# Gender Differences in Healthy Aging and Alzheimer's Dementia:

## *a $^{18}$F-FDG-PET study of brain and cognitive reserve*

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Alzheimer's Disease Neuroimaging Initiative (ADNI) database*

Network for Efficiency and Standardization of Dementia Diagnosis (NEST-DD) database

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## Abstract

**Cognitive reserve (CR) and brain reserve (BR) are protective factors against age-associated cognitive decline and neurodegenerative disorders. Very limited evidence exists about gender effects on brain aging and on the effect of CR on brain modulation in healthy aging and Alzheimer's Dementia (AD).**

**We investigated gender differences in brain metabolic activity and resting state network connectivity, as measured by $^{18}$F-FDG-PET, in healthy aging and AD, also considering the effects of education and occupation. The clinical and imaging data were retrieved from large datasets of healthy elderly subjects (HE) (225) and AD patients (282).**

**In HE, males showed more extended age-related reduction of brain metabolism than females in frontal medial cortex. We also found differences in brain modulation as metabolic increases induced by education and occupation, namely in posterior associative cortex in HE males and in the anterior limbic-affective and executive networks in HE females.**

**In AD patients, the correlations between education and occupation levels and brain hypometabolism showed gender differences, namely a posterior temporo-parietal association in males and a frontal and limbic association in females, indicating the involvement of different networks.**

**Finally, the metabolic connectivity in both HE and AD aligned with these results, suggesting greater efficiency in the posterior default mode network for males, and in the anterior frontal executive network for females.**

**The basis of these brain gender differences in both aging and AD, obtained exploring cerebral metabolism, metabolic connectivity and the effects of education and occupation, is likely at the intersection between biological and sociodemographic factors.**






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## Introduction

The concepts of brain reserve (BR) and cognitive reserve (CR) were originally introduced to explain the discrepancy between the quantity of pathological findings and their clinical expression in Alzheimer's Dementia (AD) patients [Katzman et al., 1988; Stern, 2002]. **Several factors impact on both BR and CR, including lifelong exposure to stimulating cognitive, social and physical activities, as well as high socio-economic status, educational and occupational attainments [Steffener and Stern, 2013]. These lifelong experiences could influence the integrity of the brain itself, through a process that has been called brain maintenance, resulting in a higher BR, and also in a greater CR, allowing people to maintain function in the face of brain changes or pathology. Several research studies demonstrated brain plasticity and structural changes in healthy subjects in response to life-long experiences, and reduced pathology in neurodegenerative conditions [see for review Barulli and Stern 2013].**

**The concept of both CR and BR** has greatly developed both at the theoretical and experimental levels thanks also to in vivo neuroimaging methods that provided insights into the anatomo-functional correlates both in healthy aging and in diseases [Stern, 2012; Tucker and Stern, 2011].

Exploring the brain aging in healthy individuals, several magnetic resonance imaging (MRI) studies reported evidence of age-related atrophy particularly in the frontal lobes [Kalpouzos et al., 2009; Lemaitre et al., 2012; Raz et al., 1997; Salat et al., 2004]. In addition, studies using positron emission tomography imaging with Fluorine-18-Flourodeoxiglucose ($^{18}$F-FDG-PET) also showed an association between age and decreased brain metabolism in the frontal cortex [Chételat et al., 2013; Kalpouzos et al., 2009; Pardo et al., 2007]. The protective effect of **lifelong cognitive experiences** on the aging brain has received consistent evidence by structural MRI investigations demonstrating positive correlations with increased gray and white matter volumes in frontal and temporo-parietal cortices [Arenaza-Urquijo et al., 2013; Foubert-Samier et al., 2012; Liu et al., 2012].

Following this line of research, few studies evaluated also the gender effects on aging-related brain changes in healthy individuals, using MRI or $^{18}$F-FDG-PET. The evaluation of gray matter (GM) atrophy with MRI showed, however, heterogeneous results, either a non-significant effect of gender on regional GM atrophy [Good et al., 2001; Lemaître et al., 2005; Salat et al., 2004; Smith et al., 2007], or increasing brain atrophy



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associated with aging in several brain regions in males as compared to females [Coffey et al., 1998; Gur et al., 1991; Raz et al., 2004; Sullivan et al., 2004; Takahashi et al., 2011; Thambisetty et al., 2010]. In particular, a greater reduction in cortical thickness and volume of the frontal lobe in males seems to suggest a gender related vulnerability [Cowell et al., 1994; Murphy et al., 1996; Xu et al., 2000]. Similarly to MRI evidence, also $^{18}$F-FDG findings of gender effects on cerebral metabolism are controversial, making it difficult to draw firm conclusions regarding the existence of gender effects on age-related changes in cerebral metabolism. Briefly, some $^{18}$F-FDG-PET studies reported significant decreases in brain metabolic activity in turn for males or females, while others did not find gender differences in elderly population [Fujimoto et al., 2008; Kakimoto et al., 2015; Kawachi et al., 2002; Willis et al., 2002; Yoshizawa et al., 2014]. These inconsistent findings are possibly due to methodological aspects such as the characteristics and size of the considered samples and the statistical analyses. Only one 18F-FDG-PET study explored the gender differences in the correlation between education and brain resting state metabolism in a group of healthy adults, taking into account 123 individuals [Yoshizawa et al., 2014]. A positive correlation between education and cerebral glucose metabolism was found in temporal and medial frontal regions, but without any evidence for gender effect.

Other neuroimaging researches focused on the effects of CR in neurodegenerative disorders and in particular on AD. MRI showed reduced brain volumes and micro-structural changes in AD brain correlated with high education and occupation levels [Liu et al., 2012; Solé-Padullés et al., 2009; Teipel et al., 2009], and FDG PET a more severe brain hypometabolism in highly educated AD patients for a given level of clinical impairment [Alexander et al., 1997; Garibotto et al., 2008; Morbelli et al., 2013; Perneczky et al., 2006]. Garibotto et al. (2008) reported a significant correlation between higher education and occupation and a more severe $^{18}$F-FDG-PET hypometabolism in temporo-parietal associative cortex and precuneus in subjects with AD and mild cognitive impairment (MCI) [Garibotto et al., 2008]. These results were also confirmed by Morbelli, Perneczky et al. (2013), studying the effects of education on brain metabolism in patients with amnestic MCI who later converted to AD at follow-up [Morbelli et al., 2013]. Furthermore, in the field of molecular PET imaging, Garibotto et al. (2013) using [11C]-MP4A PET for the in vivo assessment of acetylcholinesterase activity, reported a correlation between higher levels of education and occupation and



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preserved cholinergic activity in temporal medial structures, both in AD and MCI [Garibotto et al., 2013]. Overall, these findings suggest that high levels of education and occupation allow some patients coping with brain degeneration, maintaining cognitive functions and possibly delaying the clinical manifestation of dementia. Up to now, there is, however, very limited evidence on the gender differences in the expression of CR in AD patients. To our knowledge, only one 18F-FDG-PET study investigated gender differences on reserve in 93 AD patients [Perneczky et al., 2007]. Fifty males as compared to forty-three females showed a greater reduction of brain metabolism in right inferior frontal cortex, superior temporal lobe, insula and hippocampus. Nevertheless, Perneczky and colleagues did not take into account the possible effects of education and occupation.

The topic is of upmost interest since gender differences were found in AD both in the epidemiology and in the expression of AD-related neurodegeneration. The prevalence and incidence of AD are higher in women as compared to men [Mielke et al., 2014]. The greater longevity of women as compared to men [Seshadri et al., 1997] and the interaction between gender and the apolipoprotein ε4 allele [Corder et al., 2004] seem to be potential sources of the reported epidemiological differences. Additionally, also psychosocial and cultural discrepancies (e.g. as different educational and occupational attainments) between men and women have been considered as candidate causal factors [Mielke et al., 2014]. Notably, a study on post mortem specimen by Barnes et al. (2005) showed that the link between neuropathology and its clinical expression is stronger in women as compared to men. Specifically, they found that for every unit increase in pathology, women were significantly more likely to show clinical dementia (Odds-ratio: 22.67) as compared to men (Odds-ratio: 2.82) [Barnes et al., 2005]. Neuroimaging studies have also shown gender differences in AD-related neuropathology. Rowe and colleagues reported higher amyloid load (as assessed by PET with Pittsburgh compound B (PiB)) in AD males than in females with comparable cognitive impairment [Rowe et al., 2010]. Overall, these findings suggest that, although AD-related brain changes are more severe in men [Perneczky et al., 2007; Rowe et al. 2010], women are more likely to express these as clinical dementia [Barnes et al., 2005], and these findings might be crucially related to a lower or different amount of cognitive reserve.

**Considering the above very limited reports on gender differences both in healthy aging and AD brain we investigated in a large dataset of healthy elderly subjects (HE) and AD patients, whether**



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**differences exist between males and females in brain metabolic activity, also including the effects of education and occupation**. In addition, we also assessed gender differences in brain metabolic connectivity in the main resting state functional networks (i.e. the Default Mode Network (DMN), the frontal executive control network (ECN) and the Language network (LN)), an approach never previously applied. This investigation might add important knowledge in the gender neurobiology of brain aging and AD dementia.

## Material and methods

***Participants***

*Healthy elderly subjects (HE).* 225 HE (115 males and 110 females) were selected for this study from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. The ADNI is an American public-private partnership launched in 2003 to collect longitudinal data on MCI and AD patients as well as on HE subjects. The main goal of this initiative is to evaluate the combined prognostic value of several AD biomarkers and of clinical and neuropsychological assessments (see www.adni-info.org for more information). Only subjects older than 60 years (age range 61.2-85.9 years) were included in this study in order to explore aged population and with an age-range comparable to that of the AD patients. $^{18}$F-FDG-PET scans for the assessment of brain metabolism, data on education and occupation, and full neuropsychological evaluation were available in each individual.

