CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

Online Research @ Cardiff

Citation for final published version:

Serra, Laura, Giancaterino, Giulia, Giulietti, Giovanni, Petronsini, Laura, Di Domenico, Carlotta, Marra, Camillo, Caltagirone, Carlo, Bassi, Andrea, Cercignani, Mara ORCID: https://orcid.org/0000-0002-4550-2456 and Bozzali, Marco 2022. Cognitive reserve modulates brain structure and cortical architecture in the Alzheimer’s Disease. Journal of Alzheimer's Disease file

Publishers page:

information services
gwasanaethau gwybodaeth

Cognitive reserve modulates brain structure and cortical architecture in the Alzheimer's Disease

Laura Serra[1], Giulia Giancaterino[1], Giovanni Giulietti[1], Laura Petrosini[2], Carlotta Di Domenico[1], Camillo Marra[3], Carlo Caltagirone[4], Andrea Bassi[4], Mara Cercignani[5], Marco Bozzali[6,7]

1Neuroimaging Laboratory, Fondazione Santa Lucia, IRCCS, Via Ardeatina, 306, 00179, Rome, Italy.
2 Laboratory of Experimental and Behavioural Neurophysiology, Fondazione Santa Lucia, IRCCS, Rome, Italy
3Institute of Neurology, Catholic University, Largo Agostino Gemelli, 8, 00168, Rome, Italy.
4 Department of Clinical and Behavioural Neurology, Fondazione Santa Lucia, IRCCS, Via Ardeatina, 306, 00179, Rome, Italy.
5 Cardiff University Brain Imaging Centre, School of Psychology, Cardiff University, Cardiff CF24 4HQ, Wales, United Kingdom
6 Brighton & Sussex Medical School, University of Sussex – Brighton, United Kingdom
7 Rita Levi Montalcini' Department of Neuroscience University of Torino, Via Cherasco, 15, 10126, Turin, Italy.

Running head: Cognitive reserve and brain architecture in AD.

***Correspondence to: Dr. Laura Serra, Via Ardeatina 306, 00179 Rome, Italy. Telephone number: #39-06-5150 1493; Fax number: #39-06-5150 1213; Email address: l.serra@hsantalucia.it**

Abstract

Aim: Cognitive reserve (CR) explains the individual resilience to neurodegeneration. The present study investigated the effect of CR in modulating brain cortical architecture. **Methods:** 278 individuals (110 AD, 104 a-MCI due to AD, 64 HS) underwent a neuropsychological evaluation and 3T-MRI. Cortical thickness (CTh) and fractal dimension (FD) were assessed. Years of formal education were used as an index of CR by which participants were divided in High and Low CR (HCR and LCR). Within-group differences in cortical architecture were assessed as a function of CR. Associations between cognitive scores and cortical measures were also evaluated. **Results:** A-MCI-HCR compared to a-MCI-LCR patients showed significant decrease of CTh in the right temporal and in the left prefrontal lobe. Moreover, they showed increased FD in the right temporal and in the left temporo-parietal lobes. Patients with AD-HCR showed reduced CTh in several brain areas and reduced FD in the left temporal cortices when compared with AD-LCR subjects. HS-HCR showed a significant increase of CTh in prefrontal areas bilaterally, and in the right parieto-occipital cortices. Finally, a-MCI-HCR showed significant positive associations between brain measures and memory and executive performance. **Discussion:** CR modulates the cortical architecture at pre-dementia stage only. Indeed, only patients with a-MCI showed both atrophy (likely due to neurodegeneration) alongside richer brain folding (likely due to reserve mechanisms) in temporo-parietal areas. This opposite trend was not observed in AD and HS. **Conclusions:** our data confirm the existence of a limited time-window for CR modulation at the a-MCI stage.

Keywords: cognitive reserve; Alzheimer's disease; Mild cognitive impairment; cortical thickness; fractal dimension.

INTRODUCTION

Alzheimer’s Disease (AD) is the most common neurodegenerative disorder worldwide. AD implies biological, genetic and environmental factors that interact with each other producing a complex clinical picture with peculiar brain abnormalities. Brain morphology is known to modify over the lifespan with major changes occurring during development and aging [1].

The neocortex is the neuroanatomical substrate for processing of higher-level cognitive functions, while the white matter connects various brain regions to allow a network-based processing of information. The cortex is organized vertically into six different layers (or laminae) distinct from each other for neuronal composition and density [2]. In general, neuronal density and cortical thickness (CTh) are inversely correlated. This is the reason why associative areas are known to be thicker than primary sensory areas [3]. Over the last year it has been introduced a method of MRI data analysis called “Cortical thickness” (CTh), which is based on a voxel-wise processing of T1-weighted volumes [4-5]. CTh describes the distance between the inner and outer boundaries of gray matter using voxels or surface characteristics as a quantitative parameter [6]. CTh abnormalities can be considered as resulting from microstructural changes occurring in the brain tissue. Previous studies showed, in Alzheimer’s disease brains, significant reductions of cortical thickness [7-8], and associations with neuropathological abnormalities [8,10]. CTh seems therefore to be a reliable proxy measure of AD neuropathology in vivo. Another measure that can be derived from T1-weighted volumes is fractal dimension (FD), which returns information on cortical complexity. FD is regarded as a quantitative index of roughness of the brain surface and is derived from folding properties of the cortex [8].

The surface area and cortical thickness are inheritable structural features, each one driven by specific genetic factors [11-12]. Additionally, these cortical features are susceptible to environmental factors [11,13]. The impact of environment on the brain structure and cognitive functions is well documented in both animal model and in human studies (please see [14] for a review). Neurogenesis, synaptogenesis, increased level of neurotrophic factors have been associated with enriched environment exposure in animal experiments [14]. In clinical research, healthy life-styles including cognitive, social, physical stimulations, healthy diet, no smoking etc., have been associated with a higher brain resilience to neuronal damage [14-15]. All these studies fell in the framework of the cognitive reserve subject [16]. It is well known that several factors can be considered as reserve-builders but the most powerful one impacting on the individual cognitive reserve (CR) is the level of formal education. Several studies showed that education enhances the level of brain connectivity both inducing a synaptic increase [14,16-17], and stimulating behaviours oriented to more intense social and cognitive activities [17]. In particular, education is an important environmental factor that pushes the motivation to engage stimulating cognitive activities during the entire lifespan [14]. Additionally, education is considered as a socialization factor that can promote more efficient learning strategies in response to request of the environment [14].

It has been hypothesized that education modifies the relationship between accumulation of neuropathology and individual cognitive performances during aging [14-15]. Most previous studies used volumetric techniques such as voxel-based morphometry to investigate such an interaction [18-19]. However, the impact of reserve on cortical thickness has been so far only poorly explored [20-22]. Querbes and co-workers [20] by extracting a normalised thickness index from a pool of brain regions, reported a high predictive value for CTh in predicting the risk of conversion to AD in patients with Mild Cognitive Impairment and higher CR levels. A more recent study showed that individuals with higher CR and preserved cortical thickness are more protected against conversion to AD in a time framework longer than 7 years from clinical onset [21]. In addition, CR and cortical thickness had an independent impact on the risk of conversion to AD in those MCI patients who converted earlier than 7 years [21]. The effect of CR on the brain structures has been clearly demonstrated in animal models (see[14] for a review). In a rat model of AD it has been observed that physical exercise, traditionally considered as a reserve-builder [23-24], increases cortical thickness in motor areas as well as performance [25].

When considering FD, a recent study [26] analysed the association between this measure of brain complexity and cognition in patients with AD and fronto-temporal dementia (FTD). This study showed a similar pattern of FD changes between the two groups, involving the cingulate gyrus and insula, while different patterns of correlation were identified between FD and cognitive performances in either patient group. In more detail, significant correlations were observed between reduced FD in the superior temporal gyrus and isthmus of the cingulum, and memory performances in AD patients, while reduced FD in the inferior temporal, medial orbito-frontal cortex were associated to verbal fluency in FTD patients [26]. To the best of our knowledge, there are no published studies that investigated the relationship between CR and cortical complexity in both, healthy and pathological populations.

Aims of the present study were: i) to assess the effect of cognitive reserve (CR) in modulating cortical brain architecture in healthy elderly individuals and in patients with AD at different stages of disease progression; ii) to evaluate the association between CR and cortical complexity on the cognitive functioning of healthy individuals and patients with AD. To this purpose, we recruited a large cohort of participants assessing their CR, cognitive functions, and cortical thickness and complexity.

METHODS

Participants

A cohort of 278 participants, 110 with a diagnosis of probable AD (M/F=42/68; mean age=73.1, SD=6.5 years; mean years of formal education=9.7, SD=4.5), 104 with a diagnosis of amnestic MCI (a-MCI) (M/F=51/53; mean age=70.0, SD=7.9 years; mean years of formal education=9.9, SD=4.6), and 64 healthy elderly subjects (HS) (M/F=25/39; mean age=64.0, SD=9.4 years; mean years of formal education=13.3, SD=3.2) were enrolled in the study. They were consecutively recruited between January 2016 and December 2019 from the Specialist Dementia Clinic of Catholic University of Rome, and from Santa Lucia Foundation, IRCCS, Rome, Italy. The diagnosis of probable AD was formulated according to the clinical criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related

Disorders Association (NINCDS-ADRDA) [27]. The diagnosis of a-MCI was performed according to current diagnostic criteria [28]. Patients could be either single- (n=54) or multiple (n=50) domain MCI, and had not to respond to the diagnostic criteria for major cognitive disorders [29], with a CDR [30] score not exceeding 0.5. As detailed below, medial temporal lobe atrophy was assessed in all subjects to confirm that they had an intermediate likelihood of underlying AD neuropathology, and to control for patients' homogeneity across high and low cognitive reserve groups. In order to exclude individuals in a preclinical phase of cognitive decline from recruitment in the "healthy subjects" group, they had not to show any significant medial temporal lobe atrophy or any cognitive scores below the normality cut-off in each assessed cognitive domain. All recruited subjects with a Hachinski score [31] higher than 4 were excluded. Major systemic, psychiatric, and other neurological illnesses were also carefully investigated and excluded in all participants. Finally, in order to reduce any potential source of variability due to hemispheric dominance subjects had to be right-handed, as assessed by the Edinburgh Handedness Inventory [32].

