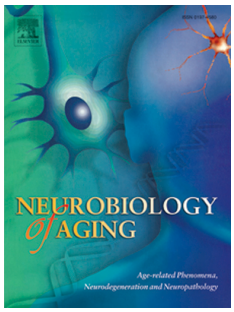


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**Basal forebrain metabolism in Alzheimer's Disease continuum: relationship with education**

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**Keywords**: basal forebrain, fluorodeoxyglucose (FDG), positron emission tomography (PET), reserve, education, Alzheimer's Disease (AD)

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**ABSTRACT**

We analyzed education, as a proxy of cognitive reserve (CR), and the cholinergic pathway in Alzheimer's disease (AD), to test the hypothesis that education might modulate the relationship between clinical symptoms and metabolic and structural changes in AD. We included 84 subjects and compared between diagnostic groups and different educational levels the glucose metabolism and volume of basal forebrain (BFM and BFV), the major cholinergic structure, and hippocampus (HM and HV), a relevant projection site for the BF. Correlations with the global cognitive status and education in the whole sample were also performed. AD dementia patients showed reduced BFV, HV and HM compared to controls. In the whole group, the global cognitive status was positively correlated with BFM and HM. Among high-educated subjects, mild cognitive impairment (MCI) showed higher BFM and HM in comparison to other diagnostic groups. Our results suggest that in MCI subjects with a higher educational level cholinergic activity is upregulated and this appears to have a compensatory effect, which may be lost in later symptomatic stages.

## 1. INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia, present in 50% to 75% of cases and mostly concerning individuals over 60 years of age (Prince et al., 2016). Before overt AD dementia, patients usually present a syndromic picture characterized by mild cognitive impairment (MCI), whilst functional autonomy is still preserved. The relationship between pathology evolution and clinical symptoms severity is not linear. Disease progression might vary according to other risk factors (e.g., (de Bruijn and Ikram, 2014)) and modulatory variables, such as the cognitive reserve (CR). CR has been introduced to describe the different individual susceptibilities to the disease and it is associated with multiple life experiences, e.g., education, occupational level and leisure activities, that exert a significant compensatory effect on the clinical expression of neurodegenerative diseases (Stern et al., 2018). The hypothesis that CR, often approximated using the years of formal education (Stern et al., 2018; Valenzuela and Sachdev, 2006), modulates the clinical presentation and progression of neurodegenerative diseases obtained strong confirmation by imaging studies, showing that among AD patients with comparable disease severity, those with higher CR exhibit more severe brain dysfunction, already in the MCI phase (Arenaza-Urquijo and Vemuri, 2018; Garibotto et al., 2008; Morbelli and Nobili, 2014). Recent evidence suggests that the modulatory effect of education might also extend to the cholinergic system (Garibotto et al., 2013; Kim et al., 2012; Marcone et al., 2012), which is selectively affected early in AD (Grothe et al., 2014; Hall et al., 2008).

The basal forebrain (BF) represents one of the main cholinergic structure in the brain and includes the diagonal band of Broca, the substantia innominata, the medial septum nuclei and the nucleus basalis of Meynert. According to Mesulam's nomenclature (Mesulam et al., 1983), as modified and implemented by Zaborszky (Zaborszky et al., 2008), the cholinergic nuclei can be classified in compartments Ch1 to Ch4: Ch1 and Ch2 mainly corresponding to the medial septum and the vertical limb of the diagonal band, Ch3 to the horizontal limb of the diagonal band and Ch4 to the nucleus basalis of Meynert. BF atrophy has been extensively observed both in MCI and dementia patients (Grothe et al., 2012; Grothe et al., 2014; Grothe et al., 2016; Hall et al., 2008; Kilimann et al., 2014; Teipel et al., 2016), and it is associated to cognitive impairment and amyloid positivity (Grothe et al., 2010; Kerbler et al., 2015; Richter et al., 2014; Teipel et al., 2014). Notably, while the neocortex and amygdala are supplied by cholinergic neurons in the posterior basal nucleus of Meynert, BF sub-regions Ch1 and Ch2 represent the main origins of cholinergic projections to the hippocampus, possibly stimulating processes of neuroplasticity and neurogenesis in this region (Colgin et al., 2003; Grothe et al., 2010). Since both BF and hippocampus are well-known areas of early neurodegeneration in AD (Jack et al., 2018; Kilimann et al., 2014; Mosconi et al., 2005; Sabri et al., 2018) and they have been both related to neuroplasticity processes, it is conceivable that these regions are those sensitive to modulatory phenomena such as CR. However, to the best of our knowledge studies investigating this hypothesis are still sparse. A previous study comparing in healthy controls, MCI and AD