*AD patients*. 282 AD patients (123 males and 159 females) were included in this study from 3 databases: 118 were retrieved from the ADNI database, 135 from the Network for Efficiency and Standardization of Dementia Diagnosis (NEST-DD) database, 29 from the Nuclear Medicine database at San Raffaele Hospital (Milan, Italy). We included in the study only patients aged 65 or older (age range 65.0-89.6 years) with a clinical diagnosis of AD according to standardized diagnostic criteria [McKhann et al., 2011].

The study was performed in compliance with the Declaration of Helsinki.

In order to match the neuropsychological assessments from different databases in both the HE and AD cases, we chose comparable tests for five neuropsychological domains, and z-scores were assessed for each test to allow comparisons (Supporting Information Table 1). Years of education were defined as the



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number of completed years of formal education. Occupational attainment (available for 225 HE and 253 AD patients: 114 males and 139 females) was rated on a 6 points scale as previously described in Garibotto et al. (2008). In detail: 1. no occupation; 2. unskilled laborer; 3. housewife; 4. skilled laborer, tradesman, lower level civil servant, employee, self-employed small business, office or sales personal; 5. mid-level civil servant or management, head of a small business, academician or specialist in a subordinate position; 6. senior civil servant or management, senior academic position, self-employed with high degree of responsibility [Garibotto et al., 2008]. In case of subjects who did more than one job during their life, the lifelong employment performed for the longest time was considered.

Descriptive statistics (means and standard deviations) were considered separately for males and females in both HE (see Table 1) and AD patients (see Table 2), using SPSS 21.0 software for Windows (SPSS Inc., Chicago, IL). Gender groups in the two cohorts were compared by means of two-sample independent sample t-tests (see Table 1 and Table2).

In addition, the AD diagnosis was supported by the presence of the AD-like hypometabolism pattern, as assessed by a single-subject validated SPM procedure of $^{18}$F-FDG-PET images [Perani et al., 2014]. We included only patients with the clinical diagnosis of Alzheimer's Dementia and the typical hypometabolism pattern as indicated in the diagnostic research criteria [McKhann et al., 2011]. We applied the SPM single-subject method that we validated in AD and in several neurodegenerative diseases with a very high sensitivity and specificity [Perani et al. 2014, 2016; Cerami et al. 2016a,b]. We thus used both clinical diagnosis and $^{18}$F-FDG-PET hypometabolism evidence to enhance the accuracy of patients' inclusion.

### *$^{18}$F-FDG-PET acquisition and image processing procedures*

Raw not preprocessed $^{18}$F-FDG-PET images were downloaded from the ADNI and NEST-DD databases. The acquisition of $^{18}$F-FDG-PET images was performed according to the European Association of Nuclear Medicine guidelines for databases from NEST-DD and Nuclear Medicine Unit of San Raffaele Hospital [Varrone et al., 2009], while for images from the ADNI database the acquisition procedure is described in the "ADNI PET technical procedures manual, version 9.5" (http://adni.loni.usc.edu/wp-content/uploads/2010/09/PET-Tech_Procedures_Manual_v9.5.pdf). We created a single averaged 15-minute



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frame for each image starting at 30 or, in the majority of cases, at 45 minutes after radio-ligand injection. We then used the Statistical Parametrical Mapping software (SPM5, www.fil.ion.ucl.ac.uk/spm), implemented in MATLAB (MathWorks Inc., Sherborn, Mass) for all the subsequent preprocessing and statistical analyses. The intensity of single-subject images was normalized dividing each image for its global mean. Then spatial normalization to a specific $^{18}$F-FDG-PET template [Della Rosa et al., 2014] and smoothing (FWHM: 8 mm) were performed in SPM5. Then the warped and smoothed images entered a whole-brain voxel-level statistical comparison with a validated database of healthy controls, age was entered as nuisance variable in this analysis in order to exclude its effect [Perani et al., 2014]. This procedure generated SPM t-maps showing regions of significant hypometabolism, with a high level of significance ($p<0.05$, with Family Wise Error (FWE) correction for multiple comparisons) [Della Rosa et al. 2014; Perani et al. 2014]. In addition, a 2$^{nd}$ level sample t-test on contrast images was implemented in SPM to identify the presence of the typical AD hypometabolism in the whole AD group, controlling for age as nuisance variable (see Supporting Information_Figure 1).

***Correlation analysis***

*HE.* A General Linear Model (GLM) was applied in SPM5 to assess, separately for males and females, the voxel-level correlations between age, education and occupation with levels of brain glucose metabolism in the whole brain, considering the smoothed and warped images of the HE group.

We performed whole brain SPM correlation analysis between age and brain metabolism separately for males and females in SPM, considering MMSE scores as nuisance variable and setting the threshold at $p < 0.05$ with FWE correction for multiple comparisons (cluster extent k=100). We considered the results of the SPM correlation analysis in the two groups as regions of interest for the extraction of the mean metabolic activity in each subject. Then, we performed offline correlation analyses between the mean metabolic activity and age.

In the correlations of brain metabolism with education and occupation separately, we considered as nuisance variables age, MMSE scores and neuropsychological z-scores for the five described cognitive domains. The



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correlations were set at $p < 0.001$, $p< 0.005$ and $p < 0.01$ thresholds (uncorrected for multiple comparisons) with minimum cluster extent k=50 voxel, to capture the most significant differences between gender groups given the exploratory nature of this voxel-based analysis based on a very large dataset (225).

*AD patients*. GLM was used to test the whole brain voxel-level correlations between educational and occupational attainments and brain hypometabolism in male and female AD patients. These correlation analyses for AD patients were tested at the 2nd level, that is on contrast images derived from single-subject comparisons. Each 1st level analysis includes the age as covariate and all the resulting contrast images have this effect factored out. MMSE scores, disease duration and neuropsychological z-scores for the five described domains, were entered as nuisance variables. The significant thresholds were set at $p < 0.001$, $p< 0.005$ and $p < 0.01$ (uncorrected for multiple comparisons) with minimum cluster extent k=50, given the exploratory nature of this voxel-based analysis based on a very large dataset (n. 282).

***Generalized Additive Models (GAM)***

We also applied GAM to explore the function shape and trajectories of age effects on regional brain metabolism in males and females, for both HE and AD groups.

In HE, we chose a Region Of Interest (ROI) including medial frontal cortex and anterior cingulum previously described as the most consistently involved in aging effect, both for atrophy [Kalpouzos et al., 2009; Lemaitre et al., 2012; Raz et al., 1997; Salat et al., 2004] and hypometabolism [Chételat et al., 2013; Kalpouzos et al., 2009; Pardo et al., 2007].

Five ROIs were considered in AD cases: 1) medial frontal cortex and anterior cingulum; 2) precuneus and posterior cingulum; 3) bilateral hippocampi and parahippocampal cortices; 4) inferior parietal cortex and angular gyrus; 5) lateral temporal cortex. The latter four ROIs represent the brain regions with the typical AD hypometabolism [Perani, 2014]. We applied GAM for each ROI separately, using the mgcv (Mixed GAM Computation Vehicle) library in R 3.3.2 software (R Core Team, 2016: www.r-project.org).

For each ROI, separately in HE and AD, we iteratively varied, by incremental power-of-ten steps (range $10^{-25}$-$10^{+15}$), the parameter affecting the degree of smoothness applied to the function smooth term (i.e. Age by



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Gender). Among the iteratively estimated GAMs, we then chose the one with the smoothing parameter giving the best model fit, based on the Bayesian information criterion (BIC). All models included the Age by Gender smooth term and Gender as a linear, between groups fixed effect. Regional brain metabolism was modelled with a Gaussian distribution and an identity link function.

***Metabolic connectivity analysis***

We further assessed and compared the brain metabolic connectivity in specific resting state functional networks, separately for males and females in both HE and AD patients. We explored the metabolic connectivity in the DMN, the ECN and the LN. This analysis relies on the assumption that brain regions whose glucose metabolism is correlated at rest are functionally associated, as defined in the seminal work by Horwitz et al. (1984) [Horwitz et al., 1984]. In this study, we applied a recent application of this method, the voxel-wise interregional correlation analysis (IRCA) in SPM, as described in Lee et al., 2008 [Lee et al., 2008]. Seed regions of interests (ROI) were defined from the functional atlas of resting state networks (as defined by Shirer et al. (2012) (http://findlab.stanford.edu/functional_ROIs.html)) [Shirer et al., 2012]. The following brain structures were taken as seed regions: the posterior cingulum and precuneus for the DMN; the left and right middle frontal and superior frontal gyri for ECN; the left angular, middle and superior temporal gyri for LN. Then, intensity normalization to the global mean was applied on the warped and smoothed $^{18}$F-FDG-PET images and mean FDG uptake was extracted from the seed ROI separately for males and females in each HE and AD group. The extracted mean ROI counts were entered as variables of interest in a multiple regression model in SPM5 testing for voxel-level correlations in whole brain. The metabolic connectivity analyses was first set at $p<0.05$ with FWE correction, showing only autocorrelations with seed regions. Then we applied a less restrictive False Discovery Rate (FDR) correction for multiple comparisons p-value=0.01 with cluster extent k=100, in order to explore gender differences at long distance interregional connectivity.



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## Results

***Subject Descriptive characteristics***

*HE.* Males and females showed highly significant differences ($p<0.001$) in educational and occupation scores, with males presenting higher levels on both the variables (education, Males: 17.10 ± 2.75 years; Females: 15.59 ± 2.51 years, and occupation scores: Males: 5.24 ± 0.86; Females: 4.51 ± 0.95). Neuropsychological assessment revealed significant differences between males and females in short and long term memory scores, which were significantly lower in males ($p<0.005$) (see Table 1).