The study was approved by the Ethics Committee of Santa Lucia Foundation and written informed consent was obtained from all participants and or their legal guardians before study initiation. All procedures performed in this study are in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Neuropsychological assessment

All participants underwent an extensive neuropsychological covering all cognitive domains: a) verbal episodic long-term memory: 15-Word List (Immediate, 15-min Delayed recall and recognition) [33]; Short Story Test (Immediate and 20-min Delayed recall) [34]; b) visuo-spatial long-term memory: Complex Rey's Figure (Immediate and 20-min Delayed recall) [34]; c) short-term and working memory: Digit span (forward and backward) and the Corsi Block Tapping task (forward and backward) [35]; d) executive functions: Phonological Word Fluency [33] and

Modified Card Sorting Test [36]; e) language: Naming objects subtest of the BADA (‘‘Batteria per l’Analisi dei Deficit Afasici’’, Italian for ‘‘Battery for the analysis of aphasic deficits’’) [37]; f) Reasoning: Raven’s Coloured Progressive Matrices [33]; g) constructional praxis: copy of simple drawings with and without landmarks [33] and copy of Complex Rey’s Figure [34]; h) general cognitive efficiency: Mini Mental State Examination (MMSE) [38-39]. For the purposes of the current study, focussed on the cognitive reserve, neuropsychological scores were not adjusted for age and education, as previously reported [18].

Classification criteria to define the level of cognitive reserve.

As previously reported [18], we divided participants on the basis of their level of formal education as proxy measure of CR. Within each group, the years of formal education were transformed into z scores, and individuals reporting a z score ≤ 0 were considered as having a low cognitive reserve (AD-L_{CR}; n= 58; a-MCI-L_{CR}=58 and HS-L_{CR}=44). Conversely, individuals with a z score > 0 were considered having a high cognitive reserve (AD-H_{CR}; n= 52; a-MCI-H_{CR}=46 and HS-H_{CR}=20). Individuals with high and low cognitive reserve were equally distributed across groups. Table 1 summarizes the principal characteristics of all subjects.

MRI acquisition

Image acquisition and pre-processing of volumetric images to assess cortical thickness

All participants underwent MRI-3T brain scanning (Siemens, Medical solutions, Erlangen, Germany) including the following acquisitions: (a) dual-echo spin echo (DE-SE) (TR=5000 ms, TE=20/100 ms); (b) fast-fluid attenuated inversion recovery (FLAIR) (TR=8170 ms, TE=96 ms, TI=2100ms); (c) 3D T1-weighted (TR=7.92 ms, TE=2.4 ms, TI=210 ms, flip angle = 15°). For the dual-echo and FLAIR scans, 52 contiguous interleaved axial slices were acquired with a 2 mm slice thickness, with a 192 x 256 matrix over a 256 mm x 256 mm field of view, covering the whole brain. The T1-weighted volumes were acquired in a single slab, with a sagittal orientation and 224 x 256 matrix size over a 256 x 256 mm^2 field of view, with an effective slice thickness of a 1mm.

The T1-weighted images were pre-processed by using the pipeline for surface-based morphometry included in CAT-12 (Computational Anatomy Toolbox 12), a toolbox of SPM 12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Briefly, a projection-based thickness estimation was used to compute the cortical thickness (CTh), the central surface and the FD [40], including the partial volume correction, the sulcal blurring and asymmetries corrections. CAT-12 permits to repair topological defects, using a method based on spherical harmonics [41] and to re-parameterize the surface mesh into a common coordinate system using a specific algorithm to reduce the area distortion [42]. Then an adapted two-dimensional diffeomorphic DARTEL algorithm was used for the spherical registration of the brain surface. Finally, a smoothing with a Gaussian kernel of 15 mm (FWHM) was applied to each dataset.

Medial temporal lobe atrophy

The Medial Temporal lobe Atrophy scale (MTA) [43] was employed on T1-weighted volumetric images to assess the severity of atrophy in each subject. This scale provides a rating score from 0 to 4, with scores > 1.5 [44] indicating significant atrophy. For each subject we averaged the scores obtained in the right and left hemispheres to obtain a single measure of medial-temporal lobe atrophy.

Statistical analyses

Demographical features, clinical and neuropsychological features

SPSS-20.0 (https://www.ibm.com/it-it/analytics/spss-statistics-software) was used to assess group differences in demographic and clinical variables by using a series of two-way 3 by 2 ANOVAs, with a 3 level Group (AD vs. a-MCI vs. HS) and a 2 level CR (High vs. Low). Variables included age, years of formal education, the MTA scores, the MMSE scores and neuropsychological scores. Twenty two-way ANOVAs were performed to assess differences in the neuropsychological scores, and to avoid the type-I error Bonferroni's correction was applied (p value threshold α=

0.05/20=0.003). Tukey test was used as post-hoc analysis of ANOVAs. Gender distribution across groups was assessed by using Chi-square.

Cortical thickness and fractal dimension analyses

The MRI data analyses were performed in the framework of the General Linear Model by using CAT-12 (Computational Anatomy Toolbox in SPM12) (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Two-sample t test models were used for voxel-wise comparisons of cortical thickness and FD between subjects with low and high CR within each diagnostic group (AD-L_{CR} *vs.* AD-H_{CR}; a-MCI-L_{CR} *vs.* a-MCI-H_{CR}; and HS-L_{CR} *vs.* HS-H_{CR}, respectively). Age, intracranial volumes (TIV, obtained as sum of grey matter, white matter and cerebrospinal fluid) and MMSE scores were always used as covariates of no interest. Although cortical thickness or other surface measures are usually not dependent on TIV, in the CR framework TIV is considered as a proxy measure of brain reserve. To reduce this potential confounding factor on the impact of cognitive reserve on ChT and FD we chose to enter TIV as nuisance variable into the analyses.

Moreover, a series of multiple regression models were used to assess the potential association between cortical thickness, FD and performance obtained at neuropsychological tests. Intracranial volumes and MMSE scores were again entered as covariates of no interest. All results were accepted if survived at correction for multiple comparisons ($p<0.05$ FWE at cluster level).

Results

Demographic and clinical characteristics

There was a significant main effect of Group in age ($F_{2,269}= 28.9$, $p<0.001$) due to the fact that both patients' groups were older than HS (all $p<0.001$ in the Tukey post-hoc analysis); no significant main effect of CR-level ($F_{1,269}=0.85$, $p=0.356$) or Group by CR-level interaction ($F_{2,269}=0.45$, $p=0.640$) were observed. With respect to years of formal education, as expected, there was a significant main effect of Group ($F_{2,269}= 76.3$, $p<0.001$), main effect of CR-level ($F_{1,269}=624.7$, p<0.001) and a significant Group by CR-level interactions ($F_{2,269}$=8.43, p<0.001). Moreover, there were significant main effects of Group ($F_{2,269}$= 168.3, p<0.001) and of CR-level effect ($F_{1,269}$=10.7, p=0.001) in the MMSE scores. The first effect was due to the fact that AD patients reported lower MMSE scores than those with a-MCI and HS, while a-MCI patients showed lower scores than HS (all p<0.001 in the Tukey post-hoc analysis). The main effect of CR-level indicated that individuals with high CR (regardless of their diagnostic group belonging) showed higher MMSE scores than individuals with low CR. No significant Group by CR-level interaction was observed ($F_{2,269}$=0.50, p=0.629) in all studied groups. Finally, there was a significant main effect of Group in the MTA scores ($F_{2,269}$= 72.1, p<0.001), because AD patients showed more atrophy in the hippocampus than a-MCI patients and HS, and a-MCI patients showed more atrophy than HS (all p<0.001 in the Tukey post-hoc analysis). No other significant differences were observed between groups and subgroups. Finally, patients with a-MCI showed a significant difference in sex distribution when stratified for their CR-level. This difference was due to the a-MCI-L_{CR} group that included more females than the a-MCI-H_{CR} group (χ^2=8.64, d.f.=1, p=0.033).

All these data are summarised in Table 1.

Neuropsychological assessment

Table 2 shows between-group differences in neuropsychological measures. There was significant main effect of Group in each administered test due to the fact that patients with AD showed lower scores than patients with a-MCI and HS, and, in turn, a-MCI patients reported worse scores than HS. In addition, there was significant main effect of CR-level (better scores shown by participants with high CR level compared to those with low CR level regardless of their diagnostic group belonging), in the immediate recall of 15-Word list, in the Digit span backward, in the phonological verbal fluency, in the Raven's Progressive Matrices, in the copy of drawings and in the copy of Rey's Figure. Finally, there were no significant Group by CR-level interactions.

Cortical thickness and Fractal dimension analyses

Patients with AD-H$_{CR}$ showed reduced CTh compared to AD-L$_{CR}$ patients in several brain areas including the left post central gyrus (BA3), the left parieto-occipital cortices (i.e., precuneus and primary visual cortex; BA7, BA17), the left cingulate cortex (BA31) and the right inferior frontal gyrus (BA44), and the medial part of the right temporal gyrus (BA20, BA21, BA37) (See Figure 1 panel A and table 3). A-MCI-H$_{CR}$ patients compared to a-MCI-L$_{CR}$ patients showed a significant decrease of CTh in the right temporal lobe, including the inferior temporal and fusiform gyri (BA38, BA20, BA21), and in the left prefrontal lobe (BA6, BA8, BA9) (Figure 1, panel B and Table 3). Moreover AD-H$_{CR}$ showed increased CTh compared with AD patients with low CR in the left anterior cingulate cortex (BA32), in the prefrontal cortex (BA9) and in the left inferior and medial temporal gyri (BA21, BA37) (see Figure 2A and Table 3). Finally, HS-H$_{CR}$ compared to HS-L$_{CR}$ showed a significant increase of cortical thickness in the prefrontal areas of the left (BA6, BA8, BA9) and right (BA10 BA11, BA44, BA47) hemisphere, and in the right parieto-occipital cortices (BA39 and BA19) respectively (see Figure 2B and Table 3).

When considering FD, we found in AD-H$_{CR}$ patients compared with AD-L$_{CR}$ patients reduced complexity in the left temporal cortices (BA22 and BA37) (Figure 3 panel A, Table 4). On the contrary, a-MCI-H$_{CR}$ showed a significant increase of the FD compared with a-MCI-L$_{CR}$ patients in the right temporal (BA38) and in the left temporo-parietal lobes (in posterior part of BA21, BA37, BA39) (Figure 3 panel B, Table 4).

No differences in FD were found when comparing HS with low and high CR.