patients the uptake of [18F]-fluorodeoxyglucose (FDG) in the BF provided evidence of a higher FDG uptake in MCI compared to the other two groups (Kim et al., 2012). Moreover, while in low-educated subjects the relationship between MMSE and FDG uptake was mainly represented by a positive linear regression curve, those with higher education showed an inverted-U relationship (Kim et al., 2012). A previous study of our group using the PET tracer Carbon11 labeled N-methyl-4-piperidyl-acetate to quantify acetylcholinesterase activity, reported in prodromal and early AD phase a significant positive association between the activity of the cholinergic projections in the left and right hippocampus and CR measurements (Garibotto et al., 2013). These findings suggest that the CR mechanisms might result in an increased or preserved function of the cholinergic system in these subjects. While volumetric changes in the hippocampus represent a validated imaging biomarker in AD (Jack et al., 2018), and BF atrophy has been extensively reported in AD and MCI subjects (Kilimann et al., 2014; Teipel et al., 2016), metabolic changes in these regions as evaluated by FDG-PET often went unnoticed possibly due to low spatial resolution of early generation PET scans or to possible inaccuracies in automated voxel-based analyses when performing statistical comparison at whole-brain level. While spatial resolution of conventional PET scanners for small or deep subcortical structures, such as the BF, is still limited (Heiss et al., 2004), the introduction of the high-resolution research tomograph (HRRT) allows a high spatial resolution (approximately 2.5mm) and a more reliable measurement of FDG uptake in these structures. Thus, in this study, by using volumetric MRI and HRRT-PET data, we aim to 1) examine in different AD-related clinical stages the significant alterations in volume and glucose metabolism in BF and hippocampus; 2) assess the association of these metabolic and volumetric indexes with the educational level.

## 2. MATERIAL AND METHODS

### *2.1 Subjects*

Cohort data for this study was obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.ucla.edu), a large longitudinal multicenter initiative with the purpose of studying and identifying helpful biomarkers for early detection and staging of AD. This database encompasses clinical, genetic, imaging and biochemical items. Such biomarkers are also useful for differential diagnosis from other causes of dementia and serve as surrogate markers for treatment efficacy. ADNI is composed of four phases: ADNI 1, ADNI GO, ADNI 2 and ADNI 3. Each new phase increases the amount of patients and study techniques included in the study.

The participants of the present study were selected from the ADNI1, ADNI2 and ADNI GO databases if they had undergone HRRT PET and a volumetric MRI. Diagnostic categories are detailed in Table 1. Subjects without information on disease severity (i.e., MCI belonging to ADNI 1 initiative) were excluded. Besides, among patient subgroups, only those with an amyloid positive profile were considered. A final sample of 84

subjects was included in the current study, including 28 healthy controls and 56 amyloid positive subjects with cognitive deficits or complaints. See Table 2 for demographic and clinical features.

**Table 1:** Table defining the diagnostic criteria for each disease stage. http://adni.loni.usc.edu/study-design/background-rationale/

| | | | |
|---|---|---|---|
| **CN** | **Normal Aging /Cognitively Normal** | ADNI 1/GO/2 | CN participants are the control subjects in the ADNI study. They show no signs of depression, mild cognitive impairment or dementia. |
| **SMC** | **Significant Memory Concern** | ADNI 2 | SMC participants score within normal range for cognition (or CDR = 0) but indicate that they have a concern, and exhibit slight forgetfulness. The informant does not equate this as progressive memory impairment nor considers this as consistent forgetfulness. |
| **EMCI** | **Early Mild Cognitive Impairment** | ADNI GO/2 | MCI participants have reported a subjective memory concern either autonomously or via an informant or clinician. However, there are no significant levels of impairment in other cognitive domains, essentially preserved activities of daily living and there are no signs of dementia. Levels of MCI (early or late) are determined using the Wechsler Memory Scale Logical Memory II. |
| **LMCI** | **Late Mild Cognitive** Impairment | ADNI GO/2 | |
| **AD** | **Alzheimer's disease** | ADNI 1/GO/2 | AD participants have been evaluated and meet the NINCDS/ADRDA criteria for probable AD. |

**Table 2.** Demographic and clinical characteristics of the diagnostic groups. HC: healthy controls, SMC: significant memory complaints, MCI: mild cognitive impairment (EMCI=early, LMC=late), AD: Alzheimer's disease. M±SD: mean± standard deviation. * MMSE score obtained at the clinical visit closest to the FDG-PET exam

| | HC (n=28) | SMC (n=7) | MCI (n=33) (19 EMCI, 14 LMCI) | AD (n=16) | Statistics |
|---|---|---|---|---|---|
| *Age in years (M ± SD)* | 73.9 ± 7.1 | 70.8 ± 6.6 | 73.3±7.4 | 75.6 ± 8.0 | p=0.52 |
| *Gender (F/M)* | 10/18 | 5/2 | 13/20 | 4/12 | p=0.208 |
| *Education, year (M ±SD)* | 16.7 ± 2.8 | 14.9 ± 3.2 | 15.5±2.3 | 16.2 ± 2.1 | p=0.18 |
| *MMSE (M ± SD)** | 28.7 ± 1.5 ¥ | 28.6 ±2.1 ¥ | 27.7±1.5 †¥ | 23.3 ± 2.5 | p<0.0001 |
| *APOE4 (0,1,2)* | 23/4/1 | 2/4/0, 1 n.a. | 16/13/3, 1 n.a. | 7/8/1 | - |
| *Amyloid (+,-)* | 14/12, 2 n.a. | 7/0 | 33/0 | 16/0 | - |