*AD patients*. Males and females significantly differed ($p<0.001$) in educational years and occupation scores, with males showing higher levels (education of 13.25 ± 4.82 years and occupation scores 4.57 ± 1.28) than females (10.45 ± 4.47 years and occupation scores mean of 3.76 ± 1.06). Male and female AD patients did not differ in age, disease duration and MMSE. Neuropsychological assessment revealed deficits across all the investigated cognitive domains in both the groups. However, males were significantly more impaired in short-memory domain as compared to females (See Table 2).

***Correlations***

*HE.* Age showed highly statistically significant negative correlations with brain glucose metabolism in both males and females (Figure 1). In particular, increasing age correlated with significant and extended reduction of brain metabolism in the medial frontal cortex and anterior cingulate gyrus in males. The average strength of the correlation in these regions in males was r=-0.563. In females, there was less extended age-related hypometabolism, involving mainly the anterior cingulate cortex, with an average strength of r=-0.520.

Positive correlations of both education and occupation with brain metabolism were found in both HE gender, but with distinct metabolic patterns (Figure 2 and Supporting Information Table 2 for anatomical details and levels of significance). In males, both education (r=0.404) and occupation (r=0.506) were correlated with increased metabolism in posterior cerebral structures (education with brain metabolism in the



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precuneus, postcentral gyrus, inferior and superior parietal lobes and left rolandic operculum; occupation with the left inferior parietal cortex, the bilateral postcentral gyri and in the left temporal lobe and insula). In females, both education and occupation correlated with increased metabolism (education r=0.487 and occupation r=0.509) in the insula, amygdala, parahippocampal gyrus, the left superior parietal lobe and in the cerebellum. In addition, education involved also the anterior medial frontal gyrus, the orbitofrontal cortex and temporal pole bilaterally, while occupation the left inferior frontal gyrus. Notably, the voxel extents resulting from the correlation analyses were significantly more extended in females (voxel numbers: education 5259; occupation 2156) than in males (voxel numbers: education 1817; occupation 947).

*AD patients.* Education and occupation significantly correlated with brain hypometabolism in both males and females, but with differences (see Figure 3 and Supporting Information Table 3 for anatomical details and levels of significance). In females, education showed significant correlations in both anterior and posterior brain structures, namely the anterior cingulate cortex, the medial frontal cortex, and the posterior cingulate and precuneus (r= -0.428). Moreover, education effect in females was also evident in limbic structures, such as the left insula and parahippocampal gyrus. In males, education correlated with hypometabolism (r= -0.365), in the left inferior and middle temporal gyri, the left angular gyrus and bilaterally in the cerebellum (i.e. VIIa Crus I). There was a more extended pattern in females (voxel number 4731) than in males (voxel number 749). In females, occupation showed limited effects and only in inferior temporal lobes (r= -0.316). In males, occupation was the most relevant proxy that correlated with hypometabolism in the precuneus bilaterally, and in the left posterior cingulate cortex, lateral temporal and parietal cortex (r= -0.415). The correlation pattern was more extended in males (voxel number 2341) than in females (voxel number 962).

***GAM results***

In HE, the effect of age on brain metabolism was significant in the frontal medial and anterior cingulate ROI. This effect was found for both genders, but with a significantly steeper, negative slope for males versus females ($p < 0.001$, Figure 1). Of note, in spite of the unconstrained order of nonlinearity

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allowed by the GAM's smooth term, the estimated relationship between age and frontal metabolism was nonetheless linear.

In AD patients, the negative relationship between age and brain metabolism was significant only in the male group, specifically in the frontal medial and anterior cingulate cortex ($p < 0.00001$), with a nearly linear relationship (see Supporting Information Figure 2A), and in the hippocampal structures ($p < 0.00001$), with a perfectly linear relationship (see Supporting Information Figure 2B).



***Metabolic connectivity results***

***HE subjects***

*DMN.* Significant metabolic correlations were found within the seed regions (i.e. autocorrelation within the PCC/precuneus itself) and the inferior and superior parietal lobules in both males and females. The resting state whole-brain metabolic connectivity of the dorsal DMN, however, showed some gender differences. Males had a significant ($p < 0.01$ FDR) and extended pattern of connectivity between the seed region and the tempo-parietal lobes and, to a lesser extent, the prefrontal cortex. Females instead showed a more limited pattern of metabolic connectivity (Figure 4A).

*ECN.* Metabolic connectivity was largely overlapped between male and female groups, showing extended correlations with the dorsolateral, the medial frontal and the superior parietal cortex. There were however some differences, since females showed a strong pattern of left-lateralized connectivity, while males a higher connectivity with orbitofrontal cortex and ventral basal ganglia (Figure 4C).

*LN.* Metabolic connectivity for the left middle and superior temporal gyrus and angular gyrus as seeds of the LN was highly overlapped between males and females within the left hemisphere (namely temporal, parietal and inferior frontal cortex). However, males as compared to females showed a more bilateral pattern of connectivity, involving right frontal, temporal, parietal cortices. (Figure 4E).

***AD patients***

*DMN.* The pattern of metabolic connectivity in AD patients was restricted to the seed regions in the parietal lobes, with limited connectivity to the dorsolateral frontal cortex. Differences between males and



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females were evident in the lateralization of the pattern. Specifically, males showed more connectivity in the right prefrontal cortex and females in the left prefrontal cortex (Figure 4B).

*ECN.* Male and female AD patients, both showed significant metabolic connectivity within the frontal cortex. The ECN, however, was more extended in females involving also parietal and temporal cortex, especially in the left hemisphere (Figure 4D).

*LN.* Metabolic connectivity for the chosen seeds was more extended in females involving the left hemisphere the dorsolateral frontal, temporal and parietal cortex. In AD males, the metabolic connectivity was limited to the left temporo-parietal cortex only surrounding the seed regions. (Figure 4F).

## Discussion

**The present findings reveal that gender brings differences in age-related reduction of brain metabolism and in the neural correlates of education and occupation in healthy aging and in dementia**. In summary, there were gender differences in elderly brains, with males showing more age-related reduction of brain metabolism than females in frontal medial regions for cognitive control. Both HE subjects and AD patients showed correlations between **levels of education and occupation and** brain metabolism and, these were positive in aging and negative in AD. Indeed, we found increases of regional brain metabolism as a major effect of these life factors in healthy aging and a more severe cerebral hypometabolism in highly educated and lifelong occupied patients with AD. The effect of gender on these correlations emerged as an involvement of different brain networks, a result confirmed also by the metabolic connectivity analyses. In summary, the present findings reveal a greater aging effect on frontal cortex in males than females. Both in HE and AD, the effect of education and occupation was greater in anterior executive control and socio-affective networks in females, and in the posterior associative brain regions and the DMN in males. The following discussion highlights the results for HE and AD separately.

***Healthy aging.*** A significant correlation between age and a decreased brain metabolism was found in frontal medial brain regions in both genders, with a lesser voxel extent in females than in males. The GAM



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confirmed that the best fitting model to explain relationship between age and frontal metabolism was linear (see Figure 1), with significant differences for females and males indicating a more severe effect of aging in males in frontal medial cortex (Figure 1).

In general, this aging evidence aligns with the frontal aging hypothesis [Tisserand and Jolles, 2003], which suggests that aging is associated with a reduction in metabolic activity in the frontal lobe and mainly in the anterior cingulate gyrus. MRI studies on healthy aging reported structural evidence of age-related atrophy in the frontal lobe [Kalpouzos et al., 2009; Lemaître et al., 2005; Raz et al., 1997; Salat et al., 2004]. Of note, previous findings from MRI studies considering gender, identified differences in age-related atrophy, with a more severe frontal GM atrophy in males than in females [Cowell et al., 1994; Murphy et al., 1996; Xu et al., 2000].

Beside confirming these MRI results on a larger independent database, we revealed gender differences also in the amount of neuronal dysfunction, measured as reduced brain metabolic activity that was more severe in males as compared to females, thus suggesting a major gender susceptibility to age-related decline. The age-related dysfunctional changes in females were limited only to the anterior cingulate gyrus while extensively encompassed also a large part of the superior frontal gyrus in males (Figure 1). As aforementioned, results from previous FDG-PET studies on this topic are controversial, reporting inconsistent findings, possibly due to methodological aspects. Our $^{18}$F-FDG-PET study in a very large cohort of HE subjects (n= 225) demonstrated that gender differences exist in the correlation between age and frontal hypometabolism. The medial frontal cortex that was more hypometabolic in males is involved in executive functioning, cognitive control and top-down modulation of information processing towards the posterior associative cortex [see for reviews Ridderinkhof et al., 2004; Rushworth et al., 2007]. The more limited frontal lobe impairment found in women as compared to men also parallels gender differences on cognitive performances in aging. In this respect, we found a female advantage in the short-term memory domain. Crucially and related, fMRI studies reported different brain activations in males and females during cognitive control and working memory tasks, suggesting a higher involvement of the prefrontal cortex in adult females and parietal cortex in males [Christakou et al., 2009; Hill et al., 2014]. Such a difference may arise from distinct lifelong neural mechanisms in males and females underpinning the same cognitive processes. Several theories were



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advanced to explain age-related cognitive and brain changes, with a selective topographical vulnerability (e.g. in the frontal lobe), hypothesizing multiple possible causative factors and their interaction such as neurochemical deficiencies (e.g. dysregulation in the dopamine system), and decrease of inhibition and speed processing [see for review Drag and Bieliauskas, 2010]. The causes of gender differences in age-related hypometabolism however, are still elusive, but crucial factors might be considered, such as education, occupation and lifestyle habits, together with biological factors, including hormones, genes and differences in neurotransmission in the brain.

