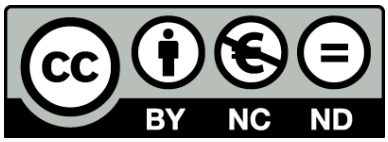
UNIVERSITAT DE BARCELONA

Characterizing cognitive aging through multimodal MRI and NIBS: influence of education and gene expression

Lídia Vaqué Alcázar



DOCTORAL THESIS

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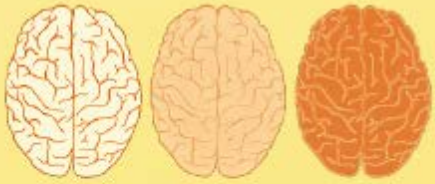

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Medical Psychology Unit
Department of Medicine
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2020

Characterizing cognitive aging through multimodal MRI and NIBS: influence of education and gene expression

Thesis presented by:

Lídia Vaqué Alcázar

To obtain the degree of doctor from the University of Barcelona in accordance with the requirements of the international PhD diploma

Supervised by:

Dr. David Bartrés Faz and Dr. Roser Sala Llonch

Medicine and Translational Research Doctoral Program

Medical Psychology Unit, Department of Medicine

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2020

'What we observe is not nature in itself, but nature exposed to our method of questioning'.

Werner Heisenberg (1901–1976).

A la meva família.

Al Kili.

Barcelona, 19th February 2020

Dr. David Bartrés Faz and Dr. Roser Sala Llonch, professors at the University of Barcelona,

CERTIFY that they have guided and supervised the Doctoral Thesis entitled 'Characterizing cognitive aging through multimodal MRI and NIBS: influence of education and gene expression' presented by Lídia Vaqué Alcázar. They hereby assert that this Thesis fulfils the requirements to present her defense to be awarded the title of doctor.

Signatures,

Dr. David Bartrés Faz Dr. Roser Sala Llonch

This Thesis has been undertaken in the Medical Psychology Unit, Department of Medicine, Faculty of Medicine and Health Sciences, University of Barcelona. The group is a consolidated research group by the Generalitat de Catalunya (grants 2014 SGR98 and 2018 SGR748) and it is part of the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) and the Institute of Neurosciences of the University of Barcelona.

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CONTENTS

FOREWORD

This Thesis, presented to obtain the degree of Doctor by the University of Barcelona, is the result of different studies carried out over a three-year period at the Medical Psychology Unit, Department of Medicine, Faculty of Medicine and Health Sciences, University of Barcelona.

This Thesis follows the published papers format, and it includes three published peer-reviewed articles and one manuscript in preparation. The four studies are presented in the following order:

1. **Vaqué-Alcázar, L.**, Sala-Llonch, R., Valls-Pedret, C., Vidal-Piñeiro, D., Fernández-Cabello, S., Bargalló, N., Ros, E. & Bartrés-Faz, D. (2017). Differential age-related gray and white matter impact mediates educational influence on elders' cognition. *Brain Imaging and Behavior, 11*(2), 318–332. Impact Factor: 3.418.

2. Bartrés-Faz, D., González-Escamilla, G., **Vaqué-Alcázar, L.**, Abellaneda-Pérez, K., Valls-Pedret, C., Ros, E. & Grothe, M. J. (2019). Characterizing the molecular architecture of cortical regions associated with high educational attainment in older individuals. *The Journal of Neuroscience, 39*(23), 4566–4575. Impact Factor: 6.074.

3. **Vaqué-Alcázar, L.,** Sala-Llonch, R., Abellaneda-Pérez, K., Coll-Padrós, N., Valls-Pedret, C., Bargalló, N., Ros, E. & Bartrés-Faz, D. (2020). Functional and structural correlates of working memory performance and stability in healthy older adults. *Brain Structure & Function, 225*(1), 375–386. Impact Factor: 3.622.

4. **Vaqué-Alcázar, L.,** Abellaneda-Pérez, K., Solé-Padullés, C., Ruffini, G., Bargalló, N., Ros, E., Sala-Llonch, R. & Bartrés-Faz, D. Effects of multifocal transcranial direct current stimulation on working memory functional patterns in healthy older adults. *In preparation.*

RELATED ACADEMIC WORK

List of additional publications of the candidate that are not included in the Thesis. These papers are the result of collaborative work with other projects during the time of the Thesis:

- Abellaneda-Pérez, K., **Vaqué-Alcázar, L.**, Vidal-Piñeiro, D., Jannati, A., Solana, E., Bargalló, N., Santarnecchi, E., Pascual-Leone, A. & Bartrés-Faz, D. (2019). Age-related differences in default-mode network connectivity in response to intermittent theta-burst stimulation and its relationships with maintained cognition and brain integrity in healthy aging. *NeuroImage, 188*, 794–806. Impact Factor: 5.812.

- Farràs-Permanyer, L., Mancho-Fora, N., Montalà-Flaque, M., Bartrés-Faz, D., **Vaqué-Alcázar, L.**, Peró-Cebollero, M. & Guàrdia-Olmos J. (2019). Age-related changes in resting-state functional connectivity in older adults. *Neural Regeneration Research*, *14*(9), 1544–1555. Impact Factor: 2.472.

- Abellaneda-Pérez, K., **Vaqué-Alcázar, L.**, Solé-Padullés, C. & Bartrés-Faz, D. (2019). Combining non-invasive brain stimulation with functional magnetic resonance imaging to investigate the neural substrates of cognitive aging. *Journal of Neuroscience Research* [epub ahead of print]. Impact Factor: 4.139.

- Sala-Vila, A., Valls-Pedret, C., Rajaram, A., Coll-Padros, N., Cofan, M., Serra-Mir, M., Perez-Heras, A. M., Roth, I., Freitas-Simoes, T. M., Domenech, M., Calvo, C., Lopez-Illamola, A., Bitok, E., Buxton, N. K., Huey, L., Arechiga, A., Oda, K., Lee, G., Corella, D., **Vaqué-Alcázar, L.**, Sala-Llonch, R., Bartrés-Faz, D., Sabate, J. & Ros, E. (2020). Effect of a two-year diet intervention with walnuts on cognitive decline. The Walnuts And Healthy Aging (WAHA) study: a randomized controlled trial. *The American Journal of Clinical Nutrition* [epub ahead of print]. Impact Factor: 6.568.

- Abellaneda-Pérez, K., **Vaqué-Alcázar, L.**, Perellón-Alfonso, R., Bargalló, N., Kuo, M.-F., Pascual-Leone, A., Nitsche, M. A. & Bartrés-Faz, D. (2020). Differential tDCS and tACS

effects on working memory-related neural activity and resting-state connectivity. *Frontiers in Neuroscience, 13*, 1440. Impact Factor: 3.648.

. Mancho-Fora, N., Montalà-Flaquer, M., Farràs-Permanyer, L., Bartrés-Faz, D., **Vaqué-Alcázar, L.**, Peró-Cebollero, M. & Guàrdia-Olmos, J. Resting-state functional connectivity dynamics in healthy aging: an approach through Network Change Point Detection. *Under review.*

GLOSSARY OF ABBREVIATIONS

AD Alzheimer's Disease

AxD Axial Diffusivity

Aβ β-Amyloid

BM Brain Maintenance

BOLD Blood-Oxygen-Level Dependent

BR Brain Reserve

CPT Continuous Performance Test

CR Cognitive Reserve

CRUNCH Compensation Related Utilization of Neural Circuits Hypothesis

CSF Cerebrospinal Fluid

CTh Cortical Thickness

DLPF Dorsolateral Prefrontal Cortex

DMN Default-Mode Network

dMRI diffusion Magnetic Resonance Imaging

DTI Diffusion Tensor Imaging

DTI Diffusion Tensor Imaging

DTIFIT Diffusion Tensor Model fit

FA Fractional Anisotropy

FLAIR Fluid Attenuation Inversion Recovery

FLAME FMRIB's Local Analysis of Mixed Effects

fMRI Functional Magnetic Resonance Imaging

FSL FMRIB Software Library

FWE Family-Wise Error

FWHM Full Width at Half Maximum

GLM General Lineal Model

GLM General Lineal Model

GM Gray Matter

GSEA Gene Set Enrichment Analysis

HAROLD Hemispheric Asymmetry Reduction in Old Adults

IQ Premorbid intelligence

MD Mean Diffusivity

MMSE Mini-Mental State Examination

MNI Montreal Neurological Institute

MRI Magnetic Resonance Imaging

MSigDB Molecular Signatures Database

NIBS Non-Invasive Brain Stimulation

PASA Posterior-Anterior Shift with Ageing

PCA Principal Component Analysis

PFC Prefrontal Cortex

RAVLT Rey Auditory Verbal Learning Test

RD Radial Diffusivity

ROCF Rey-Osterrieth Complex Figure

ROI Region Of Interest

SDMT Symbol Digit Modalities Test

STAC Scaffolding Theory of Aging and Cognition

STAC-r Scaffolding Theory of Aging and Cognition-revised

T1w T1-weighted

TBSS Tract-Based Spatial Statistics

tDCS Transcranial Direct Current Stimulation

tES Transcranial Electrical Stimulation

TFCE Threshold-Free Cluster Enhancement

TMT Trail Making Test

WHO World Health Organization

WM White Matter

WMem Working Memory

WMH White Matter Hyperintensities

CHAPTER 1

General Introduction

1.1 Cognitive aging

1.1.1 Aging: a worldwide concern

Thanks to the advances in public health and medicine, the World Health Organization (WHO) has estimated that between 2015 and 2050 the proportion of the world's population over 60 years will nearly double from 12% to 22%. The United Nations foresight by 2050 is that the proportion of elderly people will nearly double that of the young (United Nations, 2017). Despite people worldwide are living longer, unfortunately, these extra years of life are dominated by both physical and mental capacity impairments (WHO[1]).

The aging process manifests with generalized atrophy, which affects almost all organs and systems in the human body. Concretely, the nervous system and especially the brain shows multiple structural and functional variations resulting in cognitive performance disruptions. Advancing age is the foremost risk factor for the emergence of major neuropsychiatric or neurodegenerative diseases (Barnett et al., 2012). As the aging population increases worldwide, the number of people with dementia grows, which is one of the main causes of disability and dependency among elders (GBD Neurological Disorders Collaborative Group, 2017). In this line, according to WHO forecasting, in addition to the health issues associated with advancing age, by 2030 half of the worldwide economic impact of disability will be due to brain-related frailty (Mathers & Loncar, 2006). For this reason, there is a special scientific interest in developing new strategies in order to manage the course of the pathology. Nevertheless, the more encouraging progresses are focused on prevention (Kivipelto et al., 2018; Livingston et al., 2017; Satizabal et al., 2016), which supposes a challenge for the new decades of research. Hence, there is evidence supporting that 'healthy aging' can be possible, which is defined by the WHO as 'the process of developing and maintaining the functional ability that enables well-being in older age' (WHO[2]). Functional ability is about having the capabilities that enable all people to be and do what they have reason to value. Despite previous works had commonly focused on the physical well-being without considering cognitive abilities, during the last years the number of studies that have incorporated the cognitive function into the conceptual framework of successful aging has increased (reviewed in Nyberg & Pudas, 2019).