†Significantly different compared to HC p=0.024, ¥ Significant different compared to AD p<0.0001

*2.2 Measure of education*

In order to analyze metabolic and volume differences in subjects with high and low education, the whole sample was divided in two groups based on the median of years of education (Morbelli et al., 2013). Subjects with 16 or more years of education were assigned to the high education group, and those with less than 16 years to the low group.

*2.3 Amyloid status*

One of the ADNI PET core laboratories (Jagust Lab, UC Berkley, 17) made amyloid status for patients enrolled in the ADNI GO and ADNI 2 studies available. The amyloid status for ADNI 1 patients was included in a later phase if they underwent AV45. Amyloid positivity was established for a cortex-to-whole cerebellum standardized uptake value ratio (SUVR) higher than 1.11. Firstly, cortical grey matter regions of interest (frontal, anterio/posterior cingulate, lateral parietal, and lateral temporal) and reference regions were defined on the native-space MRI scans using Freesurfer (version 4.5.0) for segmentation and parcellation. The corresponding AV45 scan was then coregistered to the MRI scan and mean tracer uptake was calculated within cortical and reference regions. Finally, cortex-to-whole cerebellum SUVR was derived for each subject by dividing the average cortical AV45 uptake to the average cerebellar uptake. The procedure is further detailed online. Images for which SUVR values were not available (11C-PIB-PET) or with

borderline values were evaluated by local expert nuclear physicians according to validated visual assessment following EMA/FDA-approved Standard Operating Procedures.

*2.4 Data acquisition*

For all the 3 phases of the ADNI study, both FDG-PET and T1-weighted MRI images were acquired at screening or baseline time points. For a few patients who were present at different baseline/screening images, only those in the earliest phases were considered. Images were processed using statistical parametric mapping (SPM8, Wellcome Trust Center for Neuroimaging) software tool. Preprocessed PET and raw T1 MRI images were first manually reoriented, with the origin set at the intersection between the anterior commissure and the interhemispheric plane.

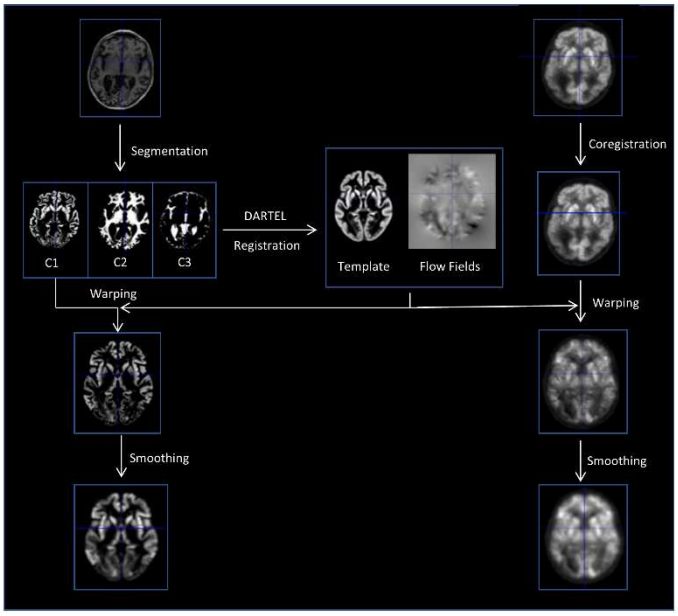
*2.5 MRI data acquisition and pre-processing*

We considered the raw 3D T1 MRI series acquired at the closest date to the FDG-PET exam. ADNI GO/2 MRI were acquired on 3T MRI scanners and ADNI 1 MRI were acquired on either 1.5T or 3T scanners using scanner-specific T1-weighted sequences. A more detailed description of the imaging protocols is reported online. MRI images were first automatically segmented into grey matter (C1), white matter (C2) and CSF (C3) using the SPM8 segmentation routine. C1 and C2 were then registered to their average template using the DARTEL toolbox (Ashburner, 2007). The resulting individual flow fields were used to warp the grey matter segments. Voxel value was modulated in order to preserve the total amount of grey matter present before warping. MRI images were finally smoothed using a Gaussian kernel of 4mm.