Relationship between neuropsychological tests and Cortical thickness and Fractal dimension

A significant positive association was found in the AD-H$_{CR}$ group between patients' performance on the 15-Word List (immediate recall) and their cortical thickness in the left posterior parietal cortex, in the left inferior temporal gyrus, and in the bilateral middle temporal gyrus. In the same group, a significant positive association was found between patients' scores on the Raven's Progressive Matrices and cortical thickness in their right frontal pole (see Figure 4, panel A). When considering the a-MCI-H$_{CR}$ group, a significant positive association was found between patients' scores at the digit span backward and CTh in the left inferior frontal gyrus (Figure 4, panel B). The HS-H$_{CR}$ group showed positive association between scores on the Raven's Progressive Matrices and CTh in the pre-central gyrus and post-central gyrus and in the precuneus bilaterally. In the same group, a significant positive association was found between scores on the Copy of Drawings test and CTh in the right precentral and inferior frontal gyri (Figure 4, panel C). No significant associations were identified in all diagnostic groups for individuals with low cognitive reserve.

When considering FD, we observed a significant positive association in a-MCI-H$_{CR}$ Digit span backward scores and FD in the left middle temporal gyrus. Finally, in the a-MCI-L$_{CR}$ group, a significant positive association was found between scores on the 15-Word List and FD in the left middle temporal gyrus (Figure 5).

No additional associations were found between FD and cognitive scores from any other group.

Discussion

The importance of studying cortical thickness and brain complexity across normal and pathological brain aging is to detect detailed interactions between cerebral functions and dysfunctions and brain architecture. Previous studies have already demonstrated the presence of reduced thickness in AD brains at different disease stages [6,9]. The novelty of the current study was to use the measure of cortical thickness and fractal dimension to detect the modulation of CR on the neuropathology of AD. Our main finding is that the effect of CR reflects on cortical thickness and fractal dimension across aging, whose modifications account for individual performance on various cognitive domains. Overall, our findings further support the Stern's hypothesis that patients with higher CR need to accumulate more neuropathology than individuals with low CR to exhibit the same level of cognitive impairment [45]. In addition, our findings in healthy elderly individuals indicate that some cortical areas play a key role in brain maintenance. Specifically, all prefrontal areas found to be ticker in HS, are involved in executive functions, which are essential for cognitive flexibility and make the brain more adaptable to social and environmental demands. Furthermore, the present study indicates that CTh and FD can be considered as reliable structural biomarkers for studying gray matter changes across AD evolution. In fact, they not only return a peculiar picture of degeneration for the different AD stage, but they are also influenced by the individual's enriching life experiences, measured through cognitive reserve.

We used here a static index of reserve, derived from the years of formal education, to assess the effect that CR has in modulating CTh and FD (a measure of cortical complexity).

Both, a-MCI and AD patients were older than HS as shown by the main effect of Group, but there was no significant effect due to the CR level or significant Group by CR level interaction. For this reason, in order to control the age effect, MRI analyses were performed within groups. We also observed a significant main effect of CR in the absence of any group interaction. This is due to the fact that subjects with high reserve have a significantly higher global cognitive function than those with low reserve, regardless of their diagnostic group belonging.

From a neuropsychological viewpoint, we found, as expected, a significant main effect of Group in several cognitive measures due to the fact that AD patients showed lower performances than a-MCI and HS, and a-MCI patients performed worse than HS in memory and executive functions. We observed also a main effect of CR level. Indeed, participants with higher CR showed better performances than those with lower CR in tests assessing episodic memory, phonological verbal fluency, reasoning and constructional praxis.

When considering the CTh in the groups of participants with high and low CR we found that, in AD patients, subjects with high CR showed thinner thickness in the left post- central gyrus, in the left parietal operculum, in the left superior parietal gyrus, in the left primary visual cortex, in the left cingulate gyrus, in the right medial temporal gyrus and in the right inferior frontal gyrus. Patients with a-MCI-H_{CR} showed reduced thickness in the left supplementary motor cortex, in the superior frontal gyrus, in the left temporal pole, inferior temporal and fusiform gyri.

More remarkably, we observed reduced cortical thickness in both patients with AD and a-MCI with high CR when compared to those with low CR. In particular, the areas in the right medial temporal gyrus (BA20/21) were thinner in both AD-H$_{CR}$ and a-MCI-H$_{CR}$. These brain areas are known to be crucial for long-term memory, and the present results reinforce the idea that they are involved since early stages of AD [46-47]. However, some cortical areas showed different anatomical patterns of atrophy in patients with AD and a-MCI. In particular, AD-H$_{CR}$ patients compared with AD-L$_{CR}$ showed reduced thickness in a cluster included the post-central gyrus (BA3), the precuneus (BA7) and the posterior cingulate cortex (BA31). The precuneus and the posterior cingulate cortex have been previously recognised as involved in memory functioning [48-50]. AD-H$_{CR}$ patients showed also thinner thickness in the BA44 (i.e., Broca's area), which is implicated in speech production. Recently, we demonstrated that this same area accounts for deficit in constructional apraxia in AD patients [51], indicating a more widespread role for the Broca's area in the pathophysiology of AD. Moreover, AD-H$_{CR}$ compared with AD-L$_{CR}$ patients showed thicker CTh in the left anterior cingulate cortex (BA32), in the prefrontal cortex (BA9) and in the left inferior and medial temporal gyri (BA21, BA37). All these regions are extensively involved in higher-level cognitive functions. We hypothesise that a thicker CTh may account for a better cognitive profile in the AD-H$_{CR}$ compared to the AD-L$_{CR}$ group. This view is reinforced by the correlations observed between the same brain regions and memory and reasoning performances in AD-H$_{CR}$ patients.

Patients with a-MCI-H$_{CR}$ showed reduced thickness in the prefrontal lobe, including the supplementary motor area (SMA) (BA6) and the dorsolateral prefrontal cortex (BA8, BA9). The SMA, is implicated in "internally-generated" planning of movements, in the planning of sequences of movements, and in motor coordination [51-52], and BA8 and BA9 have been found involved in the executive functions in both healthy controls and neurological patients [53]. In particular the dorsolateral prefrontal cortex is involved in working memory [54].

Some brain areas such as BA9 and prefrontal regions appeared to be thinner in the a-MCI-H$_{CR}$ and thicker in the AD-H$_{CR}$ group, which is counterintuitive. Longitudinal studies are needed to clarify this issue.

Conversely, individuals in the HS-H$_{CR}$ group compared to those with low CR, showed an increase of cortical thickness in the same prefrontal areas. This finding is consistent with a previous study from Kim and co-workers [55], which showed a protective effect of education on cortical thickness mainly in the prefrontal parietal and occipital regions of healthy elderly subjects.

When considering FD, we observed an opposite pattern of complexity in AD and a-MCI patients. Indeed, AD-H$_{CR}$ showed reduced FD than AD-L$_{CR}$ in the left medial temporal gyrus, while a-MCI-H$_{CR}$ showed increased FD in the same cortical area and in the right temporal pole. Interestingly, FD changes were observed in the mesial temporal lobe structures, which are critical for the memory disorders observed in AD. King and co-worker [8] observed reduced FD in patients with AD compared to healthy controls in several brain areas from the temporal lobe, in the mammillary bodies and in the superior and inferior colliculus. In line with this observation we speculate that the progression of neurodegeneration reduces the complexity of brain cytoarchitecture, resulting in a minor folding pattern, with a modulation of CR.

It is remarkable that a different trend was observed in a-MCI patients only. Indeed, in a-MCI-H$_{CR}$ patients lower CTh corresponded to a greater folding in the temporal-occipital regions of both hemispheres. This effect seems to disappear at more advanced disease stages. In fact, AD patients with high CR compared to those with low CR, showed less cortical complexity in the left temporal lobe, alongside a decrease in CTh. According with Im et al., [56] cortical thickness and FD share approximately 50% of variance and the non-total correspondence between the two morphometric measures could be found in the modality (how and where) of occurrence of brain atrophy. FD is known to depend on the volumetric changes of the white matter and the lateral ventricles, which structurally support the cerebral folds [8,56]. These subcortical atrophying processes, which are well delineated in patients AD, cannot be detected yet in subjects with mild cognitive impairment. This means that cognitive reserve modulates cortical FD in AD and MCI in relation to the different level of atrophy. However further studies on the relationship between CR and FD and brain atrophy are needed to clarify these aspects.

We observed also positive associations between cortical complexity measures and cognitive tests. Specifically, patients with AD-H_{CR} showed a positive association between cortical thickness in the mesial temporal lobe structures and long-term episodic memory and cortical thickness in the frontal pole and reasoning ability. While a-MCI-H_{CR} patients showed positive associations between thicker thickness (and also FD in turn) in the left inferior frontal gyrus and scores on verbal working memory tests; finally, HS-H_{CR} individuals showed a positive association between thickness in the fronto-parietal areas and reasoning and between the inferior frontal and precentral gyri and constructional praxis abilities. These associations highlight the strict relationship between the integrity of cortical structures and cognition and the protective effect of cognitive reserve.

In conclusion, a detailed analysis of cortical complex architecture help understanding the role played by cognitive reserve in modulating the effect of AD neuropathology on different cognitive functions. Beyond the speculative interest of this investigation, we believe that this approach is potentially useful when stratifying patients for clinical trials. Recruiting individuals with different levels of cognitive reserve means introducing a remarkable source of variability that might mitigate the effect of interest.

Acknowledgments

This research was supported in part by Ministero della Salute (Italian Ministry of Health) (Linea di Ricerca corrente 2021-2022).

Conflict of interests

None of the Authors has any conflict of interest to disclose.

Figure legends

Figure 1. Reduced cortical thickness in patients with AD and a-MCI

Panel A illustrates the reduction of cortical thickness in AD-H_{CR} compared to AD-L_{CR} patients in several brain areas including the post central gyrus, the parieto-occipital cortices, the cingulate cortex in the left hemisphere and the inferior frontal gyrus, the medial part of the temporal gyrus in the right hemisphere. In panel B a-MCI-H_{CR} patients showed a significant decrease of cortical thickness compared to a-MCI-L_{CR} patients in the right inferior temporal and fusiform gyri, and in the left prefrontal lobe. The statistical comparisons were overlapped on MRIcron ch2bet template (https://www.nitrc.org/projects/mricron).

Abbreviations: AD-H_{CR}= Alzheimer's Disease with high cognitive reserve; AD-L_{CR}= Alzheimer's Disease with low cognitive reserve; a-MCI-H_{CR}= amnestic Mild Cognitive Impairment with high cognitive reserve; a-MCI-L_{CR}= amnestic Mild Cognitive Impairment with low cognitive reserve; L=Left; R=Right.

See text for further details.

