than participants with less education in the older group, whereas no education effect was found in the younger group. Of note, a second ANOVA was done to explore the brain activity in the same ROI for the 2-back condition. The results showed an age*education interaction, $F(1, 28) = 9.10$, $p < 0,006$) and none of the main effects were significant (Age: $F(1, 28) <1$; Education: $F(1, 28) <1$). Post-hoc analyses indicated that 1) education only had an effect in the old-old group and that 2) age only had an effect in the high-educated group. Here again, participants with more education showed more brain activity than those with less education in the older group, whereas no education effect was found in the younger group. Moreover, the older participants showed more activation than the younger ones in the high educated group, whereas no age effect was found in the low-educated group.

3.4. Relationship between fMRI and VBM data

Activation analyses were repeated by including GM volume as a nuisance covariate to evaluate the impact of structural differences on the relationships between education by age interaction and n-back task-related brain activity. The effect of the education by age interaction on brain activity in the left superior, middle and medial gyri (BA 9) in the 1-back condition remained significant after covarying in a whole-brain voxel-based manner for GM volumes ([x: -45, y: 23, z: 36] t: 7.73, $p < 0.001$, k: 1805 voxels;). There were no significant correlations between the education*age interaction-related ROI for GM volume and task-related brain activation when adjusted for education*age interaction term. Figure 5 combines in a single representation the regions activated in the 1-back condition in the entire group and those where a significant effect of the education by age interaction were found on brain activity or regional brain volume correlate with education.

Please insert Figure 5 about here

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4. Discussion

In this study, we investigated the impact of educational attainment on regional GM volume and on working memory task-related brain activation in a group of older individuals and examined whether this varies as a function of age. Our structural results show that educational attainment is positively correlated with GM volume in frontal and parietal regions and the education by age interaction indicates that greater education is associated with smaller age-related volume loss in the left middle frontal gyrus and the right medial cingulate gyrus. The functional results also revealed an education by age interaction. Persons with higher education activate more their left superior middle and medial frontal gyri, but this effect is only present in the old-old group (> 67 years of age). Finally, we failed to find a significant relationship between structure and function associated with education. In the following, we will discuss each of these main findings. We will then address how this relates to current reserve models.

First, the VBM analysis performed in this study reflects a positive correlation between years of education and GM volumes in the right parietal and frontal regions. More precisely, the more educated older adults were, the greater their cortical GM volume in the right medial and middle frontal gyri, in the right middle and posterior cingulate gyri, in the right inferior parietal lobule, and in the right anterior cerebellum. Our findings are consistent with results from a previous study using VBM that showed that education was positively associated with greater GM volume in temporo-parietal and orbitofrontal regions in a sample of 331 healthy older adults (Foubert-Samier et al. 2012). Frontal and parietal regions are typically involved in memory and executive functioning and show the most important GM density changes during childhood and adolescence (Sowell et al. 2003). Therefore, they may be regions that are particularly malleable and receptive to cognitive stimulation received during these periods. It is interesting to note that we found differences only in the right hemisphere. A precise interpretation of this lateralization effect is difficult and further investigation is needed to confirm this effect with a larger sample and to provide an understanding of its mechanism. Interestingly, we found an education by age interaction positively correlated with GM volume in the left middle frontal gyrus and in the right medial cingulate gyri. The interaction is due to the fact that persons with lower education suffer from a reduced volume with age in this region whereas those with higher education show no volume loss with age. This is consistent with higher education having a protective effect against the age-related decline on brain structure and supports the brain maintenance hypothesis, which refers to the preservation of neurochemical, structural and functional brain integrity with age (Nyberg et al., 2012). The lack of an age effect on this particular region for those with higher education data suggests that education can minimize some of the effect that age has on the brain.