*2.6 FDG-PET data acquisition and pre-processing*

HRRT-PET scans were acquired on a Siemens ECAT tomograph. For the present study, PET series already available in preprocessed form were selected, i.e. 6 five-minute frames (ADNI 1) or 4 five-minute frames (ADNI GO/2) scans. Images were co-registered, averaged and reoriented into a standard voxel image grid (more details online). The reoriented PET images were first co-registered to their corresponding MRI image. PET images were then warped using the corresponding MRI scan flow field obtained from the DARTEL registration. PET images were modulated to preserve total amount of activity and finally smoothed using a Gaussian kernel of 4mm.

**Figure 1:** Flowchart of the image processing procedure.



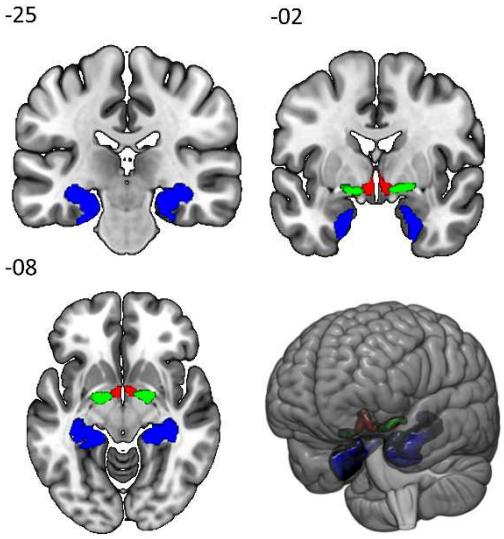

*2.7 Region of interest analysis*

Values from specific regions of interests (ROIs) were extracted using the SPM anatomy toolbox, which allows for integration of functional imaging data and probabilistic cytoarchitectonic maps (Eickhoff et al., 2005).

For the classification of the cholinergic nuclei in the BF we followed Mesulam's nomenclature, as modified and implemented by Zaborszky (Zaborszky et al., 2008), defining Ch1 to Ch4 compartments. Ch1 and Ch2 mainly correspond to the medial septum and the vertical limb of the diagonal band, Ch3 to the horizontal limb of the diagonal band and Ch4 to the nucleus basalis of Meynert (Zaborszky et al., 2008). Thus, the chosen ROIs were defined as follows: for the basal forebrain, left and right medial septal nucleus and diagonal band of Broca (Ch1-3L and Ch1-3R), left and right nucleus basalis of Meynert (Ch4L and Ch4R). For the hippocampus, we considered bilateral dentate gyrus (DG), CA1, CA2, CA3, subiculum, entorhinal cortex and HATA regions (See Figure 2 for graphical representation of BF and hippocampal ROIs). We used the "all assigned" options for voxel assignment. Mean metabolic values of the basal forebrain and hippocampus sub-regions were calculated on the co-registered, warped, modulated and smoothed HRRT PET images. Values were normalized by mean vermis activity. For the metabolic value of the whole BF (BFM) and hippocampus (HM), a mean weighted by sub-regional volume was calculated. In the analyses of grey matter volume, mean voxel values were extracted from the warped, modulated and smoothed grey matter images. Regional volume was determined by multiplying the mean voxel values with the total regional volume of the probabilistic map and subsequently correcting for total intracranial volume, which was

calculated as the sum of grey matter, white matter and CSF volumes (BF volume: BFV; hippocampal volume: HV).

**Figure 2.** Cytoarchitectonic maps of the basal forebrain (Ch1-3 (red) and Ch4 (green)) and hippocampus (blue).




*2.8 Statistical analysis*

Statistical analyses were performed on whole BF and whole hippocampus values (BFM, HM, BFV and HV) as well as on BF sub-regions. In order to compare the different diagnostic groups, we performed one-way ANOVA (LSD post-hoc test, $p < 0.05$) on glucose metabolism (BF: BFM and sub-regions; hippocampus: HM) and grey matter volume (BF: BFV and sub-regions; hippocampus: HV) using age, gender, and ApoE4 status as nuisance variables.

In order to investigate the association with education, we computed partial correlations (Pearson's correlation, $p < 0.05$) in the whole sample between this variable and metabolic/volume values (BFM, HM, BFV and HV). Age, gender, MMSE and ApoE4 status were selected as nuisance variables**.** In order to further test the hypothesis driving the concept of cognitive reserve (i.e., in subjects with high education, cognitive impairment is less severe than expected for the amount of pathological damage), we also performed a correlation analysis between education and the residuals derived from a regression analysis of MMSE on metabolic/volume variables, controlling for the nuisance variables (age, gender and APOE). Finally, in order to further test this association, an exploratory whole-brain voxel-by-voxel analysis was performed in the whole sample (SPM8, one sample t-test, proportional scaling to global value, FWE corrected p-value < 0.05) evaluating possible correlations (positive and negative) between years of education and structural/functional measurements.