Figure 2. Increased cortical thickness in patients and healthy subjects

Figure 2A illustrates patients AD-H_{CR} with increased cortical thickness compared with AD patients with low CR in the left anterior cingulate cortex, in the prefrontal cortex and in the left inferior and medial temporal gyri. Finally, individuals HS-H_{CR} compared to HS-L_{CR} showed a significant increase of cortical thickness in the prefrontal areas bilaterally, and in the right parieto-occipital cortices. The statistical comparisons were overlapped on MRIcron ch2bet template (https://www.nitrc.org/projects/mricron).

Abbreviations: AD-H_{CR}= Alzheimer's Disease with high cognitive reserve; AD-L_{CR}= Alzheimer's Disease with low cognitive reserve; HS-H_{CR}= Healthy Subjects with high cognitive reserve; HS-L_{CR}= Healthy Subjects with low cognitive reserve; L=Left; R=Right.

See text for further details.

Figure 3. Fractal dimension

Panel A shows reduction of fractal dimension in AD-H_{CR} patients compared with AD-L_{CR} patients in the left temporal cortices. Panel B shows increase of fractal dimension in a-MCI-H_{CR} compared with a-MCI-L_{CR} patients in the right temporal and in the left temporo-parietal lobes.

The statistical comparisons were overlapped on MRIcron ch2bet template (https://www.nitrc.org/projects/mricron).

Abbreviations: AD-H_{CR}= Alzheimer's Disease with high cognitive reserve; AD-L_{CR}= Alzheimer's Disease with low cognitive reserve; a-MCI-H_{CR}= amnestic Mild Cognitive Impairment with high cognitive reserve; a-MCI-L_{CR}= amnestic Mild Cognitive Impairment with low cognitive reserve.

L=Left; R=Right.

See text for further details.

Figure 4. Associations between cortical thickness and cognitive tests

Panel A reports significant positive associations in the AD-H_{CR} group between patients' performance on the 15-Word List (immediate recall) and their cortical thickness in the left posterior parietal cortex, in the left inferior temporal gyrus, and in the bilateral middle temporal gyrus. In the same group, a significant positive association was found between patients' scores on the Raven's Progressive Matrices and cortical thickness in their right frontal pole. Panel B shows positive association in the a-MCI-H_{CR} group between patients' scores on the digit span backward and cortical thickness in the left inferior frontal gyrus. Panel C illustrates in th HS-H_{CR} group positive associations between scores at the Raven's Progressive Matrices and cortical thickness in the pre-central gyrus and post-central gyrus and in the precuneus bilaterally and between scores at the Copy of Drawings test and cortical thickness in the right precentral and inferior frontal gyri.

The statistical comparisons were overlapped on MRIcron ch2bet template (https://www.nitrc.org/projects/mricron).

Abbreviations: AD-H_{CR}= Alzheimer's Disease with high cognitive reserve; AD-L_{CR}= Alzheimer's Disease with low cognitive reserve; a-MCI-H_{CR}= amnestic Mild Cognitive Impairment with high cognitive reserve; a-MCI-L$_{CR}$= amnestic Mild Cognitive Impairment with low cognitive reserve; HS-H$_{CR}$= Healthy Subjects with high cognitive reserve; HS-L$_{CR}$= Healthy Subjects with low cognitive reserve. L=Left; R=Right.

See text for further details.

Figure 5. Associations between Fractal dimension and cognitive tests

Ppositive associations in a-MCI-H$_{CR}$ is observed between Digit span backward scores and Fractal dimension in the left middle temporal gyrus. Moreover, in the a-MCI-L$_{CR}$ group, a significant positive association was found between scores on the 15-Word List and FD in the left middle temporal gyrus. The statistical comparisons were overlapped on MRIcron ch2bet template (https://www.nitrc.org/projects/mricron).

Abbreviations: a-MCI-H$_{CR}$= amnestic Mild Cognitive Impairment with high cognitive reserve; a-MCI-L$_{CR}$= amnestic Mild Cognitive Impairment with low cognitive reserve. L=Left.

Table 1. Principal demographic and clinical characteristics of the participants.

	Participants					
	AD-HCR	**AD-LCR**	**a-MCI-HCR**	**a-MCI-LCR**	**HS-HCR**	**HS-LCR**
	N=52	**N=58**	**N=46**	**N=58**	**N=20**	**N=44**
Mean (SD) Age [years][a]	73.2 (6.8)*#	72.9 (6.2)*#	70.2 (8.9)&	71.1 (7.1)&	62.2 (9.5)	64.4 (9.4)
Mean (SD) education [years] [a]	13.7 (2.5)*§	6.1 (2.6)*	14.6 (2.1)&§	6.2 (1.7)&	16.9 (0.2)§	11.6 (2.6)
Sex (M/F)[b]	19/33	23/35	30/16	21/37	7/13	18/26
Mean (SD) MMSE score [a]	22.3 (4.1)* #§	20.6 (4.1)*#	27.7 (1.7)&§	26.5 (1.9)&	29.6 (0.9)§	28.8 (1.3)
Mean (SD) MTA score [a]	2.9 (0.7)*#	2.9 (0.9)*#	2.1 (0.8)&	1.9 (0.8) &	0.7 (0.5)	0.8 (0.8)

[a] Two-ways ANOVA; [b] Chi-square; Post-hoc comparisons: *AD *vs.* HS p-value<0.05; #AD *vs*. a-MCI p-value<0.05; &a-MCI *vs.* HS p-value<0.05; § High-CR >Low-CR p-value<0.05.

Abbreviations: AD-LCR: Alzheimer's Disease patients with low cognitive reserve; AD-HCR: Alzheimer's Disease patients with high cognitive reserve; a-MCI-LCR: amnestic Mild Cognitive Impairment patients with low cognitive reserve; a-MCI-HCR: amnestic Mild Cognitive Impairment patients and high cognitive reserve; HS-LCR: healthy subjects with low cognitive reserve; HS-HCR: healthy subjects with high cognitive reserve; MMSE: Mini Mental State Examination; MTA: medial temporal lobe scale. See text for further details.

Table 2. Performance obtained by participants on neuropsychological testing.

Neuropsychological tests	**Participants**						**Two-ways ANOVA**		
	AD-H$_{CR}$	**AD-L$_{CR}$**	**a-MCI-H$_{CR}$**	**a-MCI-L$_{CR}$**	**HS-H$_{CR}$**	**HS-L$_{CR}$**	**Group effect**	**CR level effect**	**Group x CR level**
<u>Verbal episodic memory</u>									
15-Word List:									
Immediate recall (cut-off ≥28.5)	19.5 (8.3)*#§	17.7 (7.1)*#	29.2 (7.9)&§	25.5 (7.7)&	47.0 (1.8)§	40.7 (9.6)	$F_{2,269}$=173.1 **$p<0.001$**	$F_{1,269}$=13.9 **$p<0.001$**	$F_{2,269}$=1.4 $p=0.247$
Delayed recall (cut-off ≥4.6)	1.3 (1.3)*#	1.3 (1.5)*#	4.2 (2.8)&	3.7 (2.4)&	7.7 (1.5)	9.5 (2.1)	$F_{2,269}$=233.9 **$p<0.001$**	$F_{1,269}$=6.7 $p=0.010$	$F_{2,269}$=2.4 $p=0.094$
Recognition *hit rates*	8.4 (0.5)*#	8.5 (0.4) *#	11.1 (0.5)&	10.8 (0.4)&	13.8 (0.9)	13.6 (0.5)	$F_{2,269}$=43.2 **$p<0.001$**	$F_{1,269}$=0.06 $p=0.811$	$F_{2,269}$=0.11 $p=0.896$
Recognition *false*	6.3 (0.7)*	6.6 (0.7)*	3.9 (0.7)	4.2 (0.7)	1.1 (1.2)	1.2 (0.8)	$F_{2,269}$=17.9 **$p<0.001$**	$F_{1,269}$=0.12 $p=0.732$	$F_{2,269}$=0.01 $p=0.991$
Short Story:									
Immediate recall (cut-off > 3.1)	2.4 (2.0)*#	2.1 (2.0)*#	4.7 (1.6)&	4.0 (1.9)&	6.5 (1.2)	5.6 (1.5)	$F_{2,269}$=66.2 **$p<0.001$**	$F_{1,269}$=5.7 $p=0.018$	$F_{2,269}$=0.48 $p=0.621$

Delayed recall (cut-off ≥ 2.8)	0.9 (1.4)*#	0.9 (1.8) *#	4.0 (2.0)&	3.7 (2.2)&	5.9 (1.5)	5.6 (1.4)	$F_{2,269}=104.7$ **$p<0.001$**	$F_{1,269}=0.4$ $p=0.517$	$F_{2,269}=0.2$ $p=0.814$
Visuo-spatial episodic memory									
Rey's Complex Figure:									
Immediate recall (cut-off > 6.4)	3.0 (3.5)*#	2.6 (3.0)*#	10.2 (8.2)&	6.7 (4.8)&	14.8 (5.9)	14.9 (7.0)	$F_{2,269}=71.1$ **$p<0.001$**	$F_{1,269}=2.9$ $p=0.091$	$F_{2,269}=2.3$ $p=0.106$
Delayed recall (cut-off ≥ 6.3)	3.0 (3.3)*#	2.0 (2.7)*#	9.6 (6.7)&	6.8 (5.2)&	15.9 (5.4)	15.0 (6.0)	$F_{2,269}=111.3$ **$p<0.001$**	$F_{1,269}=5.4$ $p=0.021$	$F_{2,269}=0.9$ $p=0.409$
Verbal short-term memory									
Digit Span forward (cut-off > 3.7)	5.1 (1.1)*	4.8 (1.1)*	5.6 (1.0)&	5.0 (0.9)&	6.5 (1.0)	6.2 (1.2)	$F_{2,269}=31.2$ **$p<0.001$**	$F_{1,269}=8.1$ $p=0.005$	$F_{2,269}=0.36$ $p=0.697$
Digit Span backward	3.2 (1.3)*#§	2.5 (1.7)#*	4.0 (0.9)&§	3.1 (1.3)&	4.8 (0.9)§	4.4 (0.8)	$F_{2,269}=31.8$ **$p<0.001$**	$F_{1,269}=15.6$ **$p<0.001$**	$F_{2,269}=0.6$ $p=0.505$
Visuo-spatial short-term memory									
Corsi Span forward (cut-off ≥ 3.5)	3.4 (1.5)*#	3.2 (1.5) #*	4.4 (0.8)&	4.0 (0.5)&	6.5 (1.0)	6.2 (1.2)	$F_{2,269}=53.3$ **$p<0.001$**	$F_{1,269}=4.8$ $p=0.028$	$F_{2,269}=0.17$ $p=0.845$