[1] WHO (2018). Ageing and health. https://www.who.int/news-room/fact-sheets/detail/ageing-and-health
[2] WHO (2020). Ageing and life-course. https://www.who.int/ageing/healthy-ageing/en/

In this light, understanding how the health of our brain can be preserved in aging, characterizing the brain mechanisms underlying successful cognition, as well as, the lifestyle's impact on it, is extremely helpful for the research in neurodegenerative pathologies, such as Alzheimer's Disease (AD).

1.1.2 Cognitive aging and heterogeneity

Regarding the cognitive functioning in aging, there is a widespread notion that this period is related to a generalized disruption of cognition (Park & Reuter-Lorenz, 2009; Salthouse et al., 2004; Salthouse, 2009, 2010). Park et al., (2002) results clearly showed that there are gradual age-related declines in the speed of processing, working memory (WMem), and long-term memory, beginning in adulthood. However, the verbal ability, which is an experienced-based 'crystallized' skill rather than a cognitive mechanism is protected from the aging impact (Park & Reuter-Lorenz, 2009; Figure 1A). Further, accumulating evidence shows that functions like semantic memory can remain intact and even improve with age (Grady & Craik, 2000; Salthouse, 2009).

On the other hand, considering individual trajectories, the existence of performance heterogeneity in normal aging (Habib et al., 2007; Lindenberger, 2014; see Figure 1B) and in the lifespan progressions identified in longitudinal studies (Josefsson et al., 2012; Yaffe et al., 2009) have stressed that some elders are able to preserve optimal brain integrity and neural functioning in aging (Gorelick et al., 2017). The previous evidence has given rise to the existence of the 'successful cognitive aging' concept (e.g., Baltes & Baltes, 1990; Williams & Wirths, 1965; Negash et al., 2013; Pudas et al., 2013).

From a methodological standpoint, the most suitable approach to identify and characterize interindividual differences in the trajectory of cognitive aging are longitudinal studies (Ghisletta et al., 2012; Raz et al., 2005). Recent evidence indicates that such investigations, allow classifying elderly people in at least three main groups. As example, Lin et al., (2017a) analyzed episodic memory, executive function and multiple health scores from annual sessions repeated over 5 years in a sample of 354 adults and identified one group of successful agers (41%) with high and stable cognition, that were notably differentiable from the low stable agers group (38%) and decliners (21%; Figure 2A). In other study from the Betula project (Nilsson et al., 1997), data from 1,954 individuals on an

episodic memory composite score based on five tasks were analyzed. The results revealed that 18% of the participants could be classified as maintainers with high and stable performance, 13% as decliners, and the remaining 68% of the sample as those subjects showing age-typical average change (Josefsson et al., 2012; Figure 2B). Also within the Betula project, 2,509 older adults were classified based on the Mini-Mental State Examination (MMSE) performance slopes across sessions (at baseline and at years 3, 5, and 8) and it was found that 30% of them maintained cognitive function over the 8 years (Yaffe et al., 2009; Figure 2C).

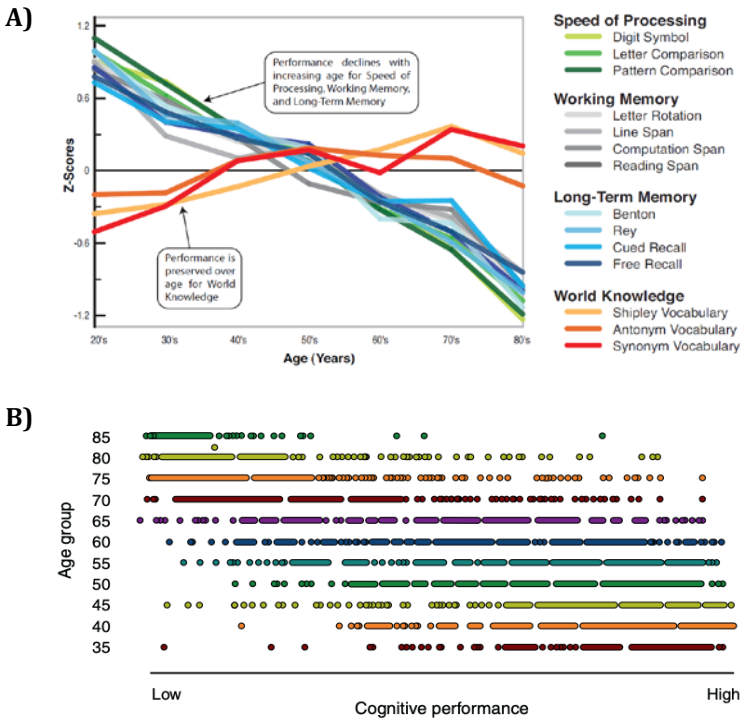

Figure 1. Successful memory aging and heterogeneity. A) Cross-sectional aging data showing behavioral performance on measures of speed of processing, working memory, long-term memory, and world knowledge. Almost all measures of cognitive function show decline with age, except world knowledge, which may even show improvements. Extracted from Park & Reuter-Lorenz (2009); originally from Park et al., (2002). B) The graph shows the results of factor analytic technique that sorted individuals into 'low' and 'high' cognitive performance (each circle denotes the average score of an individual), evidencing that several older individuals performed at high levels. Extracted from Nyberg et al., (2012); originally from Habib et al., (2007).

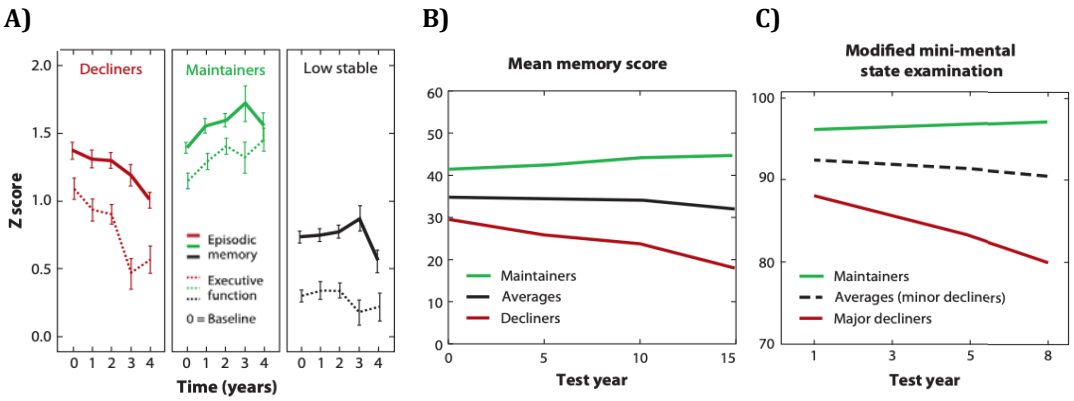

Figure 2. Identification of successful memory and cognitive aging in longitudinal studies by A) Lin et al., (2017a); B) Josefsson et al., (2012); and C) Yaffe et al., (2009). Green indicates the successful group, black the average decliners or minor decliners and red identifies the decliners. Extracted from Nyberg & Pudas (2019).

1.1.3 Lifestyle influences in cognition

Maintaining brain health across lifespan depends on a variety of factors, including genetic and lifestyle components. Moreover, it seems that the complex interaction between them predicts such substantial variability in aging trajectories (Brooks-Wilson, 2013; Staff et al., 2012; Valenzuela & Sachdev, 2006). Regarding the genetic factors, the Apolipoprotein E (APOE) ε4 allele has been frequently linked with cognitive decline trajectories in healthy aging (Josefsson et al., 2012; Yaffe et al., 2009) and it is considered the main genetic determinant for AD (Liu et al., 2013). Further, longitudinal cohort studies have indicated that genetic differences between individuals have a role in interindividual variability in cognition, although this seems to be more important in establishing performance level and less in lifespan trajectories (reviewed in Nyberg & Pudas, 2019). On the other hand, there is ample evidence for the influence of lifestyle factors on cognitive aging, suggesting that the impact of age can be viewed in the context of balance between risk and protective elements (Mattson & Magnus, 2006): the different interactions with the environment have the potentiality to move an individual into a more positive (or negative) aging trajectory (Lindenberger, 2014). Recent investigations (for a review see Mintzer et al., 2019) have highlighted the importance of sustaining healthy habits such as mental well-being, engaging in cognitively stimulating activities, social interactions, good dietary patterns (e.g.,

Mediterranean diet; Guasch-Ferré et al., 2017; see also Loughrey et al., 2017 for a review), good sleep quality (Fung & Veasey, 2017; Leng et al., 2017; Sexton et al., 2014a), weekly moderate/vigorous exercise, and not smoking (Yaffe et al., 2009) to maintain a good brain health in advancing ages. Converging evidence from longitudinal population-based investigations have established that engaging in complex mental activities during lifetime is related to a significant decrease in the prevalence of dementia (Stern, 2012). Of particular relevance to successful cognitive aging, some studies have indicated that higher performance is associated with a lower risk of cognitive decline (Habib et al., 2007; Rosano et al., 2012; Yaffe et al., 2010). In this sense, among lifestyle factors, educational attainment is one of the most commonly identified variable associated with higher cognition (e.g., Habib et al., 2007; Josefsson et al., 2012). However, beyond the years of formal education, as reviewed in Fratiglioni et al., (2004) the three main lifestyle components (cognitive, social and physical) appear to have beneficial effects on dementia risk.

1.1.4 Theoretical bases of successful cognitive aging

Cognitive reserve and brain reserve

The concept of Cognitive Reserve (CR) has been proposed to account for the frequent discrepancy between a person's underlying level of age-related brain changes (or brain pathology) and the observed cognitive profile that is expected to result of that damage (Stern, 2002, 2009, 2017). Therefore, CR is considered an 'active' model that attempts to cope with brain damage by using pre-existing cognitive processes or by means of compensation (Stern, 2017). There are many potential mechanisms implicated in this complex construct, but *resilience* is nowadays the most accepted term referring to multiple reserve-related processes to reaching normal cognition in the face of harmful biological effects of normal aging or pathology (Stern et al., 2018a). See section 1.3.1 for further details about neural bases of CR.