One-way ANOVA were then performed in high-education and low-education subgroups separately, to compare metabolic and grey matter differences among BF and hippocampal regions as well as on BF sub-regions. SMC subjects were excluded due to the small sample size (n=7, 2 low-educated and 5 high-educated subjects).

In order to specifically investigate the mechanisms at the base of cognitive resilience (i.e., maintaining high cognitive performances despite significant AD pathology), we focused on cognitively impaired subjects, performing a 2x2 ANOVA in order to investigate the interaction between education (Factor 1: high vs low) and clinical stage (Factor 2: MCI vs AD) in functional variables (BF: BFM and sub-regions; hippocampus: HM) and structural (BF: BFV and sub-regions; hippocampus: HV). In all the analyses focused on education, age, gender, ApoE4 status and MMSE score were entered as nuisance variables.

Finally, to investigate the relationship between cognitive impairment and structural/functional changes, we analyzed in the whole sample the partial correlation (Pearson's correlation, $p < 0.05$) between MMSE scores and imaging variables for BF (BFM, BFV) and the hippocampus (HM, HV). These analyses were performed in the overall group, and in high- and low-educated subgroups, separately. Covariates were age, gender and ApoE4 status, as well as years of education. Statistical analyses were performed using SPSS v23 statistical software (SPSS, Inc, Chicago, IL).

## 3. RESULTS

### *3.1 Functional and structural differences between diagnostic group*

First, we compared metabolic and volume differences in BF and hippocampal regions across all diagnostic groups. BFM was not significantly different across groups ($F(3,75) = 1.85$, $p=0.15$), however, the analyses of BF sub-regions showed significant differences in the left Ch4 ($F(3,75) = 2.85$, $p=0.043$), with MCI groups showing higher metabolic values in comparison to AD ($p=0.016$) and HC ($p=0.048$) (Table 4). The comparison showed that HM was significantly lower in AD than in the other groups ($F(3,75) = 3.01$, $p=0.036$). AD patients showed reduced BFV ($F(3,75)=3.12$, $p=0.031$) and HV ($F(3,75)=5.29$, $p=0.002$) in comparison to all other subgroups (See Table 3 for post-hoc). The analyses on BF sub-regions confirmed a reduced volume in AD compared to all the other groups, particularly in Ch4 ($F(3,75)=3.28$, $p=0026$).

| | HC n=28 | SMC n=7 | All MCI n=33 | AD n=16 | ANOVA |
|---|---|---|---|---|---|
| BFM | 0.73±0.09 | 0.74±0.04 | 0.76±0.08 | 0.71± 0.08 | p=0.15 |
| HM | 0.89± 0.13 | 0.96± 0.09$^{\dagger}$ | 0.91±0.13$^{\dagger}$ | 0.79±0.14 | **p=0.036** |
| BFV | 454±52$^{\dagger\dagger}$ | 480±56$^{\dagger}$ | 452±58$^{\dagger\dagger}$ | 405±40 | **p=0.031** |
| HV | 6696± 860$^{\dagger\dagger\dagger}$ | 6997±838$^{\dagger}$ | 6568±1052$^{\dagger\dagger}$ | 6103± 993 | **p=0.002** |

*BFM = mean BF metabolism; HM=hippocampal metabolism; BFV=BF volume; HV=hippocampal volume*. $^{\dagger}$ p<0.05 compared to AD, $^{\dagger\dagger}$ p<0.01 compared to AD, $^{\dagger\dagger\dagger}$ p<0.001 compared to AD.

**Table 3.** Comparison between metabolic and grey matter values extracted from ROIs in all subgroups.

| | HC n=28 | SMC n=7 | MCI n=33 | AD n=16 | ANOVA |
|---|---|---|---|---|---|
| Ch 1-3 left | 0.64±0.09 | 0.65±0.06 | 0.67±0.07 | 0.66± 0.09 | p=0.46 |
| Ch 1-3 right | 0.66± 0.08 | 0.68± 0.05 | 0.69±0.08 | 0.67±0.09 | p=0.37 |
| Ch 4 left | 0.73±0.10$^{\dagger}$ | 0.77±0.04 | 0.77±0.10 | 0.69±0.09$^{\dagger}$ | **p=0.043** |
| Ch 4 right | 0.79±0.10 | 0.77±0.07 | 0.82±0.10 | 0.76±0.09 | p=0.22 |

$^{\dagger}$ *p<0.05 compared to MCI*

**Table 4.** Comparison between metabolic values of BF sub-regions in all subgroups.

*3.2 Effect of education on brain metabolism and volume and correlation with cognitive status*

In a second step we investigated the relationship between education and structural/functional parameters. The results showed in the whole sample a negative correlation between education and BFV (r=-0.24, p=0.036), in agreement with previous observations showing an inverse association between CR and pathological burden, when taking into consideration disease severity (Arenaza-Urquijo et al., 2011; Borroni et al., 2009; Kemppainen et al., 2008; Sole-Padulles et al., 2009). However, no significant association was found between education and the residuals derived from a regression analysis of MMSE on BFV (ρ=0.16, p=0.15). The exploratory whole-brain voxel-by-voxel analysis performed in the whole sample showed no significant results.