Corsi Span backward	3.2 (1.9)*	2.5 (1.7)*	4.1 (1.1) &	3.6 (1.2)&	4.8 (0.9)	6.3 (0.8)	$F_{2,269}$=12.5 **p<0.001**	$F_{1,269}$=5,6 p=0.019	$F_{2,269}$=2.39 p=0.094
<u>Executive functions</u>									
Phonological verbal fluency (cut-off ≥ 17.3)	22.4 (10.0)*#§	17.4 (9.9)*#	32.9 (9.1)&§	23.2 (8.6)&	42.5 (10.5)§	36.2 (10.0)	$F_{2,269}$=73.5 **p<0.001**	$F_{1,269}$=30.9 **p<0.001**	$F_{2,269}$=1.5 p=0.226
Modified Card Sorting Test:									
Criteria achieved (cut-off ≥ 17.3)	2.3 (1.1)*#	2.0 (1.6)*#	4.2 (2.0)&	3.1 (1.8)&	6.0 (0.3)	5.6 (1.1)	$F_{2,269}$=71.7 **p<0.001**	$F_{1,269}$=6.9 p=0.009	$F_{2,269}$=1.8 p=0.174
<u>Reasoning</u>									
Raven's Progressive Matrices (cut-off ≥ 18.9)	21.6 (7.6)*#§	18.1 (7.7)*#	27.0 (4.9)&§	23.5 (5.4)&	32.1 (3.7)§	31.6 (3.3)	$F_{2,269}$=73.4 **p<0.001**	$F_{1,269}$=10.6 **p=0.001**	$F_{2,269}$=1.33 p=0.267
<u>Language</u>									
Naming of objects (cut-off ≥ 22)	25.4 (2.7)*#	23.4 (1.1)*#	28.9 (0.9)	27.9 (1.7)	29.7 (0.3)	28.9 (0.5)	$F_{2,269}$=22.9 **p<0.001**	$F_{1,269}$=3.5 p=0.062	$F_{2,269}$=0.34 p=0.716
<u>Constructional praxis</u>									

Copy of drawings (cut-off ≥ 7.1)	7.8 (3.6)*#§	6.4 (3.2)*#	10.1 (1.4)&§	8.2 (2.0)&	11.3 (0.9)§	11.0 (1.1)	$F_{2,269}=48.7$ **p<0.001**	$F_{1,269}=14.5$ **p<0.001**	$F_{2,269}=1.9$ p=0.138
Copy of drawings with landmarks (cut-off ≥ 61.8)	57.1 (21.8)*#	51.3 (21.4)	68.1 (1.7)	63.3 (9.8)	69.4 (0.6)	69.3 (0.7)	$F_{2,269}=23.6$ **p<0.001**	$F_{1,269}=3.28$ p=0.072	$F_{2,269}=0.64$ p=0.527
Rey's Complex Figure-Copy (cut-off ≥23.7**)**	21.9 (12.2)*#§	18.2 (11.6)*#	30.8 (6.5)&§	25.1 (8.1)&	33.1 (3.4) §	32.4 (2.7)	$F_{2,269}=35.6$ **p<0.001**	$F_{1,269}=8.2$ **p<0.001**	$F_{2,269}=1.4$ p=0.257

Two-ways ANOVA; Post-hoc comparisons: *AD *vs.* HS p-value<0.05; #AD *vs.* a-MCI p-value<0.05; &a-MCI *vs.* HS p-value<0.05; § High-CR >Low-CR p-value<0.05. In bold p-values surviving after Bonferroni's correction (p≤0.003).

Abbreviations: AD-L_{CR}: Alzheimer's Disease patients with low cognitive reserve; AD-H_{CR}: Alzheimer's Disease patients with high cognitive reserve; a-MCI-L_{CR}: amnestic Mild Cognitive Impairment patients with low cognitive reserve; a-MCI-H_{CR}: amnestic Mild Cognitive Impairment patients and high cognitive reserve; HS-L_{CR}: healthy subjects with low cognitive reserve; HS-H_{CR}: healthy subjects with high cognitive reserve; See text for further details.

Table 3. Cortical thickness in the participants according with their cognitive reserve's level

Group	Brain region	Side	Size	MNI coordinates			Z	p-level FWE-cluster level
				x	y	z		
AD-H$_{CR}$<AD-L$_{CR}$								
	Post central gyrus	**L**	**26555**	**-46**	**-14**	**30**	**24.2**	**<0.001**
	Inferior Frontal gyrus			-39	21	22	14.5	
	Orbitofrontal cortex			-36	33	-11	10.9	
	Primary visual cortex	**L**	**20891**	**-17**	**-75**	**4**	**18.3**	**<0.001**
	Fusiform gyrus			-30	-54	-8	15.2	
	Parietal operculum	**L**	**3821**	**-53**	**-40**	**29**	**16.1**	**0.014**
	Superior Parietal cortex	**L**	**4354**	**-31**	**-55**	**54**	**6.24**	**0.006**
	Supramarginal gyrus			-45	-47	42	5.35	
	Cingulate gyrus	**L**	**3779**	**-14**	**-45**	**35**	**6.15**	**0.015**
	Precuneus			-15	-46	54	4.08	
	Medial temporal gyrus	**R**	**3564**	**52**	**-41**	**-3**	**3.25**	**0.021**
	Inferior Frontal gyrus	**R**	**4418**	**57**	**20**	**16**	**2.65**	**0.005**

	Medial frontal gyrus			38	9	40	2.61	
a-MCI-H$_{CR}$ < a-MCI-L$_{CR}$								
	Supplementary motor cortex	**L**	**4565**	**-8**	**7**	**44**	**3.40**	**0.001**
	Superior Frontal gyrus			-7	28	58	2.93	
	Temporal pole	**R**	**5770**	**52**	**12**	**17**	**3.53**	**<0.001**
	Inferior Temporal gyrus			55	20	-35	3.21	
	Fusiform gyrus			29	2	-41	2.81	
AD-H$_{CR}$>AD-L$_{CR}$	**Supplementary motor cortex**	**L**	**8215**	**-4**	**-8**	**54**	**6.48**	**<0.001**
	Frontal pole			-7	59	29		
	Middle Temporal gyrus	**L**	**11720**	**-64**	**-51**	**1**	**6.47**	**<0.001**
	Supramarginal gyrus			-57	-27	30		
	Posterior cingulate cortex	**L**	**1564**	**-5**	**-53**	**12**	**6.32**	**<0.001**
HS -H$_{CR}$ > HS-L$_{CR}$								
	Precentral gyrus	**L**	**3015**	**-29**	**-3**	**46**	**3.24**	**0.024**
	Medial frontal gyrus			-38	25	43	2.86	

Parietal operculum	**R**	**6227**	**62**	**-19**	**18**	**4.13**	**<0.001**
Inferior Frontal gyrus			49	10	16	3.19	
Medial temporal gyrus	**R**	**4204**	**52**	**-49**	**8**	**3.59**	**0.001**
Superior Temporal gyrus			51	-34	1	2.44	
Anterior Cingulate cortex	**R**	**3131**	**5**	**20**	**32**	**3.13**	**0.016**
Paracingulate gyrus			11	36	25	2.69	
Frontal pole	**R**	**3461**	**35**	**50**	**13**	**3.11**	**0.008**
Orbitofrontal cortex			28	22	-22	2.35	

Abbreviations: AD-L$_{CR}$: Alzheimer's Disease patients with low cognitive reserve; AD-H$_{CR}$: Alzheimer's Disease patients with high cognitive reserve; a-MCI-L$_{CR}$: amnestic Mild Cognitive Impairment patients with low cognitive reserve; a-MCI-H$_{CR}$: amnestic Mild Cognitive Impairment patients and high cognitive reserve; HS-L$_{CR}$: healthy subjects with low cognitive reserve; HS-H$_{CR}$: healthy subjects with high cognitive reserve; L= left; R=Right.

See text for further details

Table 4. Fractal dimension in the participants according with their cognitive reserve's level

Group	Brain region	Side	Size	MNI coordinates			Z	p-level FWE-cluster level
				x	y	z		
AD-HCR<AD-LCR								
	Medial temporal gyrus	**L**	**3856**	**-52**	**-53**	**5**	**3.37**	**0.001**
	Superior Temporal gyrus			-61	-31	0	3.04	
a-MCI-HCR > a-MCI-LCR								
	Temporal pole	**R**	**2715**	**45**	**12**	**-32**	**3.89**	**0.019**
	Fusiform gyrus			36	-31	-26	2.75	
	Medial temporal gyrus	**L**	**3654**	**-59**	**-43**	**-11**	**4.11**	**0.002**
	Lateral Occipital gyrus			-45	-64	12	2.40	

Abbreviations: AD-LCR: Alzheimer's Disease patients with low cognitive reserve; AD-HCR: Alzheimer's Disease patients with high cognitive reserve; a-MCI-LCR: amnestic Mild Cognitive

Impairment patients with low cognitive reserve; a-MCI-H_{CR}: amnestic Mild Cognitive Impairment patients and high cognitive reserve; L= left; R=Right.

See text for further details**.**

Table 5 Relationship between neuropsychological tests and Cortical thickness and Fractal dimension

Group	Neuropsychological test	Brain region	Side	Size	MNI coordinates			Z	p-level FWE-cluster level
					x	y	z		
Cortical thickness									
AD-H$_{CR}$									
	15-Word List IR	**Posterior parietal lobe**	**L**	**1372**	**-46**	**-63**	**20**	**4.39**	**<0.001**
		Middle temporal gyrus	L		-49	-57	12	3.63	
		Inferior temporal gyrus	**L**	**563**	**-58**	**-44**	**-12**	**3.75**	**0.03**
		Middle temporal gyrys	**R**	**863**	**61**	**-48**	**4**	**4.08**	**0.006**
	Raven's Progressive Matrices	**Middle frontal gyrus**	**R**	**1216**	**46**	**7**	**47**	**3.75**	**0.001**
		Precentral gyrus	R		38	2	33	3.60	
		Frontal pole	**R**	**545**	**16**	**45**	**34**	**3.73**	**0.037**

a-MCI-H_{CR}	**Digit Span backward**	**Inferior frontal gyrus**	**L**	**531**	**-45**	**26**	**4**	**5.04**	**0.030**
HS-H_{CR}	**Raven's Progressive Matrices**	**Precentral gyrus**	**L**	**1150**	**-24**	**-30**	**55**	**4.86**	**<0.001**
		Postcentral gyrus	**L**	**2385**	**-50**	**-17**	**50**	**4.30**	**<0.001**
		Precuneus	**L**	**581**	**-8**	**-68**	**26**	**3.69**	**0.016**
		Precentral gyrus	**R**	**985**	**15**	**-30**	**59**	**4.04**	**0.001**
	Copy of Drawings	**Precentral gyrus**	**R**	**630**	**24**	**-8**	**48**	**4.53**	**0.011**
		Inferior frontal gyrus	**R**	**653**	**38**	**17**	**22**	**4.22**	**0.009**
Fractal dimension									
a-MCI-H_{CR}	**Corsi span forward**	**Middle temporal gyrus**	**L**	**649**	**-63**	**-41**	**-11**	**4.06**	**0.007**
a-MCI-L_{CR}	**15-Word List IR**	**Middle temporal gyrus**	**L**	**471**	**-51**	**-22**	**-12**	**4.04**	**0.028**

Figure 1.