In general, it has been confirmed that CR estimates are consistently related to better cognition (for a meta-analysis see Opdebeeck et al., 2016). However, former investigations have found that higher levels of CR appear to exert less of an impact on rates of cognitive change in healthy aging, that may instead resulting in a higher level of cognitive

performance (Lenehan et al., 2015; Pettigrew et al., 2018; Soldan et al., 2017; Zahodne et al., 2015). CR protective effects on cognitive performance trajectories are not fully understood. Whereas some have reported associations between higher levels of CR and reduced rates of cognitive decline (Ouvrard et al., 2016; Then et al., 2015; Verghese et al., 2003; Wilson et al., 2007), others have shown greater rate of change (Singh-Manoux et al., 2011; Soldan et al., 2017), and others still have reported baseline differences but no differences in cognitive trajectories (Everson-Rose et al., 2003; Lane et al., 2017; Wilson et al., 2005). When the studies account for clinical impairments as AD, it has been identified that higher CR is related to a lower risk of dementia (Karp et al., 2009; Stern et al., 1994) but greater rates of cognitive decline after the clinical onset (Andel et al., 2006; Soldan et al., 2017; Stern et al., 1995), in accordance with Stern (2009) model (Figure 3). However, current discrepancies emerged in the field, proposing that higher CR (measured as years of education, see next paragraph) was related to earlier onset of accelerated cognitive decline and unrelated to rate of acceleration when time is treated as years before death (Wilson et al., 2019).

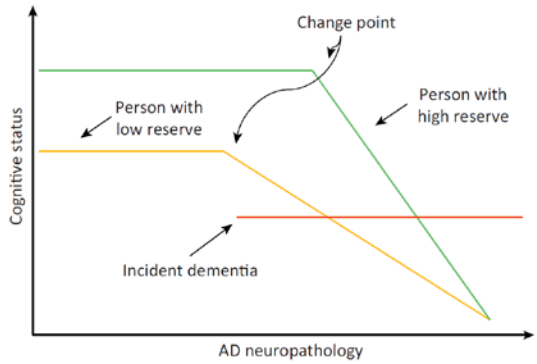

Figure 3. Theoretical illustration of CR. Based on epidemiological and neuroimaging findings, CR may mediate between AD pathology and its clinical manifestation. Extracted from Barulli & Stern (2013); originally from Stern (2009).

It is important to highlight that there are differences across individuals because of the fact that CR is dynamic and influenced by the interaction between the innate characteristics (genome) and lifetime exposures (Stern et al., 2018a). Lifetime experiences account for variances in CR across subjects and explain the differential susceptibility to functional impairment in the presence of the same level of neural insult (Barulli & Stern, 2013). Because there is extensive epidemiological evidence for the independent

association of different lifetime exposures with more successful cognitive aging (e.g., Lövdén et al., 2005), CR is typically measured via a set of potential proxies including: 1) educational attainment; 2) current or estimated premorbid intelligence (IQ); 3) lifetime occupation; 4) lifetime cognitively stimulating activities; 5) participation in leisure activities; 6) physical activity, 7) social engagement, 8) socioeconomic status, and 9) early life experiences (for a review about early-life risk factors for AD, see Borenstein et al., 2006). These variables are not mutually exclusive, often overlap, and may continue to be enhanced throughout the lifespan (for a life model of CR see Richards & Deary, 2005). However, it is essential to note that proxies listed above must be used cautiously and not be treated as direct measures of CR (Stern et al., 2018a). Probably, years of education is the most commonly used proxy variable of CR, which has been associated with greater cognitive function among middle-age and older adults (Berggren et al., 2018; Gonzalez et al., 2013; Lenehan et al., 2015; Mungas et al., 2018; Zahodne et al., 2011) and lower risk of dementia (Kukull et al., 2002; Stern et al., 1994; Tyas et al., 2001). Nonetheless, several studies have failed to find a robust association between the educational attainment and longitudinally assessed cognitive changes in older age (e.g., Seblova et al., 2019; Vemuri et al., 2014; Wilson et al., 2009, 2019; Zahodne et al., 2011). Recent findings have suggested that influences on CR vary over time and the contribution of years of education to CR is limited to its influence in cognition (Wilson et al., 2019).

In more recent years, the residual approach has been proposed as a new method in order to quantify CR. That is, CR is measured as the variance in cognition that is not explained by demographic and brain predictors (Reed et al., 2010). Despite this system supposes a more direct quantification of CR being able to capture their dynamics, there are disadvantages, as the elevated risk of including in this measure many things other than reserve (Stern et al., 2018a) and the differences across studies due to the fact that CR measure depends on the variables in the model (Hohman et al., 2016; Reed et al., 2010; Zahodne et al., 2015).

On the other hand, the concept of Brain Reserve (BR) supposes the 'passive' model of reserve. Thus, BR encompasses the brain anatomical characteristics (e.g., the size of the brain or specific structures) that provide an advantage in front of age-related damage or pathology (Satz et al., 2011). It is considered 'passive' because beyond a certain threshold the functional impairment is inevitable (Barulli & Stern, 2013). Theoretically, BR covers all

the anatomical aspects of the brain that could be measured using neuroimages techniques among others. However, this approach needs to carefully consider that structural markers might reflect a combination of BR and Brain Maintenance (BM) when measured longitudinally (Stern et al., 2018a).

Brain maintenance

Maintenance refers to 'the process of preserving a condition' (OED[1]), focusing on a relative lack of age-related brain changes at neurochemical, structural and functional level, that allows some people to show little age-related cognitive decline or even performance stability by the preservation of neural resources (Nyberg et al., 2012). BM is related to a healthier brain measured by aspects such as less structural decline (i.e., volume and/or thickness loss, microvascular lesions, protein aggregates accumulation). The BM concept is consistent with the notion that during late life, the neural resources can remain preserved through the action of protective mechanisms of cellular repair (Cabeza et al., 2018) and may overlap to a large degree with mechanisms of brain plasticity in adulthood (Lövdén et al., 2010).

CR, BR, and BM are fundamentally related concepts. As distinctive, BM refers to the reduction of the impact of age-related brain changes (or pathology) on brain integrity and represents the process of maintaining, or even enhancing, the brain. BM theories emphasize neuroprotective mechanisms, while CR concept is related to compensatory mechanisms. By definition, the BM concept should be preferably measured in a longitudinal manner by demonstrating relative preservation of brain integrity, whereas BR represents the status of the brain at a point in time (Stern et al., 2018a).

Adding to the complexity of operational definitions that may lead to successful or preserved cognitive function in advanced age, another theoretical construct was put forward to describe those older adults who are resilient to cognitive decline despite their increasing age, the so-called 'superagers'. Originally, 'superager' refers to someone in their 80s or older who exhibits cognitive function at least at the level of cognitively average individuals in their 50s and 60s (Rogalski et al., 2013).

[1] OED online (2012) Oxford University Press. http://oed.com

Recently, another criterion is imposing on the field, referring that a 'superager' is an older adult (aged >60 years) with episodic memory performance at, or above, the mean of normative young samples (i.e., 18-35 years) and with normal-for-age performance [i.e., not 1 standard deviation (SD) below] on the other cognitive domains (Harrison et al., 2012; Sun et al., 2016). Nevertheless, this approach recognizing successful cognitive elders does not account for the longitudinal trajectories of each individual, required in order to identify BM.

- Cognitive reserve (CR): differences in cognitive processes as a function of lifetime intellectual activities and other environmental factors that explain differential susceptibility to functional impairment in the presence of neural damage.
- Brain reserve (BR): differences in brain quantitative characteristics that explain differential susceptibility to functional impairment in the presence of neurological insults.
- Brain maintenance (BM): slight age-related cognitive decline or even performance stability by the preservation of neural resources.

Lately, in the AD framework two emerging concepts have been proposed that hold strong potential to be translocated to the healthy aging field: *resistance* and *resilience* (Arenaza-Urquijo & Vemuri, 2018). Regarding the conceptual distinction between these new nomenclatures, *resilience* refers to the ability to cope in the face of brain insults, showing better-than-expected cognitive performance in relation to the level of damage (e.g., compensation to counteract the changes that occur during aging). On the other hand, *resistance* represents the capacity to avoid the appearance of age-related alterations remaining cognitively normal. These concepts can also be used to explain brain mechanisms underlying successful cognitive aging (Arenaza-Urquijo & Vemuri, 2018): in the face of age-related brain alterations, *resilience* stresses compensation processes typically linked with CR (Stern, 2009), while *resistance* focused on the lack of age-related changes (Nyberg et al., 2012).

1.2 Brain imaging changes in aging

One of the most commonly used techniques in the study of cognitive neuroscience in aging is Magnetic Resonance Imaging (MRI), which is a non-invasive technique that uses a

magnetic field and radiofrequency waves to create detailed pictures of organs and structures inside the body (Mori & Barker, 1999). In the last decades the availability of diverse MRI methods has significantly improved the knowledge in the human cognitive neuroscience literature, in terms of how the brain experiences changes with age at anatomical and functional level (Grady, 2012; Tromp et al., 2015). Brain MRI can be broadly divided into structural, diffusion and functional acquisitions. In one hand, structural MRI allows for high-quality imaging of the brain with good anatomic detail and offers more sensitivity and specificity than other imaging modalities for many types of neurological conditions, while diffusion acquisitions measure the brain fibers' integrity. On the other hand, the functional images have a high timing resolution able to measure brain activity. It is essential to remark the importance of multimodal studies that account for the three of them in order to simultaneously characterize the brain structure and function (see Chapter 3 for more deeply definitions of each neuroimaging technique).

1.2.1 Age-related changes in gray matter

There is a fairly extensive literature on decline in Gray Matter (GM) structures (volume and/or thickness) and at least some portion of the age-related changes in cognitive function is caused by these structural failures (Fjell et al., 2013, 2014a). Another crucial feature is that the ventricular cerebrospinal fluid (CSF) volume increases exponentially with advancing age (Driscoll et al., 2009; Pfefferbaum et al., 2013). Even in groups of healthy elderly persons at very low risk of AD, volumetric reductions and ventricular expansion were detectable over 1 year (Fjell et al., 2013). As regards the topographic pattern of GM degeneration (Figure 4A), numerous studies have reported a prominent reduction of the Prefrontal Cortex (PFC), particularly dorsolateral and dorsomedial regions (Allen et al., 2005; Driscoll et al., 2009; Fjell et al., 2009, 2014a; Grieve et al., 2005; Jernigan et al., 2001; Raz et al., 2004, 2005; Resnick et al., 2000, 2003; Salat et al., 2004; Tisserand et al., 2002), with many also implicating lateral parietal and lateral temporal areas (Allen et al., 2005; Driscoll et al., 2009; Fjell et al., 2009, 2014a; Good et al., 2001; Grieve et al., 2005; Raz et al., 1997, 2004, 2005; Resnick et al., 2000, 2003; Salat et al., 2004). Nevertheless, there are discrepancies in the literature regarding the general pattern of atrophy or preservation in structures such as the hippocampus (Grieve et al., 2005; Raz et al., 2005), cingulate and insula (Grieve et al., 2005; Resnick et al., 2003), and the orbitofrontal cortex (Fjell et al.,

2009; Raz et al., 2005). In a more recent study from Lee et al., (2018), the analysis of a population of 2,944 cognitively normal individuals classified into six groups according to their age (20s/30s, 40s, 50s, 60s, 70s, and over-80s) revealed widespread and severe cortical thinning trajectories based on age. However, it seems that the precuneus, inferior temporal and lateral occipital regions were relatively preserved against age effects until later in life, stressing that there is selectively vulnerable brain regions for age-related cortical thinning (Figure 4B). Therefore, despite cross-sectional studies usually find linear age-relationships reporting that aging affects the cortex diffusely (McGinnis et al., 2011; Salat et al., 2004), sometimes tendencies toward accelerating or reduced estimated decline with increasing age have been reported for specific regions. For instance, Fjell et al., (2014b) showed that there is an accelerated decline of the entorhinal and the lingual cortex with age and decreasing decline in anterior parts of the cingulate.