The effect of education has been then evaluated dividing subjects in low/high education subgroups according to the median years of education (16 years). First, we performed a 2 (high vs low education) x 3 (HC vs MCI vs AD) ANOVA on age. The analysis showed no significant effect on age of educational level ($F(1,71)=0.53$, p=0.47), diagnostic groups ($F(2,71)=0.056$, p=0.95), or interactions across these factors

(f(2,71)=2.7, p=0.07). Moreover, no differences were found between high and low education subgroups in APOE positivity (X2(1)=2.5, p=0.11) or gender (X2(1)=1.3, p=0.2). No significant differences were found in age and gender between diagnostic groups considering low educated (age: F(2,22)=1.21, p=0.32; gender: Fisher's exact test: 0.31 p=1.00) ) and high educated subjects (age: F(2,49)=2.24, p=0.12; gender: Fisher's exact test: 0.79 p=0.68). In low educated subjects we found a trend to significance in APOE e4 positivity (Fisher's exact test: 6.35, p=0.05), while no significant differences were found in highly educated subjects (Fisher's exact test: 3.4, p=0.19).

In the high education subgroup, MCI patients showed higher metabolic values compared to HC in BFM (F(2,44)=4.18, p=0.022, LSD post-hoc p=0.006), Ch1-3L (F(2,44)=5.16, p=0.010) and Ch4L F(2,44)=4.89, p=0.012) (See Figure 3), which was associated to a possible trend toward significance in Ch4L grey matter volume (F(2,44)=3.07, p=0.056). Moreover, high-educated MCI subjects showed higher hippocampal metabolism in comparison to HC, although not reaching significance (F(2,44)=3.14, p=0.05). No significant differences were present between the low education subgroups.

The 2x2 ANOVA comparing metabolic and structural values between MCI and AD divided accordingly to high/low education showed a significant interaction between group and education in BFM (F(1, 40)=4.29, p=0.045) (See Figure 3). These results were confirmed in Ch1-3L (F(1, 40)=4.69, p=0.036), Ch1-3R (F(1, 40)=6.62, p=0.014) and Ch4R (F(1, 40)=4.27, p=0.045). No significant interactions were found in hippocampal variables.

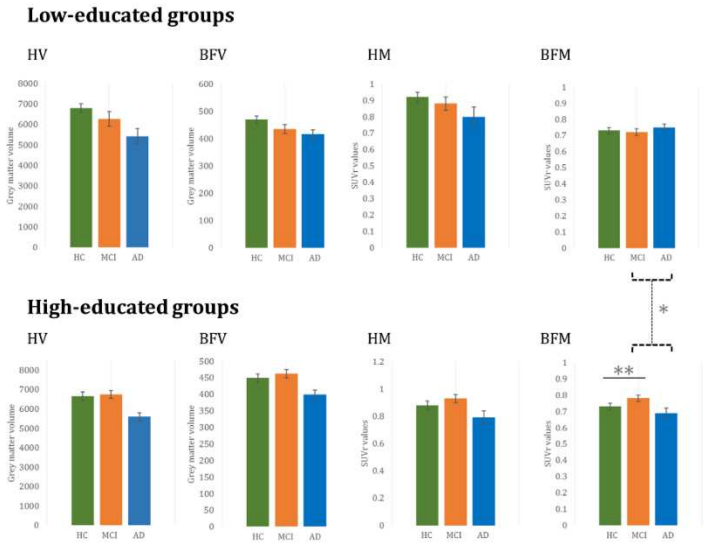



**Figure 3.** Bar charts representing the mean values of grey matter volume (HV, BFV) and metabolism (HM, BFM) extracted from BF and hippocampus. Dotted lines represent the significant interaction in BFM between group (MCI in orange, AD in blue) and education (low, high) is shown. Error bars represent

standard errors *HV: hippocampal volume, BFV: basal forebrain volume, HM: hippocampal metabolism, BFM: basal forebrain metabolism,* *p<0.05, **p<0.01.

The correlation analyses between the cognitive status (i.e., MMSE score) and metabolic/volume variables in the whole group, showed that MMSE score was positively correlated with BFM (r = 0.226, p=0.046) and HM (r = 0.376, p = 0.001). Besides, MMSE score significantly correlated to HV (r=0.326, p = 0.004). These results were confirmed only in the high-educated group (BFM: r = 0.37, p = 0.007; HM: r = 0.45, p = 0.001; HV: r = 37, p = 0.006), while no significant results emerged in low-educated subjects.