The second main result in HE was the significant gender differences in the correlations of educational and occupational attainments with brain metabolic activity. We found in males and females positive correlations between both education and occupation and increased brain metabolism, indicating the significant effect of education and occupation on healthy aging brain [Arenaza-Urquijo et al., 2013; Yoshizawa et al., 2014]. **These findings are compatible with the concept of BR. As above reported, healthy aging is typically associated with reduced metabolism, particularly in frontal lobes. Thus, the reported correlations of higher education or occupation with higher metabolism in specific brain regions could represent increased BR as a function of these life experiences.**

However, the correlations for males and females emerged in spatially distinct patterns (see Figure 2). On one hand, females showed a higher BR, as increased metabolism, in anterior medial frontal and orbitofrontal cortex, as well as in limbic structures. These brain regions are part of neural networks involved in cognitive control [see for reviews Koechlin and Hyafil, 2007; Ramnani and Owen, 2004] and in social-emotional processes [see for reviews Behrens et al., 2009; Lindquist et al., 2012]. Aging is typically associated with reduced metabolism in the frontal lobes, thus the reported correlations of higher education or occupation with relatively higher metabolism in these areas could represent increased BR as a function of these life experiences. On the other hand, in males, the BR was strong in parieto-temporal association cortex subserving cognitive functions such as visuospatial processes, attention, memory and action-related functions [see for reviews Cabeza et al., 2008; Husain and Nachev, 2007]. The differential involvement of these functional networks aligns with evidence from cognitive studies showing that females outperform males on emotion and social processing, such as labelling of facial expressions



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and memory for highly emotional events [see for review Christov-Moore et al., 2014], while males are better on visuospatial performances and on tasks for visuo-spatial memory [Hamilton, 2008; León et al., 2016; Postma et al., 2004]. Some MRI and fMRI studies reinforce these cognitive evidence reporting lifelong gender differences on GM volume and on the amount of task-induced BOLD changes in specific brain networks. For example, according to Gur et al. (2002) females have higher GM volume in orbitofrontal cortex, a core brain region involved in emotion processing, and ratio of orbitofrontal to amygdala volume, suggesting greater modulation of amygdala inputs by orbitofrontal cortex [Gur et al., 2002]. Moreover, several fMRI studies reported gender differences in the neural activations associated with the same tasks. For example, when dealing with cognitive control of emotions or social and empathic processes females compared to males showed more increased BOLD signal in the limbic structures and orbitofrontal cortex [Canessa et al., 2009; Canessa et al., 2011; Koch et al., 2007], while males in the parietal and dorsolateral prefrontal cortex [Koch et al., 2007]. Similarly, when performing a mental rotation task, males compared to females showed higher activation in the parietal cortex, while females showed additional activation in the inferior frontal gyrus [Hugdahl et al., 2006]. These brain structural and functional differences may reflect gender differences in strategies for cognitive processes.

Crucially, the aging effects here described in frontal regions were more limited in females, possibly as a result of the major influence of the life experiences in these structures (see Figure 2). We found a pattern of positive correlations with education and occupation in anterior frontal regions related to cognitive control in females, coherent with the Posterior Anterior Shift Aging (PASA) model [Davis et al., 2008]. According to the PASA model, an increased and compensatory frontal engagement in healthy individuals with high cognitive functioning might allow the maintenance of normal cognitive performances associated with specific cognitive demands. As for males, our results fit with previous evidence of age-related and task independent increases in parietal activity [Cabeza et al., 2004; Madden et al., 2007], indicating compensatory function of parietal activity in aging, that we found stronger in males. The neurobiological reasons for these identified differences in BR between males and females need however, to be determined. Crucial aspects



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might be the different levels of education and occupation between genders that were present in our HE series, with males showing significant higher scores than females on both education and occupation levels. Specifically, we hypothesize that the different levels and type of education and occupation, as well as different lifelong strategies for cognitive processes might explain at least in part the observed gender effects on BR. Social and educational gender differences throughout the entire life, together and in interaction with other biological factors, may lead to gender specific reorganization of brain structural and functional networks. This in turn leads to different neuroprotective and compensatory mechanisms of reserve in older age.

Furthermore, our results show that despite elderly females reach lower levels of education and occupation during life, they obtained higher scores in short and long term memory, as compared to males. A similar finding was reported by van Exel and colleagues [van Exel et al., 2001] who described better cognitive functions in females despite their lower level of education. The origin of the specificity of compensatory cerebral activities in females and males, as well as the differences in cognitive performance are in need of further investigation, considering also other constitutional and biological (e.g. hormonal and genetic) sex specificities.

***Alzheimer's Dementia.*** The analyses on the whole AD group revealed significant correlations between high levels of educational and occupational attainments and more severe brain hypometabolism, as shown by previous $^{18}$F-FDG-PET studies [Garibotto et al., 2008; Morbelli et al., 2013; Perneczky et al., 2006]. Accordingly, in our study, both female and male patients showed a more severe hypometabolism with increasing education and occupation levels in posterior AD-related brain regions (see Figure 3).

Of note, we report here for the first time that the effect of education and occupation, on brain hypometabolism in AD are different according to gender. Specifically, in males, occupation and, to a minor extent, education had a relevant effect on the posterior cerebral associative cortices (i.e. posterior cingulum/precuneus and temporo-parietal cortex), especially on the left hemisphere. In AD females, education had a comparable effect on brain metabolism in the same posterior brain regions, but also a strong effect in the medial frontal executive networks and in temporo-limbic structures involved in socio-affective



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processes (see Figure 3). This specific regional involvement in females encompassing mainly anterior brain networks, parallels the effects we found in HE females, namely a major sparing of the age related hypometabolism in frontal medial cortex in comparison to males. This finding in AD females might be interpreted as a further efficient mechanism that helps supporting cognitive performances in dementia. In the light of these results, we hypothesize that gender differences in the correlations of education and occupation with brain hypometabolism in AD might reflect different effects coping with neurodegeneration, with the recruitment of frontal executive neural resources in females.

To our knowledge, only another study investigated gender differences in brain glucose metabolism in AD patients, taking into account the concept of reserve and finding more severely reduced brain metabolism in males than in females [Perneczky et al., 2007]. Differently from our study, Perneczky et al. performed a group-comparison analysis between females and males restricted to only few a priori brain regions of interest. Moreover, they considered only education as a confounding variable, together with age and clinical severity, thus assessing only the passive model of reserve. Finally, also the sample size may contribute to the observed different results as compared to our study. In fact, they considered 93 AD patients, while we evaluated 282 AD cases.

In addition, applying the GAM in the AD group, the results showed the effect of aging on brain metabolism in the frontal medial regions and in the hippocampal structures (see Supporting Information Figure 2), only in AD males. This further confirms a higher susceptibility to aging effect in frontal lobe in males as compared to females. We suggest that frontal medial cortex and limbic hippocampal structures are more resistant in females coping better with aging and AD.

***Brain metabolic connectivity*.** Finally, the whole-brain metabolic connectivity analyses in three functional brain networks, namely the DMN, ECN and LN, revealed gender differences in both healthy aging and AD. In the HE, males showed a more extended pattern of metabolic connectivity in dorsal DMN as compared to females (see Figure 4A). This result is consistent with the finding of reserve-related increased metabolism in posterior regions in HE males. In AD patients, the dorsal DMN metabolic connectivity in parietal and temporal cortices was reduced in both genders, but the effect was particularly marked in male patients (see



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Figure 4B). Overall, this is consistent with the severe damage of the dorsal DMN frequently reported in AD patients [Buckner et al., 2008; Greicius et al., 2004]. Notably, both female and male AD patients showed preserved metabolic connectivity between posterior cingulum/precuneus and dorsolateral frontal cortex, but with different lateralization (left for females and right for males) that again might be associated with gender differences in lifelong cognitive strategies. Indeed, several studies reported that during the same task, males showed more activation in right cerebral hemisphere, while females in left hemisphere [Frings et al., 2006; Speck et al., 2000]. A similar gender asymmetry was reported also in resting state fMRI functional connectivity with visual network more right-lateralized in males and attentional networks more left-lateralized in females [Agcaoglu et al., 2015].

As for the ECN, Morbelli, Perneczky and colleagues (2013) showed an enhanced brain connectivity in AD patients with high educational levels [Morbelli et al., 2013]. In our study, metabolic connectivity in ECN was largely overlapped between males and females in the HE group (see Figure 4C). In AD patients, metabolic connectivity within the frontal cortex was even more extended than in HE (see Figure 4D). This aligns with the assumption of Grady et al. [Grady et al., 2003] for which recruitment of additional prefrontal regions in AD patients may reflect a compensative strategy to maintain cognitive function. Of note, female AD patients showed a more extended metabolic connectivity in fronto-parietal network (Figure 4D).