When analyzing activation in the whole group, we found n-back task-related activation in frontal and parietal regions and an increased activation in these regions in response to increasing load level. These results are in line with previous studies that reported n-back-related activation of a fronto-parietal network (for a meta-analysis, see Owen et al. 2005), and increased activation of this network with higher levels of load (Braver et al. 1997; Jonides et al. 1997). The evaluation of the effect of education on activation indicates results that are partly consistent with our predictions for compensation. We found increased activation with age in the left superior, middle and medial frontal gyri (BA 9) but only in persons with higher education. Furthermore, among the oldest participants, higher levels of education were related to greater n-back task-related activation. In contrast, no education effect was found in younger participants and no age effect was found in those with low education. The same pattern was found for the 1-back and 2-back conditions, yet, while it resulted from the whole-brain analysis for the 1-back condition, it was only found following an ROI analysis in the 2-back condition. Altogether, these results do not support the potential protective role of education on activation since we did not find that education reduced the age effect on activation. Rather, we observed that there was an increased activation with age only in those with higher education. This suggests that higher education might support the involvement of compensatory processes and these processes might be more involved at an older age when biological aging increases its detrimental impact on the brain.

The finding that higher education is related to larger recruitment of the left dorso lateral prefrontal cortex (BA 9) can be related to current findings on the neuroanatomical basis of working memory. This region was found to be associated with the manipulation of verbal and spatial information in working memory (Barbey, Koenigs & Grafman, 2013). Furthermore, larger activation of the dorso lateral prefrontal cortex (BA 9) was found for the 1-back condition in high performing older individuals compared to younger adults suggesting that this is a region that can be recruited to support working memory performance in aging (Mattay et al., 2006). Thus, one tentative explanation is that higher education allows the oldest individuals to compensate for age-related changes by over-activated at the lowest load. As the right dorso lateral prefrontal cortex (BA 9) is active when looking at the whole group, one related account of the activation pattern found here is that higher education in late aging is associated with a greater recruitment of this region contralaterally. Accordingly, the hemispheric asymmetry reduction in older adults' model

(HAROLD; Cabeza 2002) proposes that older adults compensate by recruiting the contralateral regions. If this interpretation is confirmed, our results would suggest that this pattern is a characteristic of individuals with higher education.

It is important to highlight that education was not associated with better performance on the task irrespective of load and age. This is in contrast with both our structural and functional findings. The absence of a measurable behavioral benefit from education is at odd with our interpretation that education minimizes the age effect on volume loss in the frontal lobe and that it allows compensatory recruitment of the same regions. There may be numerous explanations for this inconsistency. One possibility is that those structural differences are of insufficient magnitude to support visible behavioral effects. Another possibility is that it is explained by a threshold effect. The positive effect of education may start to have a measureable impact on behavior only when the system is more stressed for instance at a later age or in those who develop suffer from a brain disease. One of our hypotheses was that different task loads would be associated with different patterns of activation in participants with high vs. low levels of education and that these educational differences in load-related brain activation patterns would be amplified in late aging. It is of note that we did not find a distinctive load-related brain activation pattern between older participants with higher vs. lower levels of education and no differentiated effect of education were found in the load-related brain patterns between younger and older participants. One possibility is that our tasks were not of a sufficient difficulty level to exhaust resources from low-level individuals and that a larger range of load would have been required to evidence a load by education interaction.

The innovative aspect of this study was to include both structural and functional analyses in relation to education, as this allowed us to look at their relationship and potential influences. One interesting question is whether differences related to education occur in the typical working memory network and another is whether structural and functional associations co-occur in the same regions. Interestingly, some of the regions which show larger volumes as a function of education are part of the working memory activated network: the right medial frontal and middle cingulate gyri and the right inferior parietal lobule are activated by the task and are also regions that are enlarged in individuals with higher levels of education. Even though no performance advantage was found in relation to education, one would expect working memory to benefit from this increased brain volume in the working memory network and that higher education would thus favor better working memory capacities when given more demanding conditions. We found that the regions that are more activated in the higher educated older participants are regions where no correlation was found between GM volume and educational attainment. It suggests that education affects the brain activity in regions that are structurally not sensitive to its influence. When comparing regions that correlate structurally vs. functionally with education, we find that regions that are more active as a function of education do not correspond to the regions of greater GM volume. It suggests that the functional differences supported by education might be independent from education-related structural differences. This interpretation is consistent with our finding that regional GM volumes appear to not account for education-related differences found in task-related brain activation. The positive correlation between age by education interaction and working memory task-related activation in the left prefrontal regions remains present when controlling for individual regional GM volume differences.