## 4. DISCUSSION

In this study we showed that education modulates the relationship between the clinical symptoms and the structural and functional integrity of two regions significantly affected by AD neurodegenerative processes (i.e., BF and hippocampus). Notably, we provided evidence of a significant interaction between education and clinical stage, suggesting a differential effect of this modulatory variable according to the clinical severity.

The current lack of effective treatment for AD emphasizes the importance of investigating CR, as it could play a significant role in delaying or slowing down the onset and progression of disease (Barulli and Stern, 2013). Of note, when analyzing separately subjects with higher and lower educational level, we observed only in the high-educated ones an increase in BF metabolic activity that was present specifically in the early symptomatic stage, namely in MCI, compared to HC and AD. These findings might suggest an underlying upregulation in cholinergic activity in the early symptomatic phases, particularly in subjects with high education, possibly representing a compensatory capacity that might be lost with disease progression. FDG-PET (Ashraf et al., 2015), fMRI studies (Clement and Belleville, 2010; Gigi et al., 2010), as well as studies investigating the cholinergic neurotransmission (Garibotto et al., 2013) in MCI and HC support this hypothesis, which is further reinforced by the significant results of the interaction analysis between educational level and clinical stage. To the best of our knowledge, only a single study, and in a limited patient population, addressed this issue studying BF with HRRT-PET, observing, in agreement with our data, a positive correlation between BF metabolism and MMSE, together with a metabolic increase in MCI (Kim et al., 2012). The increase of BF metabolism in MCI subjects and the association with MMSE performances might be thus interpreted as possible compensative mechanisms for AD-induced dysfunction of structures targeted by the cholinergic innervation. Importantly, the presence of cholinergic plasticity has been previously reported, with the observation of a compensatory response maintaining or upregulating choline acetyltransferase activity namely in patients with MCI, and an association with CR has been postulated,

even if not specifically investigated (DeKosky et al., 2002; Ikonomovic et al., 2003; Mufson et al., 2015). Despite the mild effectiveness of acetylcholinesterase inhibitors reported in AD patients (Wattmo et al., 2011), a recent functional magnetic resonance study (Richter et al., 2018) showed that acute rivastigmine treatment in MCI induced significant functional brain changes during an episodic memory task in the same brain regions recruited by healthy controls, suggesting a trend towards a 'normalization' of neocortical activity.

The relationship between cholinergic system integrity and cognitive status is here illustrated by the positive correlation between BF metabolism and MMSE score, the latter also positively associated to hippocampal metabolism and volume. Hippocampal atrophy and hypometabolism as measured by FDG-PET represent well-known biomarkers of AD neurodegeneration (Jack et al., 2018) and as expected, our data confirmed in AD patients a significant functional and structural hippocampal damage. Notably, high-educated MCI subjects showed higher metabolism in this region compared to HC.

Hippocampal hyperactivity has been already reported in subjects without dementia, however this has been interpreted as an indicator of forthcoming hippocampal failure, as subjects with more rapid cognitive decline presented the greatest hippocampal activation in a fMRI task at T0 and the highest loss of activation in the same region at two-year follow-up (O'Brien et al., 2010). In our sample this hyper-activation has been found specifically in high-educated subjects. Overall these results are in line with the prevalent model of CR, suggesting that higher educated subjects might put in place additional compensatory mechanisms, despite a steeper clinical decline due to the accumulation of more pathological changes (Meng and D'Arcy, 2012).

It is important to underline that we measured volume and metabolism of the BF, which is usually studied for its cholinergic properties, but it is not an exclusively a cholinergic structure. Indeed, the BF cortical projections include a relevant proportion of GABAergic neurons in the areas examined, which could contribute to the effects we observed, either directly or through a cholinergic modulation (Rosato-Siri et al., 2006; Sarter and Bruno, 2002; Yang et al., 2014). It should be noted, however, that the GABAergic population is less affected by the neurodegenerative process in AD, as compared with the cholinergic component (Palmer, 1996; Rossor et al., 1982)). In addition, the relationship between GABAergic vulnerability and AD pathology remains unclear, as GABAergic dysfunction could involve both compensatory and direct pathological responses (see (Govindpani et al., 2017)).