Lastly, we evaluated the metabolic connectivity in the LN, which is considerably involved in the debate on gender differences [Wallentin, 2009]. In HE, males compared to females showed a less left lateralized LN, thus involving bilaterally, frontal, temporal, parietal and cingulate cortices (see Figure 4E). These results supporting the hypothesis of reduced hemispheric asymmetry in older age with neural recruitment in the opposite hemisphere as proposed by the HAROLD (hemispheric asymmetry reduction in older adults) and by the CRUNCH (compensatory-related utilization of neural circuits hypothesis) models [Cabeza et al., 2002; Reuter-Lorenz and Cappell, 2008], suggest a major compensative strategy to maintain the same performance levels in males. In contrast, HE females showed a more asymmetric LN as compared to males. A better performance on language tasks, such as verbal recognition and fluency tests, was reported for females as compared to males in adult and elderly individuals [de Frias et al., 2006; McCarrey et al., 2016]. Consistently, structural MRI studies reported greater volume in brain regions of the LN (i.e., Broca's



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area and Wernicke's area) in adult females [Harasty et al., 1997; Luders et al., 2009]. This cognitive and structural evidence aligns with the LN metabolic connectivity in HE, suggesting a more efficient language network in females than in males. Consistently, female AD patients showed a more extended metabolic connectivity pattern than AD males (see Figure 4F). This result might reflect higher neuroprotective effects on LN in females than in males.

***Conclusions***. **Our findings support the significant gender differences in the effects of education and occupation on brain metabolism and cognitive resilience both in healthy aging and AD**. In HE individuals, we found age-related reduction of brain metabolism in frontal regions, more severe in males than in females. Accordingly, females showed metabolic increases related to higher levels of education and occupation and more metabolic connectivity in the anterior frontal and limbic brain networks, whereas for males in structures of the posterior DMN**.** These patterns,characterizing HE females and males, are in some way consistent with the results in the AD cohort in which males showed a more severe hypometabolism, related to high levels of education and occupation, in posterior associative regions, while females in frontal and temporo-limbic networks.

In this context, we want to highlight the importance of considering that in the past century, due to socio-economic factors, women had fewer opportunities for higher education and occupational attainments and were more engaged in familial and social activities. **These historical aspects with socially constructed gender roles, behaviours, activities, and attributes that that given society considers appropriate for men and women, are here mirrored in our elderly and AD samples**. **The gender differences we found might be explained by the levels and types of education and occupation and also by other sociodemographic factors that might contribute to differences between females and males in lifelong cognitive strategies and consequentially in brain function and networks. In addition, we should also consider other fundamental underlying biological determinants, such as genes and sex hormones that in turn contribute to brain modulation. The basis of gender differences in our findings is likely at the intersection between several sociodemographic and biological factors.**



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## Conflict of Interest

The authors have no actual or potential conflicts of interest.



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## References

Agcaoglu O, Miller R, Mayer AR, Hugdahl K, Calhoun VD (2015): Lateralization of resting state networks and relationship to age and gender. Neuroimage 104:310–325.

Alexander G, Furey M, Grady C, Pietrini P, Brady D, Mentis M, Schapiro M (1997): Association of premorbid intellectual function with cerebral metabolism in Alzheimer's disease: implications for the cognitive reserve hypothesis. Am J Psychiatry 154:165–172.

Arenaza-Urquijo EM, Landeau B, La Joie R, Mevel K, Mézenge F, Perrotin A, Desgranges B, Bartrés-Faz D, Eustache F, Chételat G (2013): Relationships between years of education and gray matter volume, metabolism and functional connectivity in healthy elders. Neuroimage 83:450–457.

Barnes LL, Wilson RS, Bienias JL, Schneider J a, Evans D a, Bennett D a (2005): Sex differences in the clinical manifestations of Alzheimer disease pathology. Arch Gen Psychiatry 62:685–691.

Barulli D, Stern Y (2013): Efficiency, capacity, compensation, maintenance, plasticity: emerging concepts in cognitive reserve. Trends Cogn Sci 17:502–509.

Behrens TEJ, Hunt LT, Rushworth MFS (2009): The computation of social behavior. Science 324:1160–1164.

Buckner RL, Andrews-Hanna JR, Schacter DL (2008): The brain's default network: Anatomy, function, and relevance to disease. Ann N Y Acad Sci 1124:1–38.

Cabeza R, Anderson ND, Locantore JK, McIntosh AR (2002): Aging gracefully: compensatory brain activity in high-performing older adults. Neuroimage 17:1394–1402.

Cabeza R, Ciaramelli E, Olson IR, Moscovitch M (2008): The parietal cortex and episodic memory: an attentional account. Nat Rev Neurosci 9:613–625.

Cabeza R, Daselaar SM, Dolcos F, Prince SE, Budde M, Nyberg L (2004): Task-independent and Task-specific Age Effects on Brain Activity during Working Memory, Visual Attention and Episodic Retrieval. Cereb Cortex 14:364–375.

Canessa N, Motterlini M, Alemanno F, Perani D, Cappa SF (2011): Learning from other people's experience: A neuroimaging study of decisional interactive-learning. Neuroimage 55:353–362.

Canessa N, Motterlini M, Di Dio C, Perani D, Scifo P, Cappa SF, Rizzolatti G (2009): Understanding others'



Corresponding author: Prof. Daniela Perani

regret: a FMRI study. PLoS One 4:e7402.

Cerami C, Dodich A, Greco L, Iannaccone S, Magnani G, Marcone A, Pelagallo E, Santangelo R, Cappa SF and Perani D (2016a): The Role of Single-Subject Brain Metabolic Patterns in the Early Differential Diagnosis of Primary Progressive Aphasias and in Prediction of Progression to Dementia. J Alzheimer's Dis pp 1–15.

Cerami C, Dodich A, Lettieri G, Iannaccone S, Magnani G, Marcone A, Gianolli L, Cappa SF and Perani D (2016b): Different FDG-PET metabolic patterns at single-subject level in the behavioral variant of fronto-temporal dementia. Cortex 83, pp.101-112.

Chételat G, Landeau B, Salmon E, Yakushev I, Bahri MA, Mézenge F, Perrotin A, Bastin C, Manrique A, Scheurich A, Scheckenberger M, Desgranges B, Eustache F, Fellgiebel A (2013): Relationships between brain metabolism decrease in normal aging and changes in structural and functional connectivity. Neuroimage 76:167–177.

Christakou A, Halari R, Smith AB, Ifkovits E, Brammer M, Rubia K (2009): Sex-dependent age modulation of frontostriatal and temporo-parietal activation during cognitive control. Neuroimage 48:223–236.

Christov-Moore L, Simpson EA, Coudé G, Grigaityte K, Iacoboni M, Ferrari PF (2014): Empathy: gender effects in brain and behavior. Neurosci Biobehav Rev 46 Pt 4:604–627.

Coffey CE, Lucke JF, Saxton JA, Ratcliff G, Unitas LJ, Billig B, Bryan RN (1998): Sex differences in brain aging: a quantitative magnetic resonance imaging study. Arch Neurol 55:169–179.

Corder EH, Ghebremedhin E, Taylor MG, Thal DR, Ohm TG, Braak H (2004): The biphasic relationship between regional brain senile plaque and neurofibrillary tangle distributions: Modification by age, sex, and APOE polymorphism. Ann N Y Acad Sci 1019:24–28.

Cowell PE, Turetsky BI, Gur RC, Grossman RI, Shtasel DL, Gur RE (1994): Sex differences in aging of the human frontal and temporal lobes. J Neurosci 14:4748–55.

Davis SW, Dennis NA, Daselaar SM, Fleck MS, Cabeza R (2008): Que PASA? The posterior-anterior shift in aging. Cereb Cortex 18:1201–1209.

Della Rosa PA, Cerami C, Gallivanone F, Prestia A, Caroli A, Castiglioni I, Gilardi MC, Frisoni G, Friston K, Ashburner J, Perani D (2014): A Standardized [18F]-FDG-PET Template for Spatial Normalization



Corresponding author: Prof. Daniela Perani

in Statistical Parametric Mapping of Dementia. Neuroinformatics 12:575–593.

Drag LL, Bieliauskas L a (2010): Contemporary review 2009: cognitive aging. J Geriatr Psychiatry Neurol 23:75–93.

Foubert-Samier A, Catheline G, Amieva H, Dilharreguy B, Helmer C, Allard M, Dartigues J-F (2012): Education, occupation, leisure activities, and brain reserve: a population-based study. Neurobiol Aging 33:423.e15-25.

de Frias CM, Nilsson L-G, Herlitz A (2006): Sex differences in cognition are stable over a 10-year period in adulthood and old age. Neuropsychol Dev Cogn B Aging Neuropsychol Cogn 13:574–587.

Frings L, Wagner K, Unterrainer J, Spreer J, Halsband U, Schulze-Bonhage A (2006): Gender-related differences in lateralization of hippocampal activation and cognitive strategy. Neuroreport 17:417–421.

Fujimoto T, Matsumoto T, Fujita S, Takeuchi K, Nakamura K, Mitsuyama Y, Kato N (2008): Changes in glucose metabolism due to aging and gender-related differences in the healthy human brain. Psychiatry Res 164:58–72.

Garibotto V, Borroni B, Kalbe E, Herholz K, Salmon E, Holtoff V, Sorbi S, Cappa SF, Padovani a., Fazio F, Perani D (2008): Education and occupation as proxies for reserve in aMCI converters and AD: FDG-PET evidence. Neurology 71:1342–1349.

Garibotto V, Tettamanti M, Marcone A, Florea I, Panzacchi A, Moresco R, Virta JR, Rinne J, Cappa SF, Perani D (2013): Cholinergic activity correlates with reserve proxies in Alzheimer's disease. Neurobiol Aging 34:2694.e13-2694.e18.

Good CD, Johnsrude IS, Ashburner J, Henson RNA, Friston KJ, Frackowiak RSJ (2001): A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage 14:21–36.

Grady CL, McIntosh AR, Beig S, Keightley ML, Burian H, Black SE (2003): Evidence from functional neuroimaging of a compensatory prefrontal network in Alzheimer's disease. J Neurosci 23:986–993.

Greicius MD, Srivastava G, Reiss AL, Menon V (2004): Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci U S A 101:4637–4642.