Interpreted in the conceptual framework of reserve, finding education-related structural differences in older individuals supports the brain reserve hypothesis (see Stern 2002 for a review), which claims that differences in reserve proxies, for instance educational attainment or adult-life occupational work complexity, are associated with anatomical differences in brain structure (Katzman 1993; Satz 1993). It is possible that the volume of these regions was larger to start with in people with higher levels of education. In turn, and along with the maintenance hypothesis (Nyberg et al. 2012), this might reflect a smaller degree of age-related atrophy in persons with more education. The age by education interaction provides further support for the maintenance hypothesis at least in the left middle frontal gyrus and in the right medial cingulate gyrus. In this case, education was found to protect against the age-related volume loss. Finally, finding education-related functional differences in older individuals supports the cognitive reserve hypothesis (Stern 2002; 2009; Stern et al. 2005; Tucker & Stern 2012), which predicted that higher level of cognitive reserve, for instance higher educational attainment or IQ, is associated with greater neural efficiency, greater neural capacity, and the ability to compensate via the recruitment of additional brain regions. It therefore appears that education has far ranging effects on both structure and function which might explain why it has such a strong protective effect against age-related neurodegenerative diseases.

Some limitations of this study should be addressed. First, education is complex and its effect on the brain might reflect a range of different factors associated with differences in education. Effects related to various levels of education might reflect effects from crystallized knowledge learned during schooling or cognitive skills and metacognitive abilities and strategies learned from formal education. They may also reflect the influence of other variables that are known to correlate with educational attainment in particular the quality of the early environment and early and long-life socioeconomic status (SES) and lifestyle. Although this study was not designed to disentangle the impact of these different factors, there is a need to better understand what component of education determines the various brain differences. Second, we did not find a correlation between education and behavior. While this might be interpreted as a limitation, one might also argue that it facilitates our interpretation of the fMRI data, as these can be interpreted as reflecting the activity of processes required to succeed in the task since the accuracy of all the participants was relatively high. Third, the small sample size has an impact on our ability to detect an education effect on brain volume when corrections for multiple comparisons are applied. Nevertheless, we are comforted by the finding that several regions are positively correlated with educational attainment and by the fact that many of them were previously found to be associated with education in healthy older adults (Foubert-Samier et al., 2012). Finally, our design is cross-sectional and therefore, the direction of causality between education and regional brain volume or brain activation cannot be demonstrated.

In summary, compared to older adults with lower levels of education, we reported more cortical GM volume in the right frontal and parietal regions and we found that in some regions of the frontal lobe, lower education was associated with more age-related volume differences. The n-back task was associated with a large and consistent network of fronto-parietal activity. Interestingly, we found that the education was associated with greater working memory-related activity in the left prefrontal cortex but it was only found among older adults. The pattern is indicative of compensation as those with higher education increased their level of activation with age whereas this was not found in those with lower education. These findings suggest that education is associated with a better integrity of brain structure and with a better resistance to cope with age-related structural changes in late aging. The finding that the larger activation with education only occurs at an older age perhaps suggests that larger compensatory recruitment will only occur later in life, when the system is under pressure. Our findings that age might interact with reserve processes open interesting avenues. They are consistent with recent models which propose that the effect of training or environmental enrichment on the brain is likely to vary as a function of individual characteristics (Belleville et al, 2014). Future researches are needed to determine if these findings can be replicated with larger cohorts and other reserve proxies.

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Tables

Table 1

Demographic and clinical characteristics for all participants included in the final sample.

	Participants (n = 32)
Age (years)	68.59 (6.50)
Education (years)	15.25 (2.92)
MoCA (/30)	27.40 (1.93)
GDS (/15)	2 (2.37)
Digit span	65.34 (12.06)
TMT A (second)	35.95 (11.16)
TMT B (second)	76.38 (26.83)

Note. Numbers in parentheses are standard deviations. MoCA: Montreal Cognitive Assessment; GDS: Geriatric Depressive Scale.

Table 2

Behavioral performances in the n-back task.

1-back		2-back	
Acc (%)	RT (ms)	Acc (%)	RT (ms)
89.27 (7.88)	829.48 (164.4)	85.78 (10.02)	955.82 (216.33)

Note. Numbers in parentheses are standard deviations. RT: Reaction Time. Acc: Accuracy.

Table 3

GM volume positively correlated with educational attainment when adjusted for age, gender, and TIV.