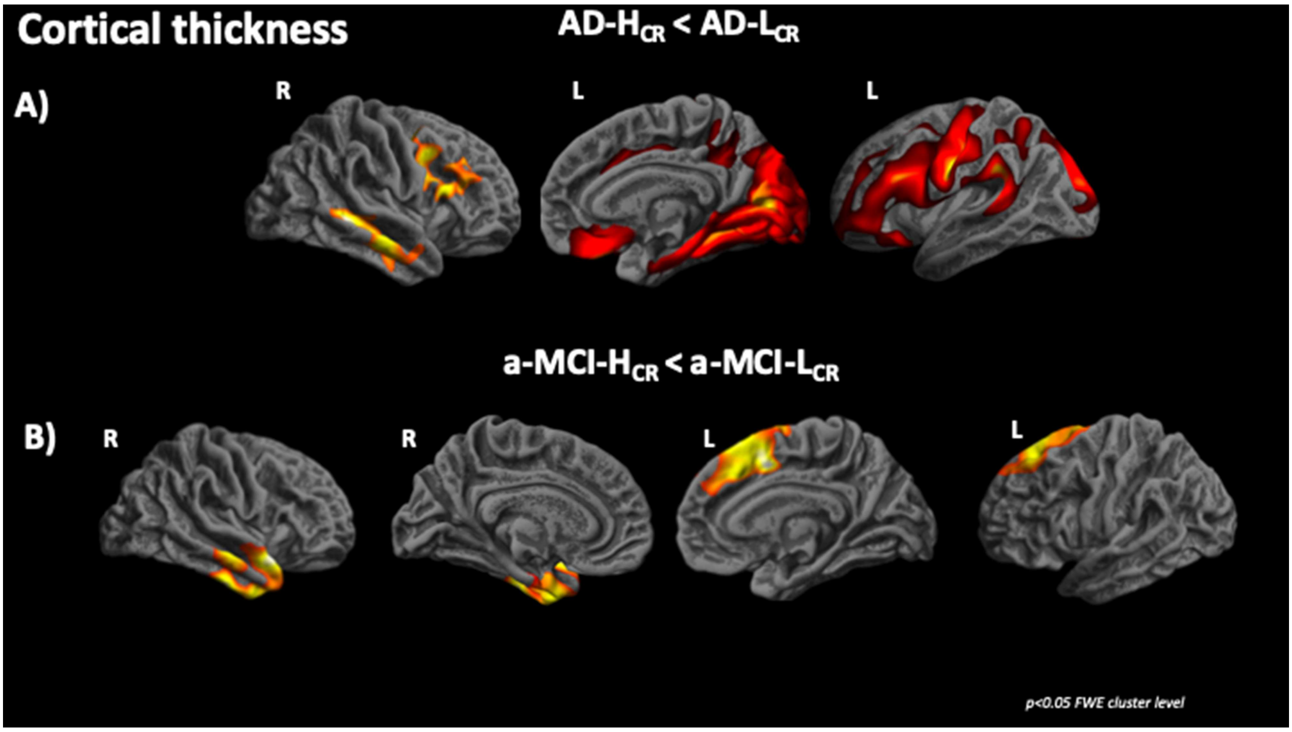

Cortical thickness
AD-H$_{CR}$ < AD-L$_{CR}$
A)
R
L
L
a-MCI-H$_{CR}$ < a-MCI-L$_{CR}$
B)
R
R
L
L
p<0.05 FWE cluster level

Figure 2.

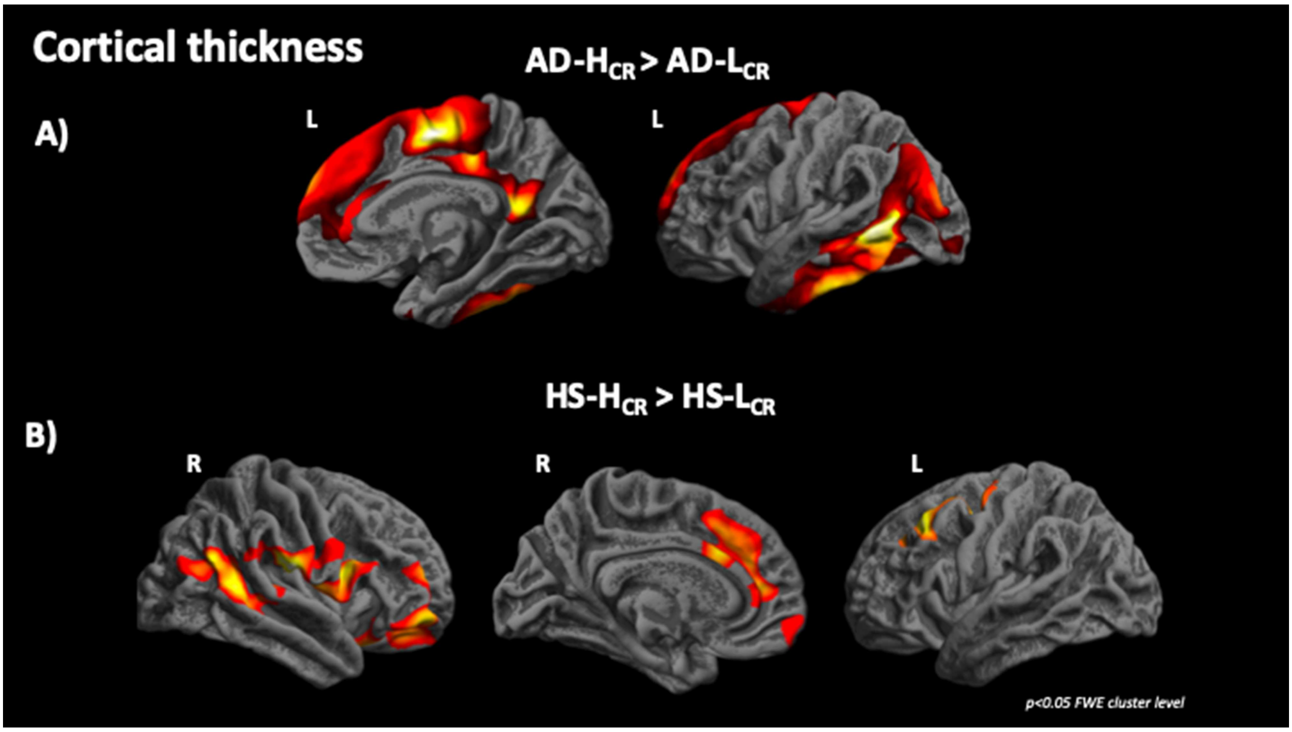

Cortical thickness
A)
$AD\text{-}H_{CR} > AD\text{-}L_{CR}$
L
L
B)
$HS\text{-}H_{CR} > HS\text{-}L_{CR}$
R
R
L
p<0.05 FWE cluster level

Figure 3.

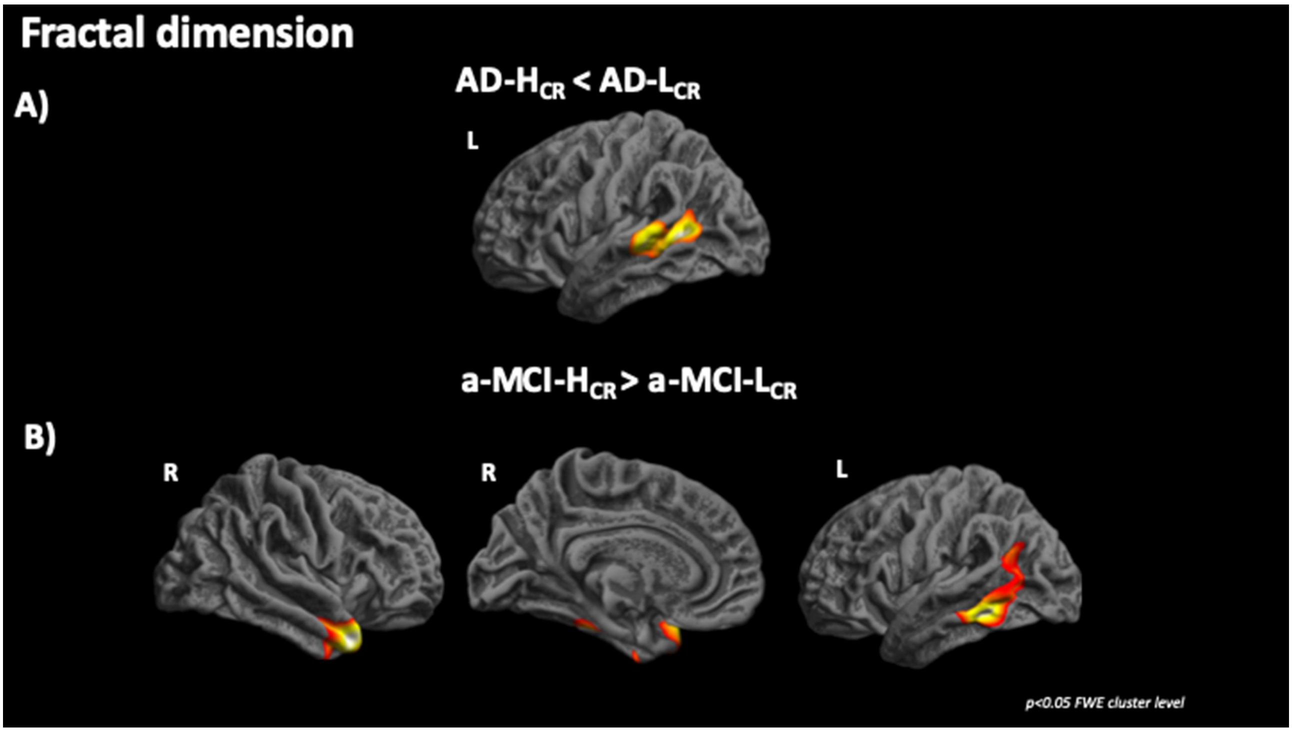

Fractal dimension
A)
$AD\text{-}H_{CR} < AD\text{-}L_{CR}$
L
$a\text{-}MCI\text{-}H_{CR} > a\text{-}MCI\text{-}L_{CR}$
B)
R
R
L
p<0.05 FWE cluster level

Figure 4.