Given these mixed results regarding the pattern of structural integrity loss in the process of aging, it has been challenging to address the question of whether there are organizing principles underlying the relatively greater selective vulnerability of some regions as opposed to others. Changes in a fronto-striatal network supporting cognitive control and executive functions has been proposed as a hallmark of healthy aging (Buckner, 2004; Head et al., 2005), emphasizing that late-maturing regions are most vulnerable to age changes, often referred to as the 'last in, first out' or 'retrogenesis' hypothesis (Fjell et al., 2009, 2014a; Grieve et al., 2005). It seems that these areas that take longer to mature because they follow more complex developmental trajectories (Shaw et al., 2008), also are more vulnerable to the negative effects of aging. Thus, the 'last in, first out' model has many similarities to the traditional 'frontal theory of aging' (Robbins et al., 1998; West, 1996). In addition, there is a growing realization that the genetically programmed neurodevelopment events cause lifelong impact on the organization of the cerebral cortex observable decades later (Chen et al., 2011, 2012, 2013; Fjell et al., 2015), revealing the need of more research focused on identifying the genetic determinants, as well environmental aspects, influencing age-related cortical progressions.

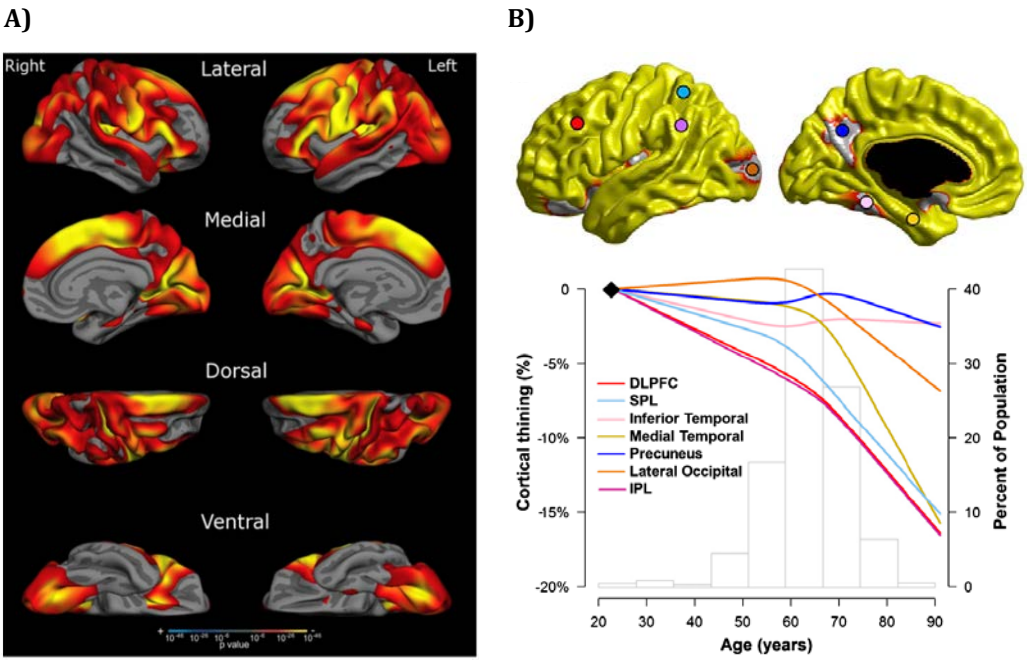

Figure 4. Age-related changes in GM. A) Linear regression of CTh and age (sample aged 18-96 years). Areas of relatively prominent thinning are shown in red-yellow, including the inferior, middle and superior frontal gyri, precentral gyrus, inferior parietal lobule, superior temporal gyrus, and occipital cortex. Extracted from McGinnis et al., (2011). B) Reduction ratios in mean CTh (expressed as a percentage - %) of cognitively normal individuals in seven representative ROIs (DLPFC, precuneus, superior parietal lobule, IPL, medial temporal, inferior temporal, and lateral occipital regions) by age. Extracted from Lee et al., (2018). Abbreviations: GM, Gray Matter; CTh, Cortical Thickness; ROI, Region Of Interest; DLPFC, Dorsolateral Prefrontal Cortex; IPL, Inferior Parietal Lobule.

Interestingly, the classic patterns of age-related atrophy show an overlapping with the Default Mode Network (DMN) regions. The DMN is a cohesive brain network (Raichle et al., 2001), composed by a specific set of brain areas that decrease activity during performance of a wide range of tasks, while are typically active during periods of rest or introspection (Snyder & Raichle, 2012) playing an important role in human brain function (Buckner, 2012). The structural architecture of the DMN include core areas as the precuneus/posterior cingulate cortex, the medial frontal cortex, and other regions, such as the parietal, angular gyri and the hippocampus (Buckner et al., 2008). Regarding the age-related changes affecting the DMN, both structural and functional aspects are compromised in normal aging (Addis et al., 2011; Andrews-Hanna et al., 2007; Lustig et al., 2003; Sala-Llonch et al., 2012; Vidal-Piñeiro et al., 2014). Given the hippocampus (as an area

conforming the DMN), there is evidence that their rates of atrophy increase with increasing age (Du et al., 2006; Pfefferbaum et al., 2013; Raz et al., 2005). Further, rates of yearly cortical volume reduction in the DMN areas are higher than in most other cortical regions, indicating that this network is critically vulnerable to normal aging (Fjell et al., 2014a). This specific vulnerability in the cortical systems constituting the DMN, was earlier hypothesized by Buckner et al., (2009) to explain the predilection of these areas to β-Amyloid (Aβ) deposition. Interestingly, Sun et al., (2016) found that the cerebral cortex of 'superagers' was thicker than that of typical older adults within the areas including, those of the DMN (see Figure 5). One speculation is that DMN areas are critically vulnerable to subtle age-related lesions and pathology accumulating through life because these regions are characterized by a high degree of life-long plasticity (Mesulam, 1999; Rapoport & Nelson, 2011).

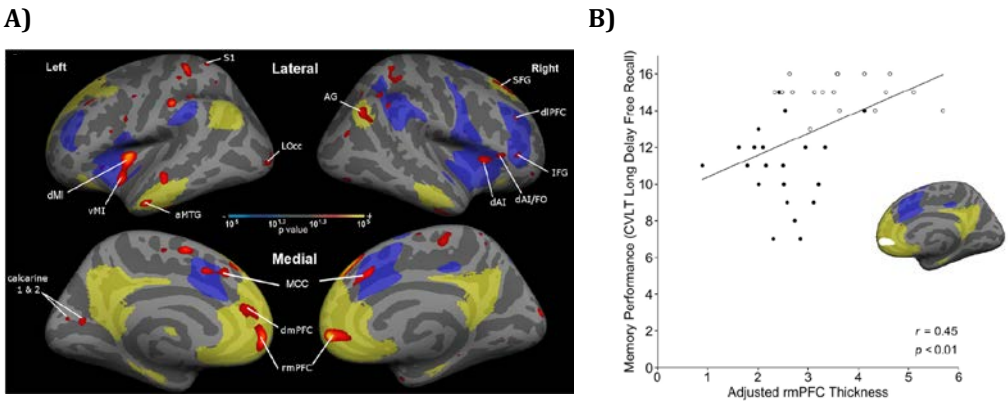

Figure 5. Regions of preserved CTh in 'superagers' within the DMN and Salience network. A) Statistical map showing regions where the cortex of 'superagers' (N=17) is thicker than in TOAs (N=23), (p<0.05; depicted as a red-yellow). The networks of interest are highlighted in yellow (DMN) and blue (Salience). B) Scatterplot illustrates the correlation between memory performance in the entire older adult group ('superagers' indicated by hollow points) and adjusted CTh in the rmPFC. The brain map adjacent to scatter plot shows regions where the cortex of 'superagers' is thicker than in typical older adults (as shown in A). Extracted from Sun et al., (2016). Abbreviations: CTh, Cortical Thickness; DMN, Default Mode Network; TOA, Typical Older Adult; rmPFC, rostral medial Prefrontal Cortex; CVLT, California Verbal Learning Test.

1.2.2 Age-related changes in white matter

Diffusion Tensor Imaging (DTI) acquisition (for further methodological details, see Chapter 3) has become one of the most popular MRI techniques in brain research (reviewed

in Assaf & Pasternak, 2008). DTI provides maps of microscopic structural information of oriented White Matter (WM) tissue in vivo, which is finding utility in studies of aging (Moseley, 2002). The available findings in this field, point out that decrease in Fractional Anisotropy (FA) is a significant trait in aging (Abe et al., 2008; Ardekani et al., 2007; Bennett et al., 2010; Bennet & Madden, 2014; Charlton et al., 2006; Davis et al., 2009; Head et al., 2004; Kennedy & Raz, 2009; Laukka et al., 2013; Malloy et al., 2007; O'Sullivan et al., 2001; Pfefferbaum et al., 2005; Salat et al., 2005; Salat, 2011; Sexton et al., 2014b; Sullivan & Pfefferbaum, 2006; Westlye et al., 2010). The WM trajectory follows an inverted U-shape curve over the lifespan (see Figure 6), detectable across modalities (using both volume and diffusion measures). That is, total WM volume peaked at approximately 50 years of age, supporting the notion of this tissue's volume growth until middle age and then starts to decline (Allen et al., 2005; Jernigan et al., 2001). However, global FA peaks at around 30 years followed by a small yet stable linear decrease until approximately 65 years with a subsequent accelerating decline (Sexton et al., 2014b; Westlye et al., 2010).