Activated areas (Brodmann area)	x	y	z	Cluster size	*t* value
Right medial frontal and cingulate gyrus (6, 32)	5	8	47	144	4.78
Right inferior parietal lobule (40)	45	-48	53	264	4.53
Right middle frontal gyrus (6)	38	-6	68	122	4.31
Right posterior cingulate gyrus and anterior cerebellum	9	-54	9	144	4.13

Note. P < 0.001 uncorrected, K=100 voxels

Table 4

VBM results of the interaction effect between age and education adjusted for age, education, gender, and TIV.

Activated areas (Brodmann area)	x	y	z	Cluster size	*t* value
Right middle cingulate gyrus (24)	8	-6	39	102	4.43
Left middle frontal gyrus (46)	-50	32	23	108	4.26

Note. P < 0.001 uncorrected, K=100 voxels

Table 5

GM volume negatively correlated with age when adjusted for education, age*education interaction, gender, and TIV.

Activated areas (Brodmann area)	x	y	z	Cluster size	*t* value
Left superior, middle and inferior frontal gyri, (11, 47)	-33	36	-11	654	6.01
Right and left middle cingulate gyri, paracentral lobule, precunei (4, 5, 6, 7, 23, 24, 31) *	9	-27	41	2225	5.64
Right anterior cingulate and medial frontal gyri (9, 32)	12	38	24	169	4.76
Right superior, middle and inferior frontal gyri (11,47)	23	29	-15	649	4.76
Left cuneus and lingual gyrus (17, 18)	-11	-90	2	639	4.74
Left middle frontal gyrus (6)	-30	-2	48	142	4.71
Right middle and inferior occipital gyri (18,19)	36	-83	-2	169	4.56
Left superior and middle temporal gyri (21, 22, 39)	-56	-51	6	515	4.39
Left superior and middle temporal gyri (21, 38)	-42	9	-26	136	4.31
Right superior and middle temporal gyri (21, 22)	56	-39	6	283	4.19
Left precentral gyrus (6)	-50	-12	32	149	4.19
Left middle temporal gyrus (21)	-54	-26	-12	119	4.15

Note. $P < 0.001$ uncorrected, K=100 voxels; *Cluster that reaches the cluster-level significance ($P<0.05$, FWE).

Table 6

Activation of brain regions in the 1-back > baseline, 2-back > baseline, and 2-back > 1-back contrasts.

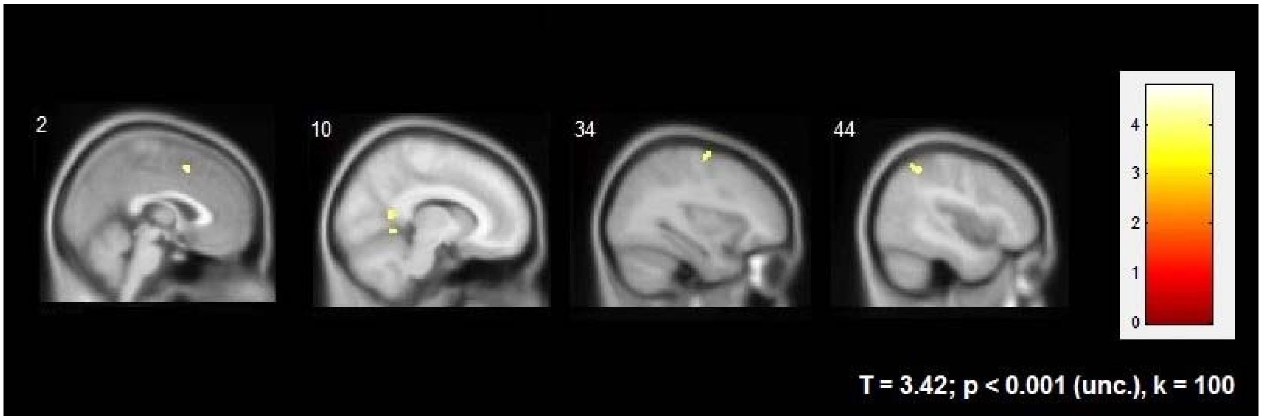
Activated areas (Brodmann area)	x	y	z	Cluster size	*t* value
<u>1-back</u>					
Right and left superior middle medial and inferior frontal gyri, superior parietal lobule, cingulate gyrus, precuneus, (1 - 9, 24, 32, 40)	-6	8	53	2538	13.04
Superior and inferior parietal lobule, supramarginal gyrus (40)	42	-52	44	261	8.65
Right superior middle frontal gyrus (9, 10)	42	29	35	128	8.24
Right inferior frontal gyrus (47)	33	29	2	107	8.17
Left superior and middle frontal gyri (9, 10)	-45	32	32	172	8.15
<u>2-back</u>					
Right and left superior middle medial and inferior frontal gyri, precentral gyrus, superior and inferior parietal lobule, cingulate gyrus, precuneus, supramarginal gyrus, insula, angular gyrus (1 - 11, 13, 32, 40, 42, 44-47)	-6	20	47	6462	12.83
Right and left cerebellum, fusiform gyrus, middle occipital gyrus (18, 19, 37)	36	-55	-49	1067	10.60
Right inferior frontal gyrus (47)	57	-49	-10	67	8.54
Right putamen	18	2	11	75	6.81
<u>Load (2-back vs. 1-back)</u>					
Right superior and middle frontal gyri (6, 8-10)	42	14	56	606	10.78
Left superior and medial frontal gyri, cingulate gyrus (6, 8, 32)	-9	20	44	238	9.05
Left superior, middle and inferior frontal gyri, precentral gyrus (6, 8, 9, 46)	-42	5	32	607	8.52
Right superior and inferior parietal lobule, supramarginal gyrus and precuneus (7, 40)	39	-58	44	290	8.00
Left superior and inferior parietal lobule (7, 40)	-30	-58	41	192	8.00
Right cerebellum	33	-67	-28	69	7.90
Right inferior frontal gyrus (47)	33	26	-4	65	7.60
Left cerebellum	-30	-64	-31	166	7.57
Left inferior frontal gyrus	-45	32	32	172	7.36