In conclusion, this study provides evidence for a modulatory effect of education on the relationship between clinical presentation and functional and structural integrity in the BF and hippocampus. In order to achieve a sufficiently high spatial resolution, we included ADNI subjects who underwent an HRRT-PET scan. Despite that automated voxel-based analysis may fail to highlight hippocampal metabolic dysfunction

(Mosconi et al., 2005), the implementation of high-resolution scan, as well as a ROI-based approach, allowed a more accurate hippocampal and BF FDG-PET quantification. A limitation of FDG-PET imaging interpretation is that it is only an indirect measuring of cholinergic activity compared to measures obtained with acetylcholinesterase markers such as [11C]-MP4A. Even using HRRT-PET, the small size of the BF may also cause measurement errors and difficulties in clearly distinguishing its sub-regions. A strength of this study is the inclusion of amyloid positive patients only. The homogeneity and the clear definition of patient sample is a crucial point, considering the significant correlation between neocortical amyloid accumulation and BF degeneration (Kerbler et al., 2015). Our study has also limitations worth discussing. The main one is the relative small sample size in each subgroup. Another is that we only considered cross-sectional data: longitudinal data would be required to show the temporal evolution of the differences we observed across diagnostic stages. A third limitation is that we observed the effect of education when adopting a median split approach, as in previous literature (Morbelli et al., 2013) but we could not show a continuous effect of education on BFM. This mismatch between the results in group and regression analyses might be possibly linked to the non-linear relation between metabolism and education, or to the small sample size and high educational level of our sample, common to other studies from the ADNI population (Mainta et al., 2018).



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**Ethics Statement**

This article does not contain any studies with human participants performed by any of the authors.

**Disclosure Statement**

The authors declare that they have no conflict of interests.

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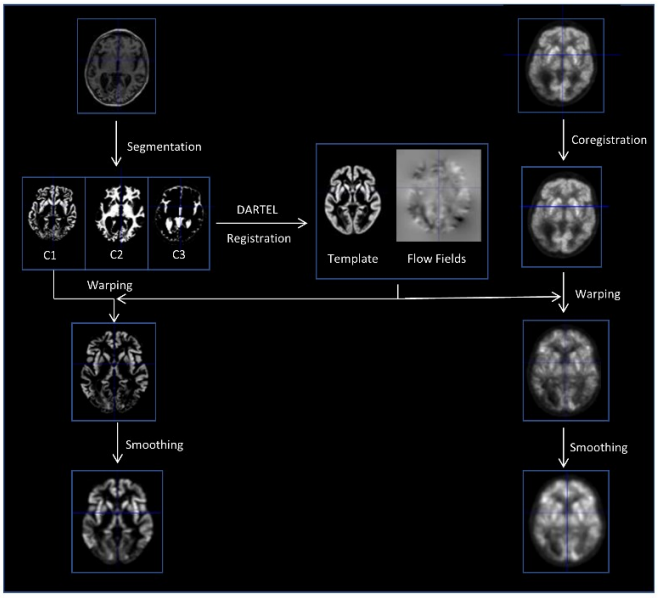
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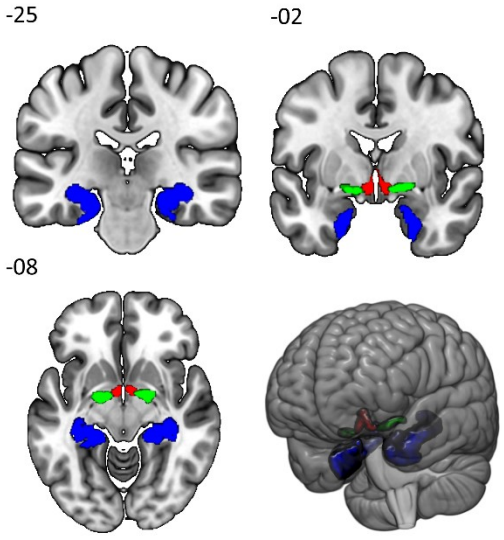

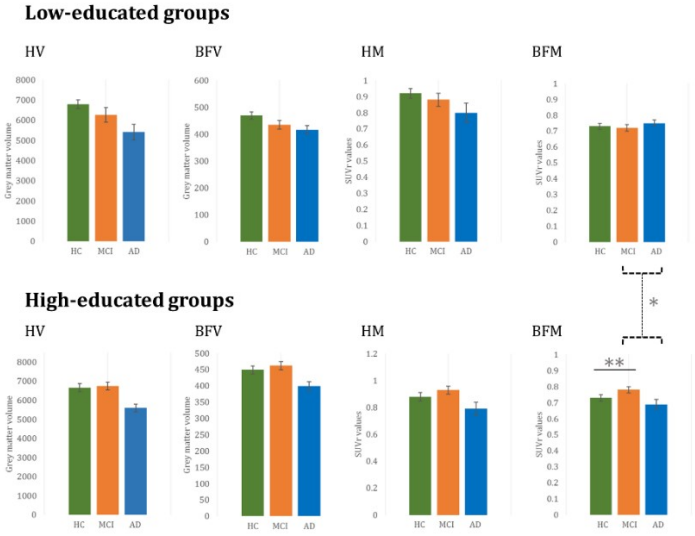


**p<0.01, * p<0.05