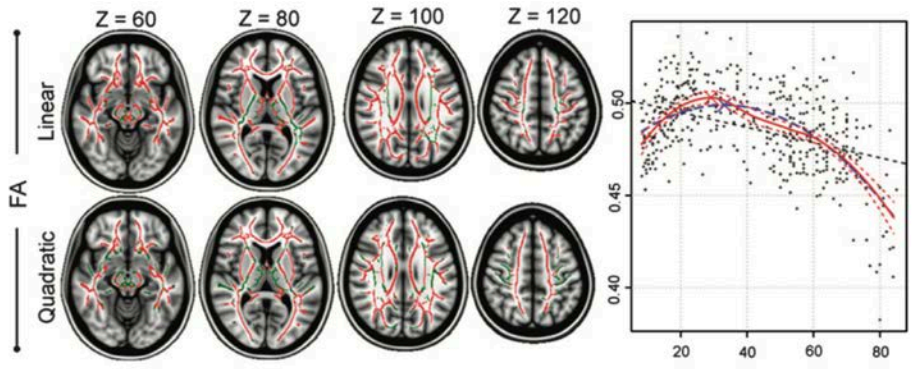

Figure 6. DTI indices across the lifespan (N=430 healthy subjects, aged: 8-85 years). *Head models:* Results from GLMs testing linear and quadratic fits for FA in the TBSS skeleton. Red areas indicate voxels with significant (p<0.05, corrected for multiple comparisons) effect of age (linear or quadratic) on the different measures over the skeleton (in green). *Scatter plot:* Black dotted line denotes the linear, blue line the quadratic and the red solid lines the LOESS fits. The red dotted lines represent the 95% confidence band of the LOESS fit. Blue and red crosses mark the estimated maxima FA. Extracted from Westlye et al., (2009). Abbreviations: DTI, Diffusion Tensor Imaging; GLM, General Linear Model; FA, Fractional Anisotropy; TBSS, Tract-Based Spatial Statistics; LOESS, Locally Weighted Polynomial Regression.

A body of neuroimaging research supports the view that there are different regional effects regarding the WM integrity in aging. Initial studies reported larger age-related FA decreases in frontal regions (Bennett et al., 2010; Bucur et al., 2008; Burzynska et al., 2010; Madden et al., 2004, 2009; Pfefferbaum et al., 2000, 2005; Salat et al., 2005, 2009; Sullivan et al., 2006). However, the subsequent investigations stressed that anterior-posterior gradient may be an oversimplification of age-related effects. According to Davis et al., (2009), WM tracts traversing frontal cortex exhibited a monotonic FA age-related decrease pattern, stable also across the frontal lobe boundary. Further, some posterior WM regions exhibited larger age-related declines in FA compared to frontal regions (Bennett et al., 2010; Salat et al., 2005). In addition, other researchers have suggested the existence of a superior-inferior gradient in which superior WM is more susceptible to age-related declines relative to inferior regions (Sullivan et al., 2010a, 2010b; Zahr et al., 2009). Together, the diversity of patterns identified, are consistent with the 'last in, first out' hypothesis (also known as 'retrogenesis'), that follows the same theory suggested for GM, postulating that late-myelinated WM fibers are most vulnerable to age-related changes (and/or disease-related degeneration), which in turn, mediate cognitive decline (Bartzokis, 2004; Brickman et al., 2012).

Studies relating DTI measures to cognitive function have been driven by diverse hypotheses about the effects of WM disruption on cognitive aging (as reviewed in Carmichael & Lockhart, 2012; Gunning-Dixon et al., 2009; Madden et al., 2011). Alterations in WM integrity have been linked with adjustments in cognition (reviewed in Gunning-Dixon & Raz, 2000; Liu et al., 2017), affecting speed of processing, WMem (Charlton et al., 2008), attention switching (Grieve et al., 2007) and episodic memory (Fletcher et al., 2013; Kennedy & Raz, 2009; Lee et al., 2012; Zhang et al., 2007). Some researchers have proposed theories of 'dis-connectivity' in age-related cognitive and affective impairments (Catani & Ffytche, 2005; Filley, 2005). This theory upholds that in a brain composed by localized but connected specialized areas, disconnection leads to dysfunction (see Figure 7). Hence, because of the fact that WM tracts conform the structural connections of the functional networks recruited in cognition, reductions in their integrity are correlated to declines in cognitive skills (Bartzokis, 2004; Davis et al., 2009; Charlton et al., 2006; Madden et al., 2004; O'Sullivan et al., 2001; Salat, 2011; Sullivan et al., 2006). Of relevance, Salthouse et al., (2011) emphasized that despite there is a relationship between WM integrity and cognitive

performance, a pair-wise correlation among these variables do not address whether the age-related differences in integrity have a causal influence on the age-related differences in cognitive performance, and mediation analyses (see section 3.5.1 in Chapter 3) are required to infer a direct or causal role on cognitive deficits in aging (Baron & Kenny, 1986; Charlton et al., 2010; Hayes, 2009; Fjell & Walhovd, 2010; Fjell et al., 2017).

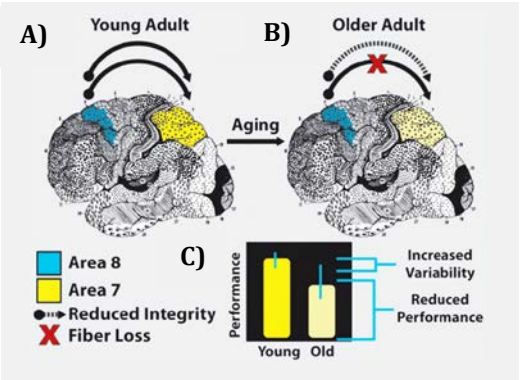

Figure 7. Integrity of structural connective is linked to functional activity. A) Efferent connections from Brodmann area 8 (in blue) to Brodmann area 7 (in yellow). The arrows indicate the anatomical connectivity between the two regions, and the color intensity indicates the functional activity within each region. B) Changes in connectivity with aging would result in a reduction of the direct influence of area 8 on area 7 (in muted yellow). *Dashed arrow:* degeneration of the optimal fiber structure resulting in altered timing and increased variability in transmission of neural signal. *Red X:* complete loss of myelinated nerve fibers would result in a 'dis-connection' between area 8 and area 7. Extracted from Salat (2011).

Classically, the WM characterization was assessed by Fluid Attenuation Inversion Recovery (FLAIR) acquisition (Barkhof & Scheltens, 2002), which is an MRI modality able to identify areas of abnormally high signal in WM, the so-called white matter hyperintensities (WMH; for further details see Chapter 3). Such WMH are presumed to have a vascular origin (Wardlaw et al., 2013) and could be reflecting pathogenic mechanisms, including cerebral ischemia, demyelination, gliosis, and axonal atrophy in adjacent fiber tracts (Bronge, 2002; Fazekas et al., 1998; Kim et al., 2008; Simpson et al., 2007). These lesions tend to accumulate with age (de Leeuw, 2001; DeCarli et al., 2005), have been associated with cognitive impairments (DeCarli et al., 2005; Nordahl et al., 2005, 2006; Prins & Scheltens, 2015; Raji et al., 2012; Tuladhar et al., 2015; Yoshita et al., 2006) and predict an increased risk of developing dementia. Nonetheless, from a methodological point

of view, it seems that DTI provides fairly detection in a more continuous way of WM dysfunction, even when the changes are quite subtle. Meanwhile, FLAIR might only be sensitive when this degeneration reaches a more advanced stage, only in those areas where this dysfunction has reached a high severity threshold (Fornage et al., 2008; Nitkunan et al., 2008; O'Sullivan et al., 2004; van Dijk 2005; Wersching et al., 2010). Therefore, it is well established that the FA DTI-derived metric provides greater specificity than FLAIR in WM-associated cognitive functioning in aging (for a review see Madden et al., 2009).

1.2.3 Age-related changes in functional MRI

The use of task-based functional MRI (fMRI) in aging has revealed a complex pattern of brain activity changes characterized by decreases, increases, or no differences between old and young subjects. There are several theories that have been proposed in order to explain the variations observed during the aging process using fMRI (Grady, 2012). These functional adjustments have been explained by way of distinct cognitive hypotheses (Eyler et al., 2011). The main challenge in this field lies with understanding the brain mechanisms that might underlie better or worse performance in elders. In general, decreased brain activity has typically been interpreted as a reflection of cognitive deficits in older adults (i.e., diminished neural capacity, Stern, 2009) and has been linked to structural changes in GM (Nyberg et al., 2010; Thomsen et al., 2004) and WM (Nordahl et al., 2006; Persson et al., 2006). On the other hand, increased Blood-Oxygen-Level Dependent (BOLD) activity has usually been understood as a compensatory mechanism (Cabeza, 2002; Grady et al., 2006; Mattay et al., 2006; Park & Reuter-Lorenz, 2009; Reuter-Lorenz & Park, 2014). However, these functional brain changes are not invariably linked to better cognitive performance (Rypma et al., 2007; Zarahn et al., 2007). In these cases, increased activations have been conceived as attempted or unsuccessful compensatory mechanisms (Cabeza & Dennis, 2013) or dedifferentiation processes (Carp et al., 2011; Park et al., 2004). The diversity of concepts proposed in the filed in order to interpret such fMRI heterogeneity in aging are described in the following sections.

Brain compensation in aging

Compensation is a complex phenomenon which states that older adults show more activity in a brain region than younger peers whilst they perform a task at the same level as younger adults (Cabeza, 2002; Cabeza & Dennis, 2013), or when this increased activity is positively correlated to performance in older adults, but not younger adults (Davis et al., 2008; Grady et al., 2005; Reuter-Lorenz et al., 2000). Thus, the term compensation is conditioned by the fact that activity increases would be directly associated with improvements in task performance and this is not always accomplished. Some investigations have suggested that these over-recruitments could be reflecting less efficient use of neural resources (Morcom et al., 2007; Rypma et al., 2007; Zarahn et al., 2007), not compensation because not necessary lead to better performance. One explanation for this is the 'partial compensation hypothesis' (de Chastelaine et al., 2011) whereby the enhancing right PFC recruitment is insufficient to meet the task demands, led by the reduction on the effectiveness of the left PFC, which would normally carry out the task (Otten & Rugg, 2001; Wagner et al., 1998). Regardless, Cabeza & Dennis (2013) suggested that the increased brain activity, which appears when task demands are greater than the cognitive resources, can be classified as: 'attempted compensation', when there are no changes in performance; 'successful compensation', when subjects perform better than those which do not activate; and 'unsuccessful compensation', when subjects perform worse. Recently, Cabeza et al., (2018) advised that to attribute increased activation to compensation process must be fulfilled the following premises, relating the more activity to a (1) gap between the available neural resources and the task demands and to a (2) beneficial link with behavior.