Note. $P < 0.05$ (FWE), K = 50 voxels

Figures

Figure 1

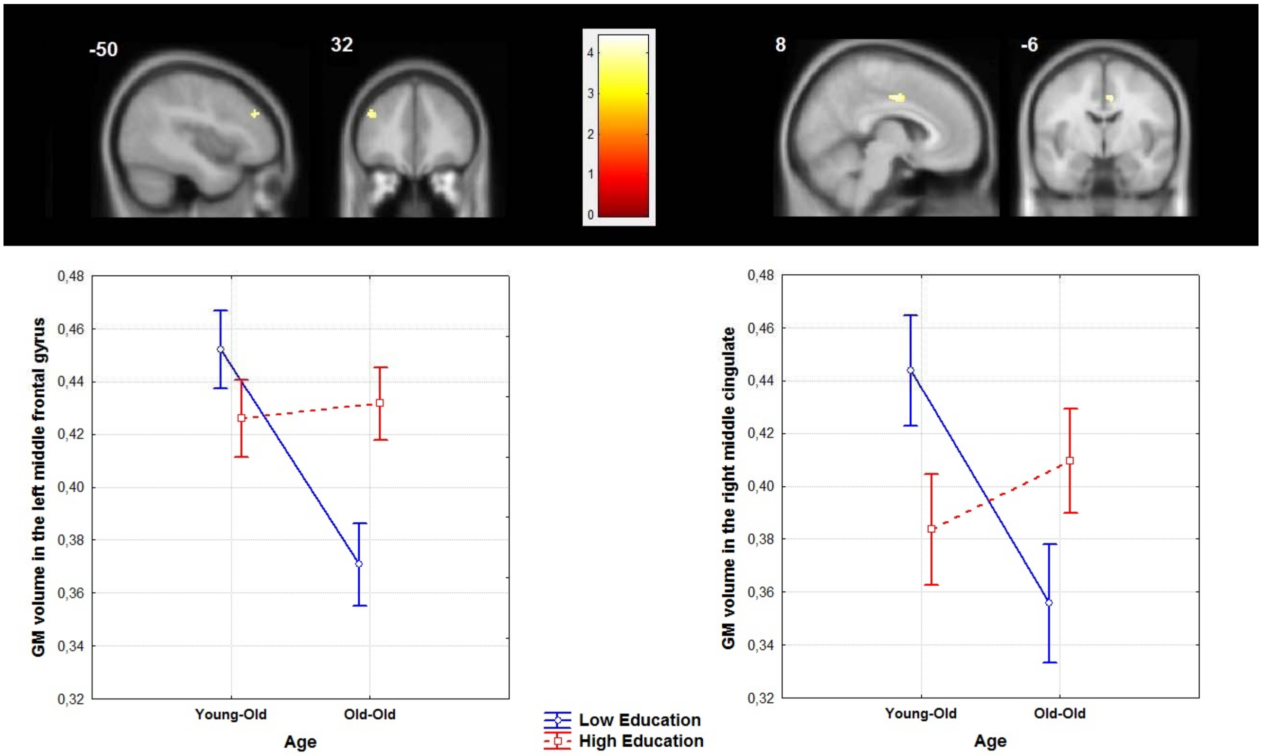
Brain areas in the right hemisphere showing the results of the voxel-wise multiple regression between years of education and GM volume.