Gur RC, Mozley PD, Resnick SM, Gottlieb GL, Kohn M, Zimmerman R, Herman G, Atlas S, Grossman R,



Corresponding author: Prof. Daniela Perani

Berretta D (1991): Gender differences in age effect on brain atrophy measured by magnetic resonance imaging. Proc Natl Acad Sci U S A 88:2845–2849.

Gur RC, Gunning-Dixon F, Bilker WB, Gur RE (2002): Sex differences in temporo-limbic and frontal brain volumes of healthy adults. Cereb Cortex 12:998–1003.

Hamilton C (2008): Cognition and Sex Differences. Palgrave Macmillan, New York.

Harasty J, Double KL, Halliday GM, Kril JJ, McRitchie DA (1997): Language-associated cortical regions are proportionally larger in the female brain. Arch Neurol 54:171–176.

Hill AC, Laird AR, Robinson JL (2014): Gender differences in working memory networks: a BrainMap meta-analysis. Biol Psychol 102:18–29.

Horwitz B, Duara R, Rapoport SI (1984): Intercorrelations of glucose metabolic rates between brain regions: application to healthy males in a state of reduced sensory input. J Cereb Blood Flow Metab 4:484–499.

Hugdahl K, Thomsen T, Ersland L (2006): Sex differences in visuo-spatial processing: An fMRI study of mental rotation. Neuropsychologia 44:1575–1583.

Husain M, Nachev P (2007): Space and the parietal cortex. Trends Cogn Sci 11:30–36.

Kakimoto A, Ito S, Okada H, Nishizawa S, Minoshima S, Ouchi Y (2015): Age-Related Sex-Specific Changes in Brain Metabolism and Morphology. J Nucl Med 57:221–225.

Kalpouzos G, Chételat G, Baron JC, Landeau B, Mevel K, Godeau C, Barré L, Constans JM, Viader F, Eustache F, Desgranges B (2009): Voxel-based mapping of brain gray matter volume and glucose metabolism profiles in normal aging. Neurobiol Aging 30:112–124.

Katzman R, Terry R, DeTeresa R, Brown T, Davies P, Fuld P, Renbing X, Peck A (1988): Clinical, pathological, and neurochemical changes in dementia: A subgroup with preserved mental status and numerous neocortical plaques. Ann Neurol 23:138–144.

Kawachi T, Ishii K, Sakamoto S, Matsui M, Mori T, Sasaki M (2002): Gender differences in cerebral glucose metabolism: A PET study. J Neurol Sci 199:79–83.

Koch K, Pauly K, Kellermann T, Seiferth NY, Reske M, Backes V, Stöcker T, Shah NJ, Amunts K, Kircher T, Schneider F, Habel U (2007): Gender differences in the cognitive control of emotion: An fMRI study. Neuropsychologia 45:2744–2754.



Corresponding author: Prof. Daniela Perani

Koechlin E, Hyafil A (2007): Anterior prefrontal function and the limits of human decision-making. Science 318:594–598.

Lee DS, Kang H, Kim H, Park H, Oh JS, Lee JS, Lee MC (2008): Metabolic connectivity by interregional correlation analysis using statistical parametric mapping (SPM) and FDG brain PET; methodological development and patterns of metabolic connectivity in adults. Eur J Nucl Med Mol Imaging 35:1681–1691.

Lemaître H, Crivello F, Grassiot B, Alpérovitch A, Tzourio C, Mazoyer B (2005): Age- and sex-related effects on the neuroanatomy of healthy elderly. Neuroimage 26:900–911.

Lemaitre H, Goldman AL, Sambataro F, Verchinski BA, Meyer-Lindenberg A, Weinberger DR, Mattay VS (2012): Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? Neurobiol Aging 33:617.e1-617.e9.

León I, Tascón L, Cimadevilla JM (2016): Age and gender-related differences in a spatial memory task in humans. Behav Brain Res 306:8–12.

Lindquist KA, Wager TD, Kober H, Bliss-Moreau E, Barrett LF (2012): The brain basis of emotion: A meta-analytic review. Behav Brain Sci 35:121–143.

Liu Y, Julkunen V, Paajanen T, Westman E, Wahlund LO, Aitken A, Sobow T, Mecocci P, Tsolaki M, Vellas B, Muehlboeck S, Spenger C, Lovestone S, Simmons A, Soininen H (2012): Education increases reserve against Alzheimer's disease-Evidence from structural MRI analysis. Neuroradiology 54:929–938.

Luders E, Gaser C, Narr KL, Toga AW (2009): Why Sex Matters: Brain Size Independent Differences in Gray Matter Distributions between Men and Women. J Neurosci 29:14265–14270.

Madden DJ, Spaniol J, Whiting WL, Bucur B, Provenzale JM, Cabeza R, White LE, Huettel SA (2007): Adult age differences in the functional neuroanatomy of visual attention: A combined fMRI and DTI study. Neurobiol Aging 28:459–476.

McCarrey AC, An Y, Kitner-Triolo MH, Ferrucci L, Resnick SM (2016): Sex differences in cognitive trajectories in clinically normal older adults. Psychol Aging 31:166–175.

McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, Klunk WE, Koroshetz WJ,



Corresponding author: Prof. Daniela Perani

Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011): The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7:263–269.

Mielke MM, Vemuri P, Rocca W a. (2014): Clinical epidemiology of Alzheimer's disease: Assessing sex and gender differences. Clin Epidemiol 6:37–48.

Morbelli S, Perneczky R, Drzezga A, Frisoni GB, Caroli A, van Berckel BNM, Ossenkoppele R, Guedj E, Didic M, Brugnolo A, Naseri M, Sambuceti G, Pagani M, Nobili F (2013): Metabolic networks underlying cognitive reserve in prodromal Alzheimer disease: a European Alzheimer disease consortium project. J Nucl Med 54:894–902.

Murphy DG, DeCarli C, McIntosh AR, Daly E, Mentis MJ, Pietrini P, Szczepanik J, Schapiro MB, Grady CL, Horwitz B, Rapoport SI (1996): Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. Arch Gen Psychiatry 53:585–594.

Pardo J V., Lee JT, Sheikh SA, Surerus-Johnson C, Shah H, Munch KR, Carlis J V., Lewis SM, Kuskowski MA, Dysken MW (2007): Where the brain grows old: Decline in anterior cingulate and medial prefrontal function with normal aging. Neuroimage 35:1231–1237.

Perani D (2014): FDG-PET and amyloid-PET imaging: the diverging paths. Curr Opin Neurol 27(4), pp 405-413.

Perani D, Cerami C, Caminiti SP, Santangelo R, Coppi E, Ferrari L, Pinto P, Passerini G, Falini A, Iannaccone S and Cappa SF (2016): Cross-validation of biomarkers for the early differential diagnosis and prognosis of dementia in a clinical setting. Eur J Nucl Med Mol Imaging 43(3), pp.499-508.

Perani D, Della Rosa PA, Cerami C, Gallivanone F, Fallanca F, Giovanna E, Panzacchi A, Nobili F, Pappatà S, Marcone A, Garibotto V, Castiglioni I, Magnani G, Cappa SF, Gianolli L, Consortium E (2014): Validation of an optimized SPM procedure for FDG-PET in dementia diagnosis in a clinical setting. NeuroImage Clin 6:445–454.

Perneczky R, Drzezga a., Diehl-Schmid J, Li Y, Kurz a. (2007): Gender differences in brain reserve: An



Corresponding author: Prof. Daniela Perani

18F-FDG PET study in Alzheimer's disease. J Neurol 254:1395–1400.

Perneczky R, Drzezga A, Diehl-Schmid J, Schmid G, Wohlschläger A, Kars S, Grimmer T, Wagenpfeil S, Monsch A, Kurz A (2006): Schooling mediates brain reserve in Alzheimer's disease: findings of fluoro-deoxy-glucose-positron emission tomography. J Neurol Neurosurg Psychiatry 77:1060–1063.

Postma A, Jager G, Kessels RPC, Koppeschaar HPF, Van Honk J (2004): Sex differences for selective forms of spatial memory. Brain Cogn 54:24–34.

Ramnani N, Owen AM (2004): Anterior prefrontal cortex: insights into function from anatomy and neuroimaging. Nat Rev Neurosci 5:184–194.

Raz N, Gunning-Dixon F, Head D, Rodrigue KM, Williamson A, Acker JD (2004): Aging, sexual dimorphism, and hemispheric asymmetry of the cerebral cortex: Replicability of regional differences in volume. Neurobiol Aging 25:377–396.

Raz N, Gunning FM, Head D, Dupuis JH, McQuain J, Briggs SD, Loken WJ, Thornton AE, Acker JD (1997): Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter. Cereb Cortex 7:268–282.

Reuter-Lorenz PA, Cappell KA (2008): Neurocognitive Aging and the Compensation Hypothesis. Curr Dir Psychol Sci 17:177–182.

Ridderinkhof KR, Ullsperger M, Crone EA, Nieuwenhuis S (2004): The role of the medial frontal cortex in cognitive control. Science 306:443–447.

Rowe CC, Ellis K a., Rimajova M, Bourgeat P, Pike KE, Jones G, Fripp J, Tochon-Danguy H, Morandeau L, O'Keefe G, Price R, Raniga P, Robins P, Acosta O, Lenzo N, Szoeke C, Salvado O, Head R, Martins R, Masters CL, Ames D, Villemagne VL (2010): Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. Neurobiol Aging 31:1275–1283.

Rushworth MF, Buckley MJ, Behrens TE, Walton ME, Bannerman DM (2007): Functional organization of the medial frontal cortex. Curr Opin Neurobiol 17:220–227.

Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RSR, Busa E, Morris JC, Dale AM, Fischl B (2004): Thinning of the cerebral cortex in aging. Cereb Cortex 14:721–730.

Seshadri S, Wolf P a, Beiser a, Au R, McNulty K, White R, D'Agostino RB (1997): Lifetime risk of



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dementia and Alzheimer's disease. The impact of mortality on risk estimates in the Framingham Study. Neurology 49:1498–1504.

Shirer WR, Ryali S, Rykhlevskaia E, Menon V, Greicius MD (2012): Decoding subject-driven cognitive states with whole-brain connectivity patterns. Cereb Cortex 22:158–165.

Smith CD, Chebrolu H, Wekstein DR, Schmitt FA, Markesbery WR (2007): Age and gender effects on human brain anatomy: A voxel-based morphometric study in healthy elderly. Neurobiol Aging 28:1075–1087.

Solé-Padullés C, Bartrés-Faz D, Junqué C, Vendrell P, Rami L, Clemente IC, Bosch B, Villar A, Bargalló N, Jurado MA, Barrios M, Molinuevo JL (2009): Brain structure and function related to cognitive reserve variables in normal aging, mild cognitive impairment and Alzheimer's disease. Neurobiol Aging 30:1114–1124.

Speck O, Ernst T, Braun J, Koch C, Miller E, Chang L (2000): Gender differences in the functional organization of the brain for working memory. Neuroreport 11:2581–2585.

Stern Y (2002): What is cognitive reserve? Theory and research application of the reserve concept. J Int Neuropsychol Soc 8:448–460.

Stern Y (2012): Cognitive reserve in ageing and Alzheimer's disease. Lancet Neurol 11:1006–1012.

**Steffener J, Stern Y (2013): Exploring the Neural Basis of Cognitive Reserve in Aging 1822:467–473.**

Sullivan E V., Rosenbloom M, Serventi KL, Pfefferbaum A (2004): Effects of age and sex on volumes of the thalamus, pons, and cortex. Neurobiol Aging 25:185–192.

Takahashi R, Ishii K, Kakigi T, Yokoyama K (2011): Gender and age differences in normal adult human brain: Voxel-based morphometric study. Hum Brain Mapp 32:1050–1058.

Teipel SJ, Meindl T, Wagner M, Kohl T, Bürger K, Reiser MF, Herpertz S, Möller H-J, Hampel H (2009): White matter microstructure in relation to education in aging and Alzheimer's disease. J Alzheimers Dis 17:571–583.

Thambisetty M, Wan J, Carass A, An Y, Prince JL, Resnick SM (2010): Longitudinal changes in cortical thickness associated with normal aging. Neuroimage 52:1215–1223.

Tisserand DJ, Jolles J (2003): On the involvement of prefrontal networks in cognitive ageing. Cortex 39:1107–1128.



Corresponding author: Prof. Daniela Perani

Tucker AM, Stern Y (2011): Cognitive reserve in aging. Curr Alzheimer Res 8:354–360.

van Exel E, Gussekloo J, de Craen AJ, Bootsma-van der Wiel A, Houx P, Knook DL, Westendorp RG (2001): Cognitive function in the oldest old: women perform better than men. J Neurol Neurosurg Psychiatry 71:29–32.

Varrone A, Asenbaum S, Vander Borght T, Booij J, Nobili F, Någren K, Darcourt J, Kapucu OL, Tatsch K, Bartenstein P, Van Laere K, European Association of Nuclear Medicine Neuroimaging Committee (2009): EANM procedure guidelines for PET brain imaging using [18F]FDG, version 2. Eur J Nucl Med Mol Imaging 36:2103–2110.

Wallentin M (2009): Putative sex differences in verbal abilities and language cortex: A critical review. Brain Lang 108:175–183.

Willis MW, Ketter T a, Kimbrell T a, George MS, Herscovitch P, Danielson AL, Benson BE, Post RM (2002): Age, sex and laterality effects on cerebral glucose metabolism in healthy adults. Psychiatry Res 114:23–37.

Xu J, Kobayashi S, Yamaguchi S, Iijima K, Okada K, Yamashita K (2000): Gender effects on age-related changes in brain structure. AJNR Am J Neuroradiol 21:112–118.

Yoshizawa H, Gazes Y, Stern Y, Miyata Y, Uchiyama S (2014): Characterizing the normative profile of 18F-FDG PET brain imaging: sex difference, aging effect, and cognitive reserve. Psychiatry Res 221:78–85.





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## Figure Legends

**Figure 1.** Aging effect in healthy elderly female and male groups. Left: Results of negative SPM linear correlations between age and $^{18}$F-FDG-PET glucose metabolism in females (pink) and males (blue). Correlations are shown at $p<0.05$ with FWE correction for multiple comparisons.

Right: Function representations of age (x-axis) and brain metabolism (y-axis) in females (pink) and males (blue), obtained by Generalized Additive Model analysis.

Continuous lines: estimate functions between age and brain metabolism; Dashed lines: 2 standard errors above and below the estimate function.

Abbreviations: ROI = region of interest; FDG-PET = positron emission tomography imaging with Fluorine-18-Flourodeoxiglucose.

**Figure 2.** Healthy elderly groups. Positive correlations between education (A) and occupation (B) and $^{18}$F-FDG-PET glucose metabolism in females (pink) and males (blue). Correlations are represented in MRIcron at $p<0.01$ uncorrected to show all gender differences in this explorative voxel-based analysis (see Supporting Information Table 2 for anatomical details and level of significance).

**Figure 3.** Patients with Alzheimer's Dementia. Negative correlations between education (A) and occupation (B) and $^{18}$F-FDG-PET glucose metabolism in females (pink) and males (blue). Correlations are represented in MRIcron at $p<0.01$ uncorrected to show all gender differences in this explorative voxel-based analysis (see Supporting Information Table 3 for anatomical details and level of significance).

**Figure 4**. Brain metabolic connectivity results. Healthy elderly subjects (A, C, E) and patients with Alzheimer's Dementia (B, D, F). Females (pink), males (blue) and overlap (violet). From top to bottom: dorsal Default Mode Network (seeds: posterior cingulum/precuneus); Executive Control Network (seeds: middle frontal and superior frontal gyri); Language Network (seeds: left angular, middle and superior temporal gyri). All the results are shown at $p<0.01$ FDR.



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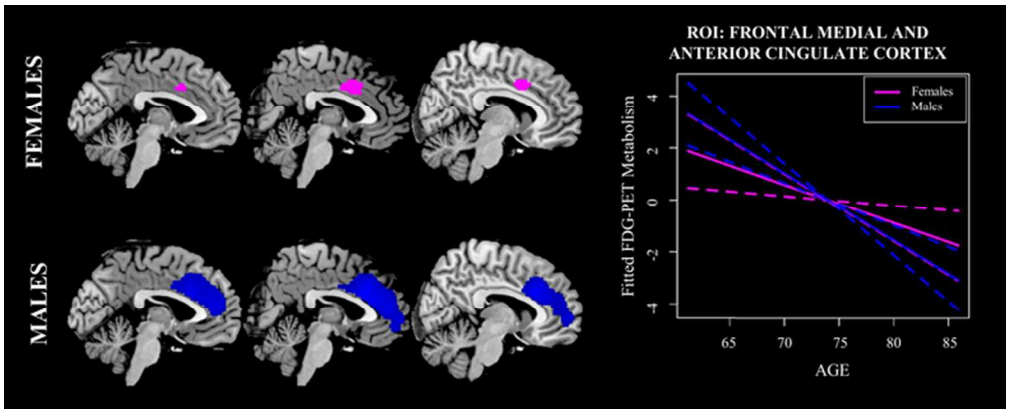


Figure 1. Aging effect in healthy elderly female and male groups. Left: Results of negative SPM linear correlations between age and 18F-FDG-PET glucose metabolism in females (pink) and males (blue). Correlations are shown at $p<0.05$ with FWE correction for multiple comparisons.
Right: Function representations of age (x-axis) and brain metabolism (y-axis) in females (pink) and males (blue), obtained by Generalized Additive Model analysis.
Continuous lines: estimate functions between age and brain metabolism; Dashed lines: 2 standard errors above and below the estimate function.
Abbreviations: ROI = region of interest; FDG-PET = positron emission tomography imaging with Fluorine-18-Flourodeoxiglucose.