In general, compensation refers to the development of new neural mechanisms as response to aging-induced system failures (Lóvden et al., 2010), which could underlie the well-established finding that older adults often show more bilateral patterns of brain activity (Cabeza, 2002; Cabeza et al., 2004; Lindenberger 2014; Madden et al., 2010; Sala-Llonch et al., 2012). Typically, the ability to engage additional brain areas in order to counteract the impaired function, has been identified as more activity in frontal regions (Cabeza, 2002; Cabeza et al., 2004; Davis et al., 2008; Park & Reuter-Lorenz, 2009; Turner & Spreng, 2012). In most cases, this frontal increased responsiveness has been associated with better cognitive performance, while the findings are mixed for other areas as the

medial temporal lobe and occipital cortex, characterized by positive and negative links with cognitive measures (Eyler et al., 2011).

Compensatory cognitive models in aging

Besides the mechanisms presented above, different models have been proposed in order to explain the functional and cognitive implications of brain activity changes in aging. Although not exclusively, the main theories described below are understood under the idea that age-related differences in neural activation can be compensatory to support cognitive performance (Festini et al., 2018; see Figure 8). Of note, it is worth noting that all these models are still matter of debate (i.e., Johansson et al., 2020; Roe et al., 2019).

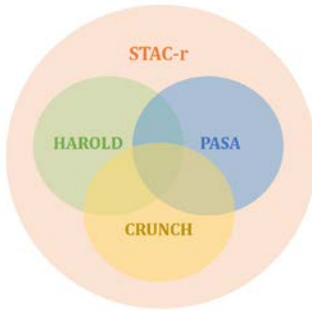

Figure 8. Depiction of the explanatory scope of the HAROLD, PASA, CRUNCH, and STAC-r models. HAROLD, PASA, and CRUNCH explain portions of the functional brain changes discussed within STAC-r. Adapted from Festini et al., (2018). Abbreviations: HAROLD, Hemispheric Asymmetry Reduction in OLDer Adults; PASA, Posterior-Anterior Shift in Ageing; CRUNCH, Compensation-Related Utilization of Neural Circuits Hypothesis; STAC-r, Scaffolding Theory of Aging and Cognition-revised.

In this regard, the Scaffolding Theory of Aging and Cognition (STAC) is a conceptual model of cognitive aging that integrated evidence from neuroimaging to explain how the combined effects of adverse structural and functional changes and putative compensatory processes produce varying levels of cognition (Park & Reuter-Lorenz, 2009). STAC provides a dynamic model where both neurophysiological variables and compensatory neural processes operate jointly to predict cognitive function over time. In a subsequent revision of this theory, Reuter-Lorenz & Park (2014) goes beyond the STAC model and proposed the STAC-revised (STAC-r), which incorporates the combination of lifespan and life-course

approaches (see Figure 9). They suggested that life-course factors serve to enhance or deplete neural resources, thereby influencing the developmental progression of brain structure and function, as well as cognition over time, and also influence compensatory processes and improve the adverse effects of structural and functional decline. In accordance, it has been suggested that higher CR estimates can stimulate plasticity and create neural scaffolds that facilitate a maintained cognition (Lövdén et al., 2010), leading to better options in order to select among the neural strategies for those people with more CR (Barulli & Stern, 2013). Also, individual differences in complex learning tasks engagement or enriched lifestyle factors (i.e., more CR) may determine the quality, quantity and effectiveness of such scaffolding (Park & Reuter-Lorenz, 2009).

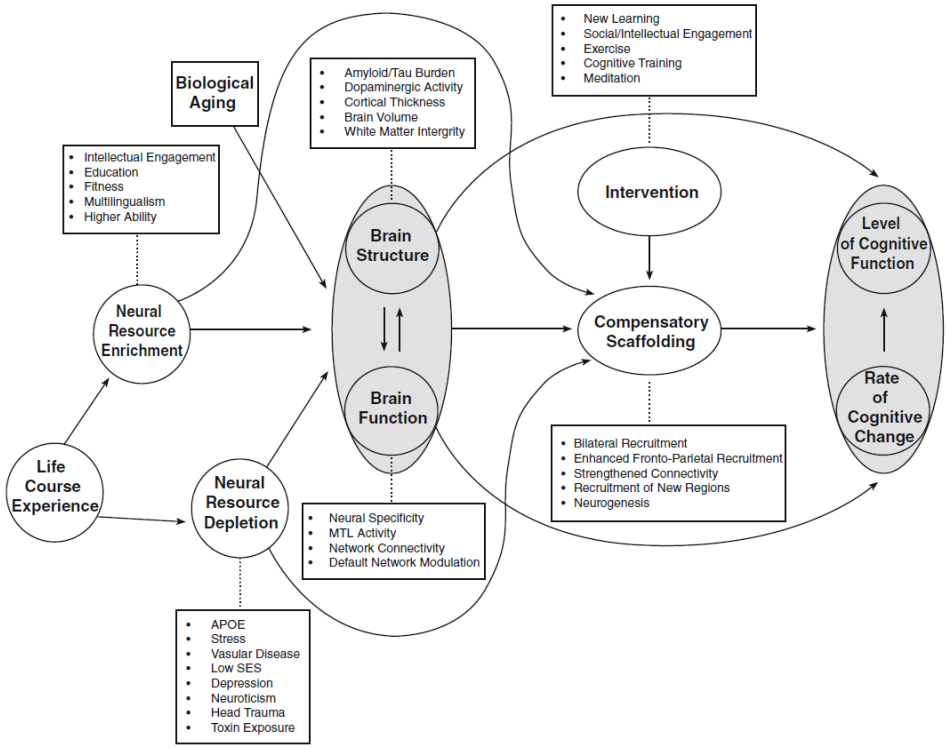

Figure 9. Conceptual model of the Scaffolding Theory of Aging and Cognition-revised (STAC-r). The conceptual model of cognitive aging integrates evidence from structural and functional neuroimaging to explain how the combined effects of adverse and compensatory neural processes produce varying levels of cognitive function, focusing on the construct of compensatory scaffolding. Life-course factors serve to enhance or deplete neural resources, thereby influencing the developmental course of brain structure and function, as well as cognition over time, and also influence compensatory processes and mitigate the adverse effects of structural and functional age-related decline. Extracted from Reuter-Lorenz & Park (2014).

HAROLD model - The Hemispheric Asymmetry Reduction in OLDer Adults (HAROLD) model was introduced by Cabeza (2002). According to this model, older adults show less lateralized PFC activity than younger adults while performing the same cognitive task. In their first study, Cabeza (2002) evidences that young adults and low-performing older adults showed unilateral frontal activity, whereas high-performing older adults showed bilateral frontal activity during an episodic memory retrieval task, suggesting that bilateral activation patterns are associated with successful task performance (Cabeza et al., 2004; see Figure 10A). This bilateral activation has been interpreted as a particular adaptive neurocognitive strategy adopted with age to preserve optimal performance (Reuter-Lorenz & Park, 2014).

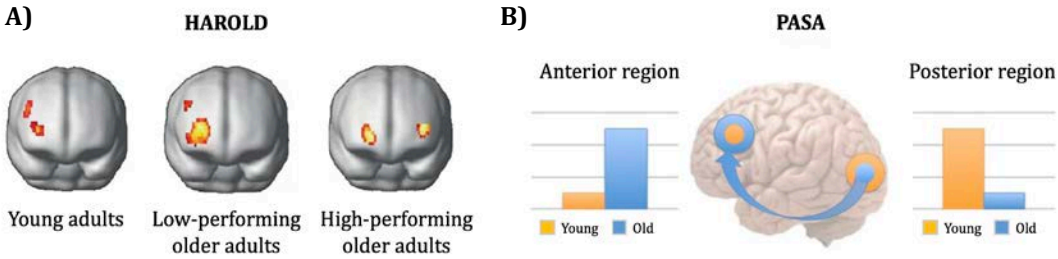

Figure 10. A) According to HAROLD model, functional pattern of young adults and low-performing older adults shows unilateral frontal activity during an episodic memory retrieval task, whereas high-performing older adults representation evidences bilateral frontal activity. Extracted from Cabeza et al., (2018); originally from Cabeza (2002). B) PASA predicts that with age comes a shift, such that older participants show greater activity in the left DLPFC and less activity in the left visual cortex during memory tasks, while younger adults show the reverse pattern. Adapted from Grant et al., (2014). Abbreviations: HAROLD, Hemispheric Asymmetry Reduction in OLDer Adults; PASA, Posterior-to-Anterior Shift in Aging; DLPFC, Dorsolateral Prefrontal Cortex.

PASA model - The Posterior-Anterior Shift in Ageing (PASA) emphasizes two observations in older adults compared with younger adults: less activation of posterior (i.e., occipital) brain regions, along with greater activation of anterior (i.e., frontal) areas (Cabeza & Dennis, 2013; Davis et al., 2008; Dennis & Cabeza, 2008; see Figure 10B). As such, the model derives from the evidence that age-related frontal over-activation is often positively correlated to performance and negatively correlated to occipital activity (Davis et al., 2008). In the same line, Gutchess et al., (2005) demonstrated that compared to younger adults, the elderly people over-activated frontal regions and under-activated medial temporal lobe regions during memory encoding. Those older adults who exhibited the lowest activation

of posterior regions tended to have greater frontal activation, again suggesting the possibility of a compensatory mechanism. In addition, this observation could be interpreted as that older adults experience more subjective task difficulty, which drives different activation patterns across age groups (Davis et al., 2008). A review work from Eyler et al., (2011) regarding the fMRI correlates of successful cognitive aging, points out that individuals who show the PASA pattern have better cognitive performance.

CRUNCH model – The Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH) defends that as task demand increases, older adults reach their capacity for engaging more neural resources until the activity reaches its peak level and this compensatory mechanism is no longer effective (inflection point, *crunch point*; Reuter-Lorenz & Cappell, 2008; Reuter-Lorenz & Lustig, 2005; Reuter-Lorenz & Mikels, 2005; see Figure 11A-C). Because of reduced neural resources in aging (Stern, 2009), the demand-activity function is hypothesized to show greater activity in the same regions as younger adults at lower levels of task difficulty but lower activity at higher levels of the task (Spreng et al., 2010). Data consistent with this idea have been reported during the performance of WMem tasks (for further details see the next section), at low loads (i.e., before the *crunch point*), age-related over-activation was observed in the right dorsolateral PFC (DLPFC), with equivalent behavioral performance between both age groups. At high loads, (i.e., beyond the *crunch point*), older adults showed lower DLPFC activity than younger adults, as well as poorer performance (Mattay et al., 2006). Similar findings were also reported for both the PFC and parietal cortex (PC; Cappell et al., 2010; Schneider-Garces et al., 2010). Thus, should be noted that the tasks measuring WMem (e.g., N-back task) represent an ideal experimental condition to test this model because the cognitive load can be easily modified, and have allowed revealing that those older adults with relatively high levels of performance increase brain activity in task-relevant brain regions as a function of load, whereas low-performing older adults show flat or inverted-U shape activation profiles (Nagel et al., 2009, 2011; Nyberg et al., 2009; Steffener & Stern, 2012; see Figure 11D). In a global view, this hypothesis based on load-dependent activity could explain why studies have reported both under- and over-activations in aging (Grady, 2012).