The coordinate in MNI space is indicated above each section. Color intensities reflect *t*-values.

Figure 2

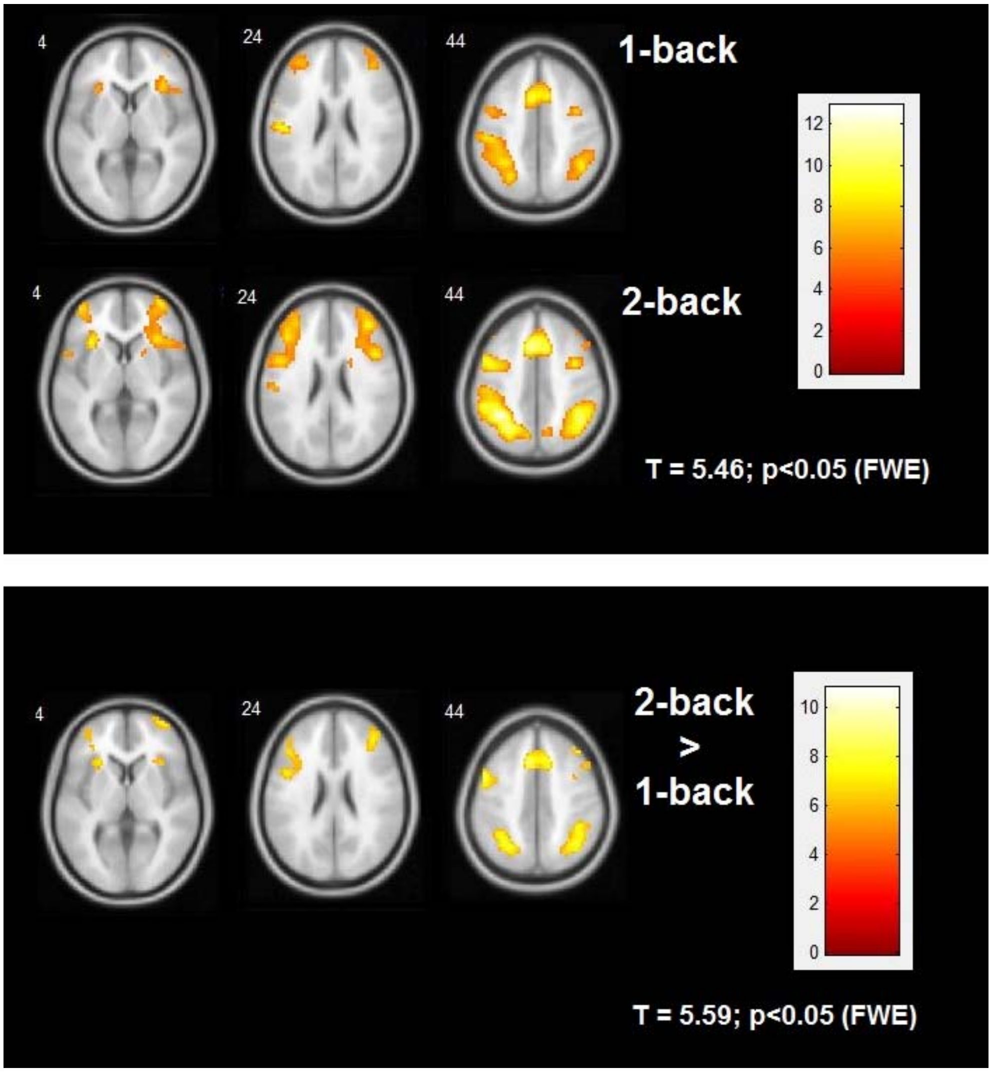
Top panel: Brain areas showing the results of the voxel-wise multiple regression between Age*Education interaction and GM volume. Bottom panel: Plot means with standard errors bars from GM volume in each region showing a significant Age*Education interaction.