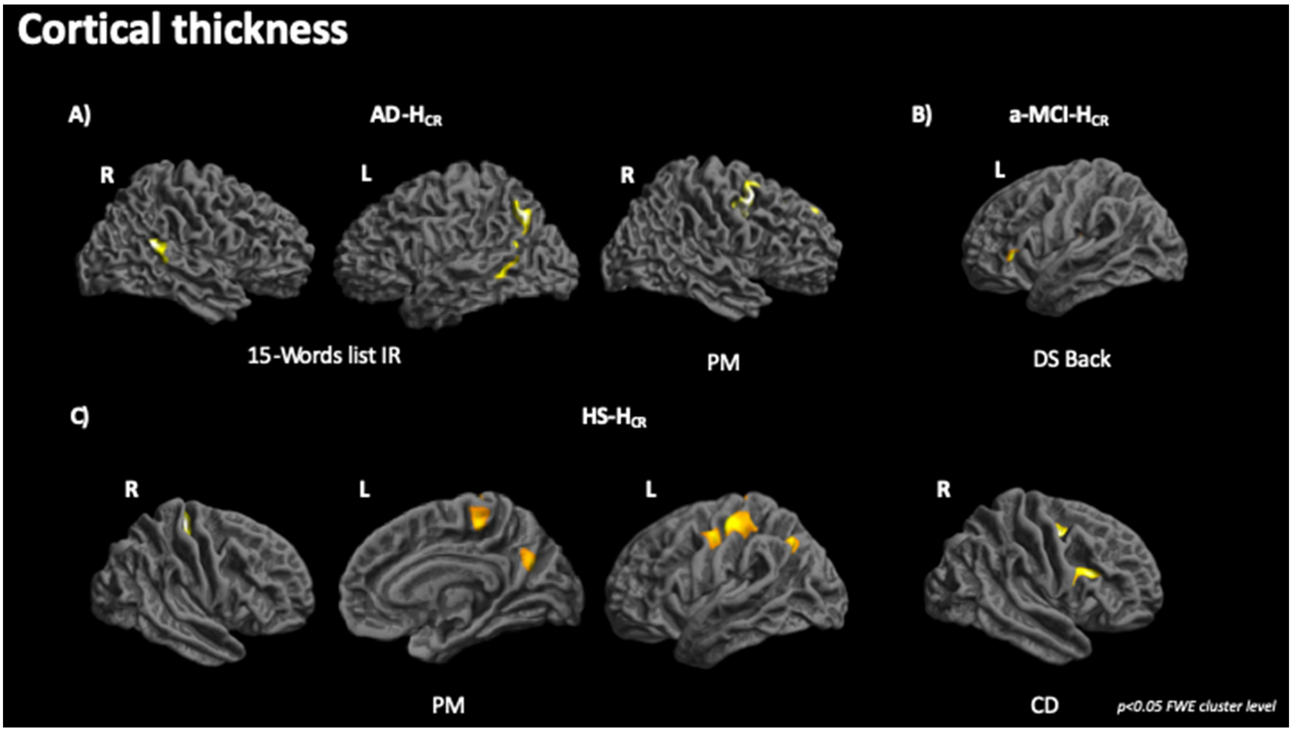
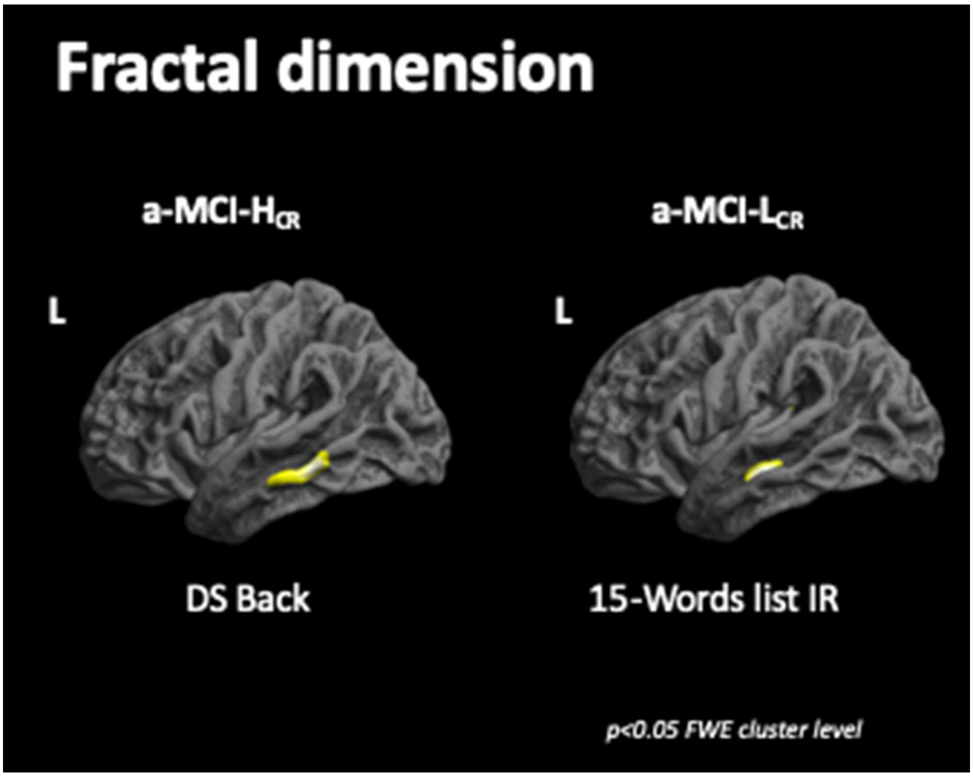

Cortical thickness
A)
AD-H_CR
R
L
R
15-Words list IR
PM
B)
a-MCI-H_CR
L
DS Back
C)
HS-H_CR
R
L
L
R
PM
CD
p<0.05 FWE cluster level

Figure 5.


Fractal dimension
a-MCI-H$_{CR}$
a-MCI-L$_{CR}$
L
L
DS Back
15-Words list IR
p<0.05 FWE cluster level

References

[1] Sandu AL, Staff RT, McNeil CJ, Mustafa N, Ahearn T, Whalley LJ, Murray AD (2014) Structural brain complexity and cognitive decline in late life—a longitudinal study in the Aberdeen 1936 Birth Cohort. *Neuroimage* **100**, 558-63.

[2] Collins CE, Airey DC, Young NA, Leitch DB, Kaas JH (2010) Neuron densities vary across and within cortical areas in primates. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 15927–15932

[3] la Fougère C, Grant S, Kostikov A, Schirrmacher R, Gravel P, Schipper HM, Reader A, Evans A, Thiel A (2011) Where in-vivo imaging meets cytoarchitectonics: the relationship between cortical thickness and neuronal density measured with high resolution [18 F]flumazenil-PET. *Neuroimage* **56**, 951–960.

[4] Dahnke R, Yotter RA, Gaser C (2013) Cortical thickness and central surface estimation. *Neuroimage* **65**,336–348.

[5] Wagstyl K, Ronan L, Goodyer IM, Fletcher PC (2015) Cortical thickness gradients in structural hierarchies. *Neuroimage* 111:241–250.

[6] Pettigrew C, Soldan A, Zhu Y, Wang MC, Moghekar A, Brown T, Miller M, Albert M; BIOCARD Research Team (2016) Cortical thickness in relation to clinical symptom onset in preclinical AD. *Neuroimage Clin.* **12**,116-22.

[7] King RD, George AT, Jeon T, Hynan LS, Youn TS, Kennedy DN, Dickerson B (2009) Characterization of atrophic changes in the cerebral cortex using fractal dimensional analysis. *Brain Imaging Behav.* **3**,154–166.

[8] King RD, Brown B, Hwang M, Jeon T, George AT (2010) Fractal dimension analysis of the cortical ribbon in mild Alzheimer's disease. *Neuroimage* **53**, 471–479.

[9] Lerch JP, Pruessner JC, Zijdenbos A, Hampel H, Teipel SJ, Evans AC (2005) Focal decline of cortical thickness in Alzheimer's disease identified by computational neuroanatomy. *Cereb Cortex.* **15**,995-1001.

[10] Duarte-Abritta B, Villarreal MF, Abulafia C, Loewenstein D, Curiel Cid RE, Castro MN, Surace E, Sánchez SM, Vigo DE, Vázquez S, Nemeroff CB, Sevlever G, Guinjoan SM (2018) Cortical thickness, brain metabolic activity, and in vivo amyloid deposition in asymptomatic, middle-aged offspring of patients with late-onset Alzheimer's disease. *J Psychiatr Res*. **107**, 11-18.

[11] Rogers J, Kochunov P, Lancaster J, Shelledy W, Glahn D, Blangero J, Fox P (2007) Heritability of brain volume, surface area and shape: an MRI study in an extended pedigree of baboons. *Hum Brain Mapp.* **28**,576-83.

[12] Panizzon MS, Fennema-Notestine C, Eyler LT, Jernigan TL, Prom-Wormley E, Neale M, Jacobson K, Lyons MJ, Grant MD, Franz CE, Xian H, Tsuang M, Fischl B, Seidman L, Dale A, Kremen WS (2009) Distinct genetic influences on cortical surface area and cortical thickness. *Cereb Cortex.* **19**,2728-35

[13] Zilles K, Palomero-Gallagher N, Amunts K (2013) Development of cortical folding during evolution and ontogeny. *Trends Neurosci.* 36, 275-84.

[14] Serra L, Gelfo F, Petrosini L, Di Domenico C, Bozzali M, Caltagirone C (2018) Rethinking the Reserve with a Translational Approach: Novel Ideas on the Construct and the Interventions. *J Alzheimers Dis.* **65**, 1065-1078.

[15] Perneczky R (2019). Dementia prevention and reserve against neurodegenerative disease. *Dialogues Clin Neurosci.* **21**,53-60.

[16] Stern Y, Barulli D (2019) Cognitive reserve. *Handb Clin Neurol.* **167**,181-190.