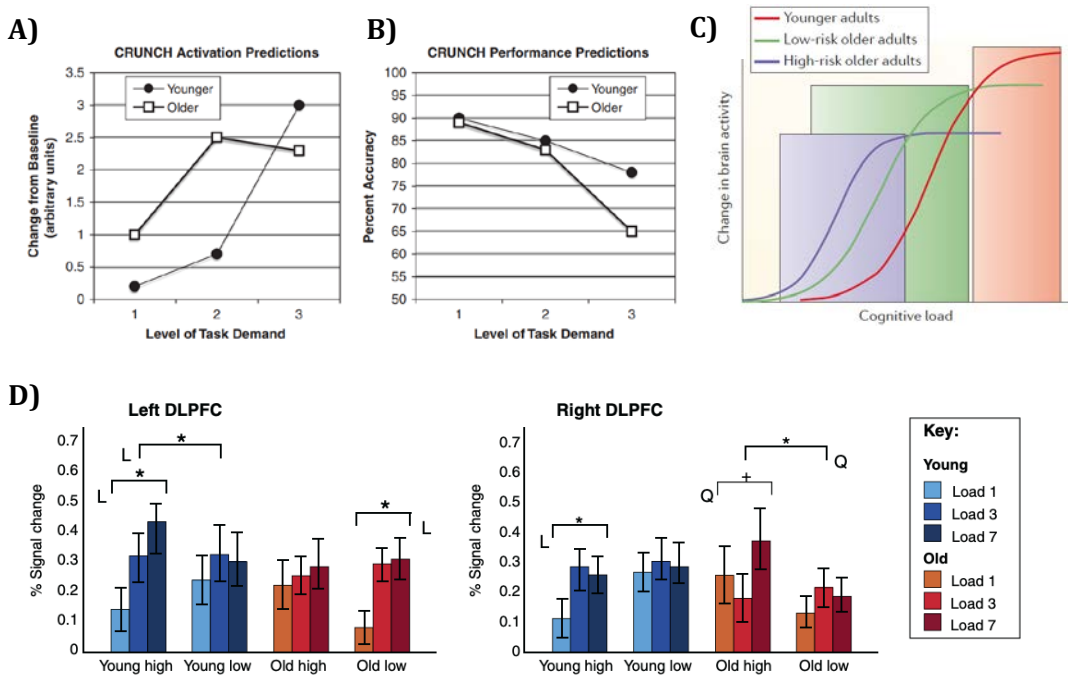

Figure 11. A) Patterns of activation predicted by CRUNCH model. For the younger adults, as the task demands rise, increase the functional recruitment. However, the older adults' recruitment at lower levels of task demand is equivalent to younger adults' level but progress to under-activation at higher levels of task demands. B) As task demands increase, older adults reach a resource ceiling and the performance level drops. Extracted from Reuter-Lorenz & Cappell (2008). C) Graphical representation of the 'S' shaped relationship between brain activity and cognitive load for young adults, low-risk and high-risk of developing AD older adults. At relatively low levels of cognitive load, older adults show higher activity relative to younger adults (green shaded area). However, activity in older adults would reach its peak and level off while younger adults' activity is still increasing. At higher load levels there would be no age difference in activity or younger adults would have higher activity (red shaded area). Also, there is higher activity in high-risk groups relative to low-risk groups at low levels of cognitive load (purple shaded area), with the reverse seen at higher levels of load. Extracted from Grady (2012). D) Age-related reduction in load-dependent modulation of WMem-related BOLD responses. The panel shows that high-performing elders exhibit a load-dependent BOLD response that mimics that of younger adults. Extracted from Nyberg et al., (2012); originally from Nagel et al., (2009). Abbreviations: CRUNCH, Compensation-Related Utilization of Neural Circuits Hypothesis; AD, Alzheimer's Disease; WMem, Working Memory; BOLD, Blood-Oxygen-Level Dependent; DLPFC, Dorsolateral Prefrontal Cortex.

Dedifferentiation

On the other hand, the concept of dedifferentiation is mainly characterized by more diffuse activation patterns (Madden et al., 1999) and less selectivity (i.e., engagement of the typical task-related areas; Grady, 2002; Townsend et al., 2006). It seems that the loss of regional process-specificity in the brain task-related areas is a common process in aging (Rajah & D'Esposito, 2005). An important finding in this filed was the work published by Park et al., (2004), where they identified that while young subjects exhibited category-specific activation in the ventral visual cortex, old participants showed less neural-differentiation during the performance of a visual task, associated with measures of task switching and WMem in old adults (Park et al., 2010). Mainly, there are two approaches to investigate dedifferentiation processes: (1) to compare whether the patters of activity across different tasks are more similar (i.e., less selective; Carp et al., 2010, 2011; Dennis & Cabeza, 2011; Park et al., 2004; Rieckmann et al., 2010) and (2) to use adaptation, which is a reduction in the response when a stimulus is presented repeatedly relative to the first presentation (Goh et al., 2010; Grady et al., 1999; Grill-Spector et al., 2006). Overall, these studies further suggest that the loss of selective brain responses may be a marker of a more general cognitive disruption. In their literature review, Rajah & D'Esposito (2005) suggested that within the PFC, different regions could underly compensatory or dedifferentiation mechanisms under different task conditions.

- **COMPENSATION** refers to the development of new neural mechanisms as response to aging-induced system failures. This functional reorganization should be related to a cognitive advantage.
- STAC: conceptual model that made prediction about how different variables were related to cognitive function, focusing on the core construct of compensatory scaffolding.
  - HAROLD: The activity pattern observed in older adults is less lateralized than the one observed in young subjects under similar conditions.
  - PASA: There are both anterior over-activation and posterior under-activation in older adults relative to younger adults.
  - CRUNCH: Age-related over-activation is a compensatory mechanism and varies with the level of task demand.
    - There is a loss of load-dependent adaptability of neural activity in aging.
- **DEDIFFERENTIATION** is mainly characterized by more diffuse activation patterns and less engagement of the typical task-related areas.

The specific case of WMem in task-based fMRI

WMem is a fundamental cognitive ability that refers to the capacity to maintain, manipulate and store information for a short period of time (Baddeley & Hitch, 1974), which is important for human goal-oriented behavior, reasoning, and decision-making. Hence, WMem is central for daily life activities and is predictive of wide-range higher-level cognitive measures (Unsworth et al., 2014). This domain can be decomposed into processes of attention, memory and inhibition of distractors and is one of the cognitive skills with clear age-related deficits (Bopp & Verhaeghen, 2020; Park & Reuter-Lorenz, 2009), entailing a functionally disabling symptom in advancing ages (Anderson & Craik, 2017; Park et al., 2002; Park & Reuter-Lorenz, 2009). In general, elderly people perform worse and more slowly than young ones (Cappell et al., 2010; Holtzer et al., 2009; Nagel et al., 2011).

The use of fMRI during WMem tasks has shown that a generally well-established set of brain areas is engaged during the performance of this cognitive function, including temporal, parietal and PFC areas (e.g., Owen et al., 2005; Rottschy et al., 2012). According to the fact that WMem is built as the combination of different cognitive processes, far to establish a unique structure as the specific region supporting this function, WMem has been linked to most areas of the brain (see Eriksson et al., 2015 for a review). The PFC has been suggested to be critically involved in normal WMem functioning, concretely related to resilient information maintenance, as evidenced by neuroimaging studies (Courtney et al., 1997; D'Esposito & Postle, 1999) and through applying non-invasive brain stimulation over this region (Brunoni & Vanderhasselt, 2014). Together with the PFC, PC is also strongly involved in WMem functioning, subtending executive processes (Collette et al., 2005; Koenigs et al., 2009) and selective attention control aspects (Awh et al., 2006). In addition, the PC activity seems to be correlated to the WMem capacity (Vogel & Machizawa, 2004). Also, the superior temporal area (Owen at al., 1996) together with the two regions described above (PFC and PC) seem to be core elements in verbal WMem (Buchsbaum & D'Esposito, 2008). Moreover, there are other regions usually linked with the WMem network, such as the cerebellum (Nee et al., 2013; Stoodley & Schmahmann, 2009) and basal ganglia (Wager & Smith, 2003). In neuroimaging, the striatum is commonly related to WMem tasks, due to their role as gating mechanisms between maintained versus up-dated representations in the PFC (O'Reilly, 2006).

Substantial changes in brain activation associated with WMem across the adult lifespan are well documented (e.g., Grady, 2008; Rajah & D'Esposito, 2005; Reuter-Lorenz & Capell, 2008). As aforementioned, it remains unclear how underlying brain activity varies as a function of age, some neuroimaging studies suggest that increased brain activity in older adults may reflect a compensatory mechanism, whereas decreased activity may indicate degeneration of function (e.g., Cappell et al., 2010; Park & Reuter-Lorenz, 2009; Reuter-Lorenz & Cappell, 2008; Sala-Llonch et al., 2015). For example, age-related hyper-activations of the PFC are typically reported (for review see Grady, 2008), which have been interpreted as reflecting compensation by reduced efficiency of executive processes (Motes & Rypma, 2010; Rypma & D'Esposito, 2000; Rypma et al., 2005) as result of anatomical degeneration (Bennett et al., 2012). While some studies have showed bilateral activity in PFC (Heinzel et al., 2016; Scheller et al., 2017; Seo et al., 2014), others report only left (e.g., Berger et al., 2015; Oren et al., 2017) or right PFC activation (e.g., Döhnel et al., 2008; Lim et al., 2008). Yet others report no over-activity in this area (e.g., Luis et al., 2015). In a more recent publication, Archer et al., (2018) identified both significant linear decreases during a visual WMem task and cubic increases in deactivated task-related areas and in regions outside this network (Grady et al., 2006). Interestingly, some studies have used a multimodal approach to explore the interaction of cognition, brain function, and structure, demonstrating a significant relationship between cognitive performance and structural integrity in aged samples (Burzynska et al., 2013; Steffener et al., 2012). Likewise, Burianová et al., (2015) evidenced that a more preserved structure facilitates the recruitment of functional compensatory mechanisms, thereby enabling better WMem accuracy.