The coordinate in MNI space is indicated above each section. Images are presented in neurological convention. Color intensities reflect *t*-values.

Figure 3

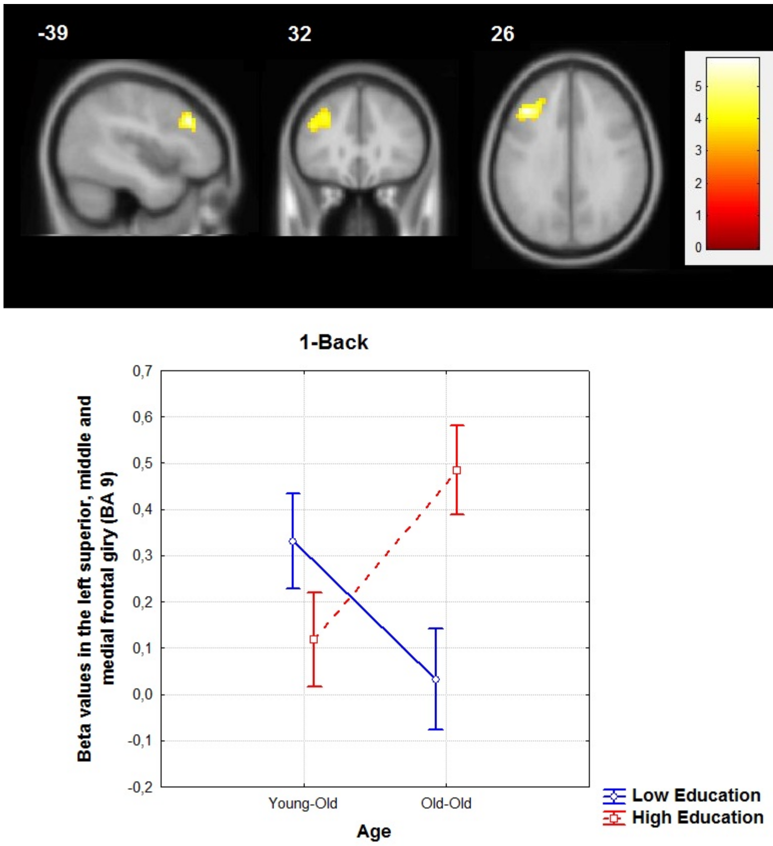
Cortical activation in the 1-back > baseline, 2-back > baseline, and the 2-back > 1-back contrasts.



The coordinate in MNI space is indicated above each section. Images are presented in neurological convention. Color intensities reflect *t*-values.

Figure 4

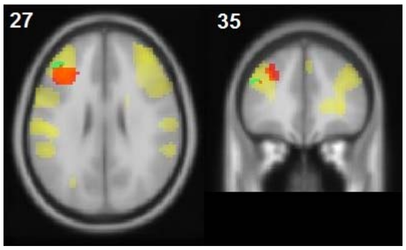
Top panel: Brain areas showing the results of the voxel-wise multiple regression between Age*Education interaction and brain activation in the 1-back. Bottom panel: Plot means with standard errors bars from GM volume in one region showing a significant Age*Education interaction.



The coordinate in MNI space is indicated above each section. Images are presented in neurological convention. Color intensities reflect *t*-values.

Figure 5

Anatomical overlap between results of VBM and fMRI analyses. In yellow, regions of significant fMRI activation during the 1-back condition (displayed at $p < 0.01$ uncorrected for visualization purposes). In red, regions where a significant effect of the Age*Education interaction on brain activation was found. In blue, regions where a significant effect of the Age*Education interaction on GM volume was found. Orange and green colors respectively represent the yellow-and-red and yellow-and-blue overlapping regions.



The coordinate in MNI space is indicated above each section. Images are presented in neurological convention.