[17] Serra L, Mancini M, Cercignani M, Di Domenico C, Spanò B, Giulietti G, Koch G, Marra C, Bozzali M (2017) Network-Based Substrate of Cognitive Reserve in Alzheimer's Disease. *J Alzheimers Dis.* **55**,421-430.

[18] Serra L, Cercignani M, Petrosini L, Basile B, Perri R, Fadda L, Spanò B, Marra C, Giubilei F, Carlesimo GA, Caltagirone C, Bozzali M (2011) Neuroanatomical correlates of cognitive reserve in Alzheimer disease. *Rejuvenation Res.* **14,**143-51.

[19] Serra L, Petrosini L, Salaris A, Pica L, Bruschini M, Di Domenico C, Caltagirone C, Marra C, Bozzali M (2019) Testing for the Myth of Cognitive Reserve: Are the Static and Dynamic Cognitive Reserve Indexes a Representation of Different Reserve Warehouses? *J Alzheimers Dis.* **72**,111-126.

[20] Querbes O, Aubry F, Pariente J, Lotterie JA, Démonet JF, Duret V, Puel M, Berry I, Fort JC, Celsis P; Alzheimer's Disease Neuroimaging Initiative (2009) Early diagnosis of Alzheimer's disease using cortical thickness: impact of cognitive reserve. *Brain.* **132**, 2036-2047.

[21] Pettigrew C, Soldan A, Zhu Y, Wang MC, Brown T, Miller M, Albert M; BIOCARD Research Team (2017) Cognitive reserve and cortical thickness in preclinical Alzheimer's disease. *Brain Imaging Behav.* **11**,357-367.

[22] Jung NY, Cho H, Kim YJ, Kim HJ, Lee JM, Park S, Kim ST, Kim EJ, Kim JS, Moon SH, Lee JH, Ewers M, Na DL, Seo SW (2018) The impact of education on cortical thickness in amyloid-negative subcortical vascular dementia: cognitive reserve hypothesis. *Alzheimers Res Ther.* **10**,103.

[23] Cutuli D, Landolfo E, Petrosini L, Gelfo F (2022) Environmental Enrichment Effects on the Brain-Derived Neurotrophic Factor Expression in Healthy Condition, Alzheimer's Disease, and Other Neurodegenerative Disorders. *J Alzheimers Dis.* **85**,975-992.

[24] Gelfo F (2019) Does Experience Enhance Cognitive Flexibility? An Overview of the Evidence Provided by the Environmental Enrichment Studies. *Front Behav Neurosci.* **9**,13:150.

[25] Anderson BJ, Eckburg PB, Relucio KI (2002) Alterations in the thickness of motor cortical subregions after motor-skill learning and exercise. *Learn Mem.* **9**,1-9.

[26] Nicastro N, Malpetti M, Cope TE, Bevan-Jones WR, Mak E, Passamonti L, Rowe JB, O'Brien JT (2020) Cortical Complexity Analyses and Their Cognitive Correlate in Alzheimer's Disease and Frontotemporal Dementia. *J Alzheimers Dis.* **76**,331-340.

[27] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, Klunk WE, Koroshetz WJ, 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.

[28] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment 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**, 270-279.

[29] American Psychiatric Association (APA) (2013) Diagnostic and Statistical Manual of Mental Disorders 5th edn. *American Psychiatric Association*.

[30] Hughes CP, Berg, L, Danziger WL, Coben LA, Martin RL (1982) A new clinical scale for the staging of dementia. *Br J Psychiatry* **140,** 566-572.

[31] Hachinski VC, Iliff LD, Zilhka E, Du Boulay GH, McAllister VL, Marshall J, Russell RW, Symon L (1975) Cerebral blood flow in dementia. *Arch Neurol* **3,** 632-637.

[32] Büsch D, Hagemann N, Bender N (2010) The dimensionality of the Edinburgh Handedness Inventory: An analysis with models of the item response theory. *Laterality* **15**, 610-628.

[33] Carlesimo GA, Caltagirone C, Gainotti G. (1996) The Mental Deterioration Battery: normative data, diagnostic reliability and qualitative analyses of cognitive impairment. The Group for the Standardization of the Mental Deterioration Battery. *Eur Neurol* **36**,378-84.

[34] Carlesimo GA, Buccione I, Fadda L, Graceffa A, Mauri M, Lo Russo S, Bevilacqua G, Caltagirone C. (2002) Standardizzazione di due test di memoria per uso clinico: Breve Racconto e Figura di Rey. *Nuova Rivista di Neurologia* **12**,1-13.

[35] Monaco M, Costa A, Caltagirone C, Carlesimo GA (2013) Forward and backward span for verbal and visuo-spatial data: standardization and normative data from an Italian adult population. *Neurol Sci*. **34**,749-754.

[36] Nocentini U, Di Vincenzo S, Panella M, Pasqualetti P, Caltagirone C (2002) La valutazione delle funzioni esecutive nella pratica neuropsicologica: dal Modified Card Sorting Test al Modified Card Sorting Test-Roma Version. Dati di standardizzazione. *Nuova Rivista di Neurologia* **12**,14-24.

[37] Miceli G, Laudanna A, Burani C, Capasso R. Batteria per l'analisi dei deficit afasici. Milano: Ass.ne per lo sviluppo delle ricerche neuropsicologiche. *Berdata*; (1991).

[38] Folstein MF, Folstein SE, McHugh PR (1975) Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. *J Psychiaîr Res*. **12**,189-198.

[39] Measso G, Zappalà G, Cavarzeran F, Crook TH, Romani L, Pirozzolo FJ, Grigoletto F, Amaducci LA, Massari D, Lebowitz BD (1993) The Mini Mental State Examination: Normative study of a random sample of Italian population. *Dev Neuropsychol;* 9:77-95.

[40] Dahnke R, Yotter RA, Gaser C (2013) Cortical thickness and central surface estimation. *Neuroimage* 65:336–348.

[41] Yotter RA, Nenadic I, Ziegler G, Thompson PM, Gaser C (2011) Local cortical surface complexity maps from spherical harmonic reconstructions. *Neuroimage* **56**:961-73.

[42] Yotter RA, Dahnke R, Thompson PM, Gaser C (2011) Topological correction of brain surface meshes using spherical harmonics. *Hum Brain Mapp*. 32(7):1109-24.

[43] Scheltens P, Launer LJ, Barkhof F, Weinstein HC, van Gool WA (1995). Visual assessment of medial temporal lobe atrophy on magnetic resonance imaging: interobserver reliability. *J Neurol* **242:** 557-560.

[44 Pereira JB, Cavallin L, Spulber G, Aguilar C, Mecocci P, Vellas B, Tsolaki M, Kłoszewska I, Soininen H, Spenger C, Aarsland D, Lovestone S, Simmons A, Wahlund LO, Westman E; AddNeuroMed consortium and for the Alzheimer's Disease Neuroimaging Initiative (2014). Influence of age, disease onset and ApoE4 on visual medial temporal lobe atrophy cut-offs. *Intern Med* **275:** 317-330.

[45] Stern Y (2009) Cognitive reserve. *Neuropsychologia* **47**: 2015-28.

[46] Rao YL, Ganaraja B, Murlimanju BV, Joy T, Krishnamurthy A, Agrawal A (2022) Hippocampus and its involvement in Alzheimer's disease: a review. *3 Biotech*. **12**:55.

[47] Bayram E, Caldwell JZK, Banks SJ (2018) Current understanding of magnetic resonance imaging biomarkers and memory in Alzheimer's disease. *Alzheimers Dement (N Y)* **4**:395-413

[48] Lundstrom BN, Ingvar M, Petersson KM (2005) The role of precuneus and left inferior frontal cortex during source memory episodic retrieval. *Neuroimage.* **27**:824-34.

[49] Koch G, Bonnì S, Pellicciari MC, Casula EP, Mancini M, Esposito R, Ponzo V, Picazio S, Di Lorenzo F, Serra L, Motta C, Maiella M, Marra C, Cercignani M, Martorana A, Caltagirone C, Bozzali M (2018) Transcranial magnetic stimulation of the precuneus enhances memory and neural activity in prodromal Alzheimer's disease. *Neuroimage*. **169**:302-311.

[50] Prawiroharjo P, Yamashita KI, Yamashita K, Togao O, Hiwatashi A, Yamasaki R, Kira JI (2020) Disconnection of the right superior parietal lobule from the precuneus is associated with memory impairment in oldest-old Alzheimer's disease patients. *Heliyon* 6:e04516.

[51] Serra L, Gabrielli GB, Tuzzi E, Spanò B, Giulietti G, Failoni V, Marra C, Caltagirone C, Koch G, Cercignani M, Bozzali M (2017) Damage to the Frontal Aslant Tract Accounts for Visuo-Constructive Deficits in Alzheimer's Disease. *J Alzheimers Dis.* **60**:1015-1024.

[52] Serra L, Mancini M, Silvestri G, Petrucci A, Masciullo M, Spanò B, Torso M, Mastropasqua C, Giacanelli M, Caltagirone C, Cercignani M, Meola G, Bozzali M (2016) Brain Connectomics' Modification to Clarify Motor and Nonmotor Features of Myotonic Dystrophy Type 1. *Neural Plast*: 2696085.

[53] Volle E, Kinkingnéhun S, Pochon JB, Mondon K, Thiebaut de Schotten M, Seassau M, Duffau H, Samson Y, Dubois B, Levy R (2008) The functional architecture of the left posterior and lateral prefrontal cortex in humans. *Cereb Cortex*. **18**:2460-2469.

[54] Kumar S, Zomorrodi R, Ghazala Z, Goodman MS, Blumberger DM, Cheam A, Fischer C, Daskalakis ZJ, Mulsant BH, Pollock BG, Rajji TK. (2017) Extent of Dorsolateral Prefrontal Cortex

Plasticity and Its Association With Working Memory in Patients With Alzheimer Disease. *JAMA Psychiatry* **74**:1266-1274.

[55] Kim JP, Seo SW, Shin HY, Ye BS, Yang JJ, Kim C, Kang M, Jeon S, Kim HJ, Cho H, Kim JH, Lee JM, Kim ST, Na DL, Guallar E (2015) Effects of education on aging-related cortical thinning among cognitively normal individuals. *Neurology* **85**:806-812.

[56] Im K, Lee JM, Yoon U, Shin YW, Hong SB, Kim IY, Kwon JS, Kim SI (2006) Fractal dimension in human cortical surface: multiple regression analysis with cortical thickness, sulcal depth, and folding area. *Hum Brain Mapp*. **27**:994-1003.