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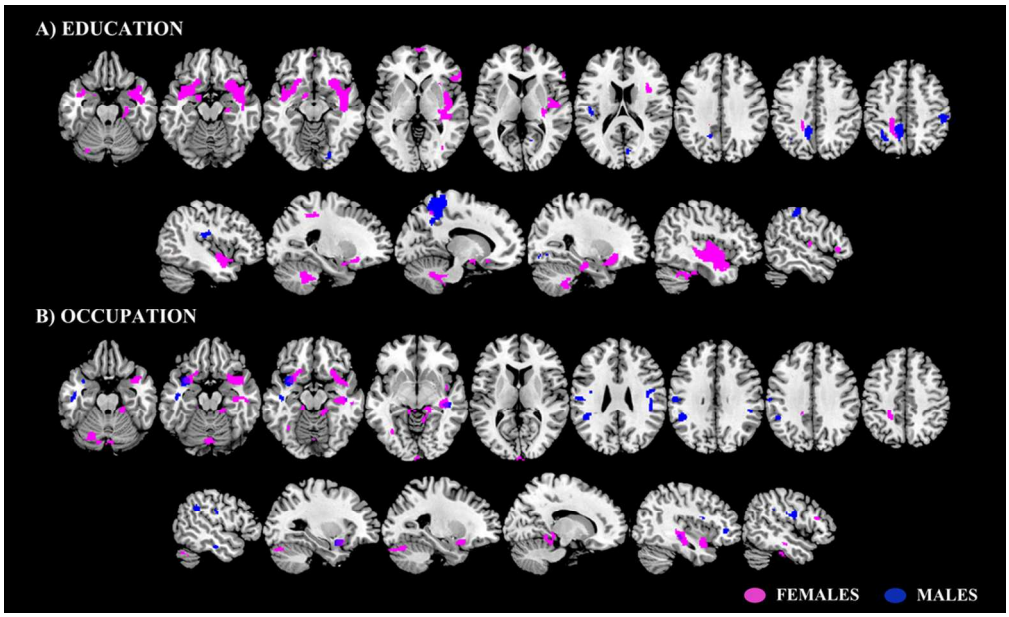


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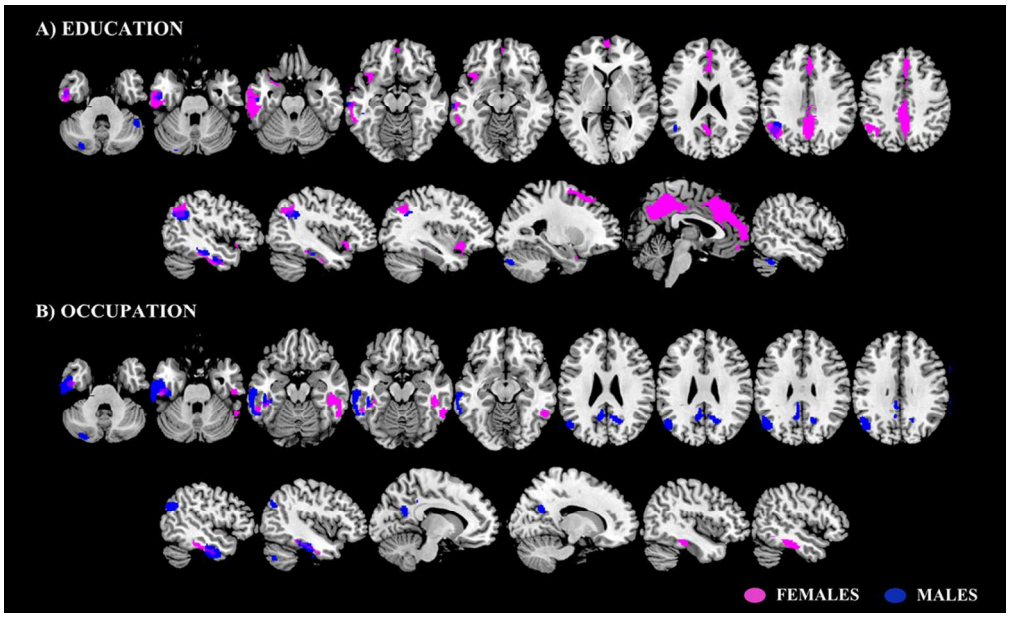


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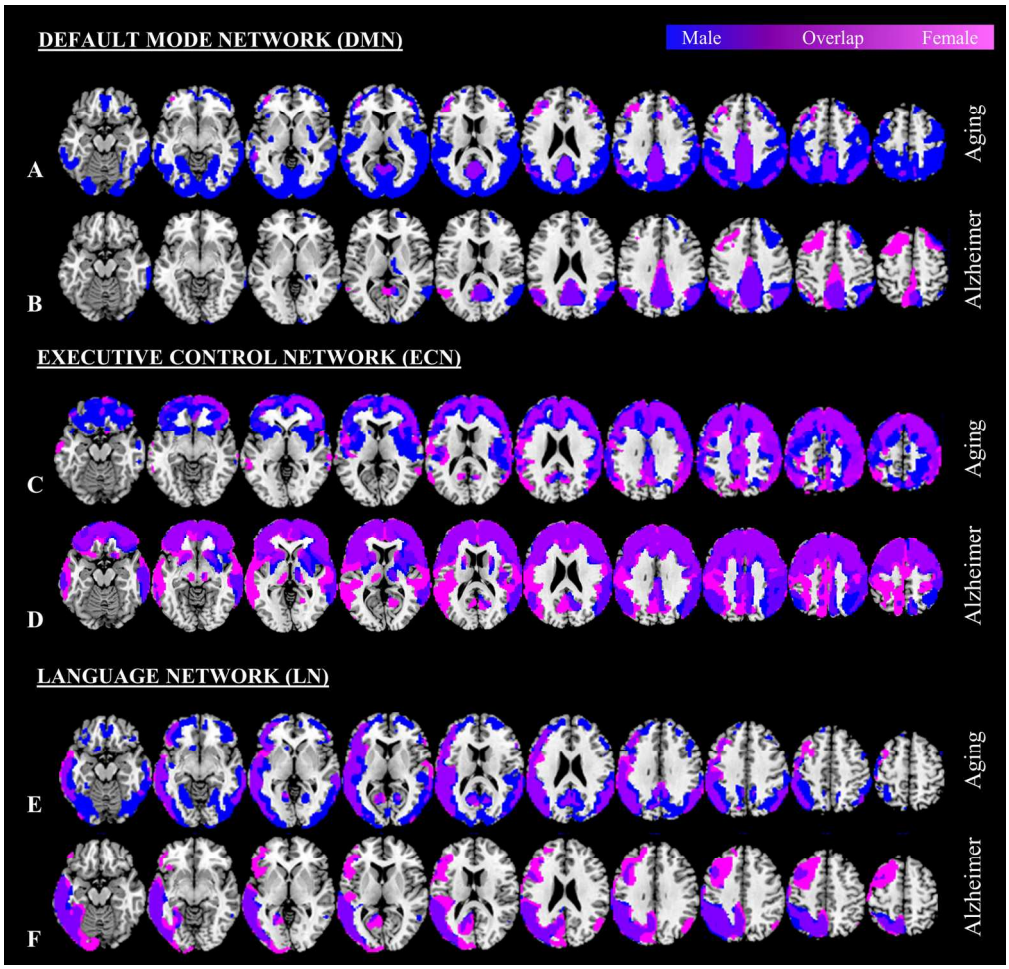


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157x151mm (300 x 300 DPI)

**Table I.** Means and standard deviations of the main characteristics for males and females in healthy elderly group and significance of t-test between genders comparisons (* = significant difference at the two sample t-test comparing males and females).

| HEALTHY SUBJECTS | WHOLE GROUP (N = 225) | MALES (N = 115) | FEMALES (N = 110) | p |
|---|---|---|---|---|
| AGE (yrs) | 73.96 ± 5.87 | 74.83 ± 6.19 | 73.06 ± 5.41 | 0.024* |
| MMSE | 29.03 ± 1.23 | 28.95 ± 1.31 | 29.12 ± 1.14 | 0.300 |
| EDUCATION (yrs) | 16.36 ± 2.74 | 17.10 ± 2.75 | 15.59 ± 2.51 | 0.0001* |
| OCCUPATION | 4.88 ± 0.98 | 5.24 ± 0.86 | 4.51 ± 0.95 | 0.0001* |
| NEUROPSYCHOLOGICAL DOMAINS | | | | |
| SEMANTIC FLUENCY | 0.00 ± 0.98 | -0.03 ± 0.99 | 0.03 ± 0.97 | 0.662 |
| LONG-TERM MEMORY | -0.03 ± 0.99 | -0.22 ± 1.05 | 0.17 ± 0.90 | 0.003* |
| SHORT-TERM MEMORY | -0.02 ± 0.99 | -0.23 ± 1.06 | 0.20 ± 0.87 | 0.001* |
| ATTENTION/EXECUTIVE | -0.02 ± 1.00 | -0.06 ± 1.06 | 0.02 ± 0.95 | 0.560 |
| VISUOSPATIAL ABILITIES | 0.02 ± 0.98 | 0.12 ± 0.86 | -0.08 ± 1.08 | 0.123 |





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**Table II**. Means and standard deviations of the main characteristics for male and female AD patients and significance of t-test between genders comparisons (* = significant difference at the two sample t-test comparing males and females).

| AD PATIENTS | WHOLE GROUP (N = 282) | MALES (N = 123) | FEMALES (N = 159) | p |
|---|---|---|---|---|
| AGE (yrs) | 74.50 ± 5.37 | 74.83 ± 5.99 | 74.25 ± 4.84 | 0.367 |
| DISEASE DURATION (yrs) | 3.52 ± 2.28 | 3.68 ± 2.32 | 3.39 ± 2.25 | 0.307 |
| MMSE | 21.83 ± 3.88 | 21.90 ± 3.63 | 21.77 ± 4.07 | 0.769 |
| EDUCATION (yrs) | 11.67 ± 4.82 | 13.25 ± 4.82 | 10.45 ± 4.47 | 0.0001* |
| OCCUPATION | 4.13 ± 1.23 | 4.57 ± 1.28 | 3.76 ± 1.06 | 0.0001* |
| NEUROPSYCHOLOGICAL DOMAINS | | | | |
| SEMANTIC FLUENCY | -1.73 ± 0.95 | -1.62 ± 0.95 | -1.82 ± 0.95 | 0.072 |
| LONG-TERM MEMORY | -2.18 ± 0.61 | -2.11 ± 0.55 | -2.24 ± 0.65 | 0.062 |
| SHORT-TERM MEMORY | -1.42 ± 1.78 | -1.72 ± 1.55 | -1.19 ± 1.91 | 0.014* |
| ATTENTION/EXECUTIVE | -2.12 ± 3.11 | -2.22 ± 3.20 | -2.04 ± 3.04 | 0.628 |
| VISUOSPATIAL ABILITIES | -0.63 ± 2.92 | -0.99 ± 3.06 | -0.35 ± 2.78 | 0.071 |





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