1.2.4 Cross-sectional vs. longitudinal approaches

Because of a hallmark of successful cognitive aging is the maintenance of abilities and their underlying neurobiology, then it seems understandable that its assessment requires longitudinal approaches in order to track within-person *changes* (Cabeza et al., 2018). Moreover, there is debate about whether changes observed in cross-sectional studies reflect real ongoing change within individuals (Salthouse, 2009) or arise from methodological artifacts (Nyberg et al., 2012; Schaie, 2009). Mainly, some of the cross-sectional design disadvantages are: (1) birth cohort effects contamination, including inter-

generational IQ increases (the 'Flynn effect', Flynn 1984, 2012; see also Trahan et al., 2014); (2) problems in order to distinguish between age-invariant and age-related differences in cognitive abilities; (3) differential recruitment bias across age groups; and (4) over- or under-estimation of the age effect in that cases where the trajectories are non-linear (Fjell et al., 2014b). It is therefore important to note, that longitudinal studies overcome some limitations of cross-sectional research, notably as the longitudinal approaches are more sensitive in identifying continuous ongoing changes in aging (Fjell et al., 2014a), have consistently demonstrate large individual differences in rates of age-related cognitive decline (Ghisletta et al., 2012; Raz et al., 2005) and can more easily determine the clinical relevance of observed brain changes and their relationship to cognitive decline in aging (Driscoll et al., 2009). In the same line, disentailing the effects of theoretical concepts introduced above, such as CR, BR, and BM, is not straightforward (Stern, 2017). In this vein, the longitudinal designs allow us to study these constructs in action, helping in establishing causal links (Barulli & Stern, 2013) and providing new insights into the field. Nevertheless, longitudinal MRI studies suppose a huge effort in order to follow the same participants over extended time periods, regarding the amount of time and funding required to carry them out, as well as the problem of the elevate number of missing participants along time points. Thus, as proposed by Fjell et al., (2014a), the optimal solution could be combining cross-sectional and longitudinal designs to investigate the effect of age on brain changes across the lifespan in order to identify multidimensional systems-vulnerability models (Walhovd et al., 2014).

1.3 Neural bases of successful cognitive aging

The well-documented differences among elderly individuals have been attributed to the effects of interacting mechanisms, such as compensation, reserve and maintenance, terms proposed in turn to describe the mechanistic theories of cognitive aging (Cabeza et al., 2018). To date, the available literature strengths that achieve successful cognitive aging may be possible in the presence of age-related brain changes (i.e., by compensatory mechanisms; Cabeza et al., 2018; Stern et al., 2018a) or in a relative lack of brain pathology (i.e., maintenance; Nyberg et al., 2012; Nyberg & Pudas, 2019).

1.3.1 Neural bases of CR

Successful cognitive aging may occur in the presence of age-related brain changes, this is based on the idea these injuries can be overtaken by means of efficient scaffolding (Park & Reuter-Lorenz, 2014) and high CR (see Chan et al., 2018). Neuroimaging studies in cognitively normal older individuals have revealed that CR modulates the relationship between cognition and measures of atrophy assessed by MRI (Brickman et al., 2011; Chan et al., 2018; Dufouil et al., 2003; Liu et al., 2012; Solé-Padullés et al., 2009; Vuoksimaa et al., 2013). Further, MRI findings stressed the existence of positive associations between CR proxies and measures of brain integrity (for a review see Bartrés-Faz & Arenaza-Urquijo, 2011; see also Bartrés-Faz et al., 2009; Lövdén et al., 2013; Piras et al., 2011). Particularly within the PFC, these associations have typically been interpreted as reflecting a higher capacity for plastic change (Arenaza-Urquijo et al., 2013, 2017; Foubert-Samier et al., 2012; Lee et al., 2016; Valenzuela et al., 2008). These discoveries may, therefore, suggest a direct neuroprotective effect of reserve (Arenaza-Urquijo et al., 2015). In contrast, CR proxies can show a greater capacity to tolerate brain damage (Stern, 2009, 2012). In clinical populations such as AD patients, CR is typically related to grater indicators of brain pathology that do not correspond with the clinical/cognitive status of patients: reduced brain metabolism, increased Aβ deposition (e.g., Kemppainen et al., 2008; Perneczky et al., 2006) or higher rates of atrophy (Solé-Padullés et al., 2009).

Lastly, the neural mechanisms of CR has been investigated employing task-related network expression as a component of the neural mechanism of CR (for a systematic review evaluating neural correlates of CR with fMRI, see Anthony & Lin, 2018) assuming that the relationship of brain integrity (i.e., BR) to the performance level can be mediated by the functional networks subtending cognition (Stern, 2017; see Figure 12A). In this regard, Stern (2006) postulated that CR can be implemented in the brain in two forms: *neural reserve* and *neural compensation*, which may operate in an overlapping manner all along the continuum between healthy aging and disease (Arenaza-Urquijo et al., 2015). The concept of *neural reserve* suggests that higher estimates of CR are related to more preexisting brain networks activation efficiency and with higher capacity (Archer et al., 2018; Bartrés-Faz et al., 2009; Fernández-Cabello et al., 2016; Habeck et al., 2003; Stern et al., 2003, 2008, 2018b), greater flexibility (Barulli et al., 2013) and higher preservation of functional mechanisms in the presence of structural damage or pathology (Solé-Padullés et al., 2009;

Steffener et al., 2009). *Neural compensation* states the engagement of alternative networks that compensate for age-related (or pathology's) disruption of preexisting networks, by recruiting alternative neural mechanisms in response to task demands (Kennedy et al., 2015; Staff et al., 2004; Steffener et al., 2011; Stern 2002). Focusing on AD, the previous theory suggests that *neural reserve* (i.e., neuroprotective mechanisms) may be at work mainly early (e.g., by slowing Aβ deposition rates, Landau et al., 2012; Wirth et al., 2014), while *neural compensation* may be more common in later stages of disease (e.g., supported by evidence showing that cognitively normal Aβ-positive subjects had glucose hypometabolism in AD-vulnerable regions, Ewers et al., 2013). This notion is also maintained regarding the aging spectrum (Anthony & Lin, 2018). Focusing on the relationships between CR estimates and AD pathology, it should be stated that there is a discussion as regards if these estimates relate to a putative neuroprotective effect (i.e., Arenaza-Urquijo et al., 2015) including an attenuation of AD biomarker deposition in early stages (i.e., Landau et al., 2012), since recent proofs suggested that CR measures did not reduce the association between higher neuropathologic burden and faster cognitive decline (Wilson et al., 2019). Related to the lifestyle exposure underlying neuroprotection, Wirth et al., (2014) suggested that the associations between cognitive and physical activity to preserved brain integrity and cognition in aging are mediated by diminishing pathological pathways thought to be involved in AD development. In addition, there are diverse imaging approaches that have been proposed to yield insights into the neural implementation of CR, (Franzmeier et al., 2017; Kennedy et al., 2015; Stern et al., 2008, 2018b). In this line, Stern et al., (2018b) in their challenge of using task-related network expression to better understand the neural implementation of CR, identified a pattern of activity whose expression correlates with CR (measured as IQ), which was suggested to be a task-invariant measure of CR (see Figure 12B for the topographic representation of these findings).

In summary, CR refers to rather macroscopic construct that is not linked to identifiable neurobiological mechanisms (Stern et al., 2018a). Therefore, the molecular pathways through which CR/BR suppose beneficial in terms of adaptability to cope with, or prevent age-related brain changes, remain unknown. *Capacity*, *efficiency*, *flexibility,* and *plasticity* have been proposed as possible mechanisms by which these constructs mediate the influence on brain aging (Cabeza et al., 2018; Stern, 2017). Furthermore, CR and BM were found to be orthogonal concepts, which implies that CR refers to the more efficient

brain functioning from given structural capacities ('software'), and suggests that BM can be seen as a measure to capture the quality of better brain aging ('hardware'; Habeck et al., 2017).

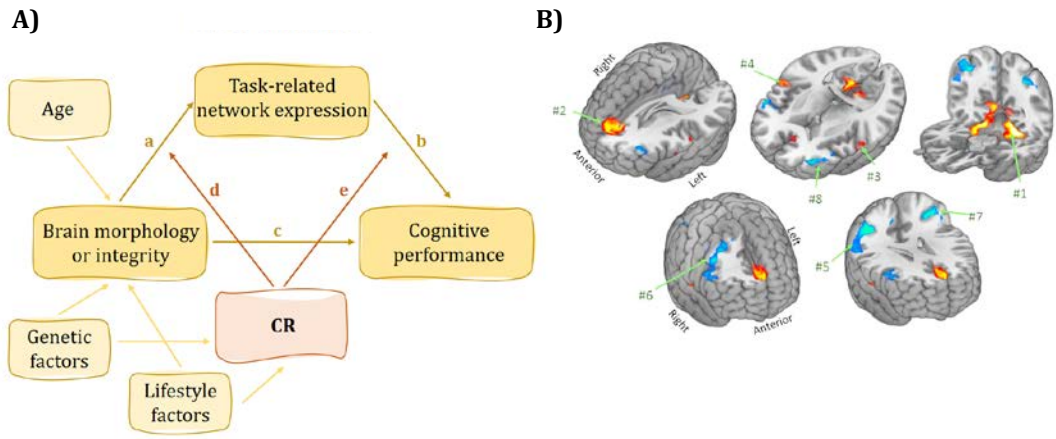

Figure 12. A) Cross-sectional model for CR and BR. The scheme assumes that brain measures impact cognitive performance via path *c*. Age, genetics and lifestyle factors are considered modifiers for brain measures and CR. In turn, CR is assumed to moderate the effect of brain characteristics on cognition, thus producing individual differences in the correlates of a given level of BR and age-related disruption. It is assumed that the effect of brain structure on cognitive performance is mediated in part by task-related brain function (paths *a* and *b*). Path *d* suggest that CR might moderate between brain status and activation such that given a certain level of brain integrity, some people's task related activation might differ as a function of CR. Path *e* recognizes that some aspects of CR might not be captured in specific task-related activations, but still might moderate between brain and cognitive function. Adapted from Stern (2017). B) Composition of the task-invariant CR pattern. Red voxels indicate positive correlation between task-activation and NART IQ, while blue voxels indicate negative correlations. The cluster numbers correspond to the anatomical labels taken from Talairach: #1 (bilateral cerebellum); #2 (bilateral medial frontal gyrus and anterior cingulate, BA 9 and BA 32); #3 (left superior temporal gyrus, BA 22); #4 (right superior temporal gyrus, BA 22); #5 (right inferior parietal lobule, BA 40); #6 (right middle and inferior frontal gyri, BA 6 and 9); #7 (left precuneus and inferior parietal lobule, BA 7 and 40); #8 (left middle and inferior frontal gyri, BA 10 and 46). Extracted from Stern et al., (2018b). Abbreviations: CR, Cognitive Reserve; BR, Brain Reserve; NART IQ, National Adult Reading Test premorbid intelligence; BA, Brodmann Area.

- **Capacity:** maximum degree to which a task-related brain activity increases according to the difficulty rising demands to keep performing a task.
- **Efficiency:** the degree to which given task-related areas must become activated in order to accomplish the same task at a comparable (or even higher) cognitive level.
- **Flexibility:** individual differences in the utilization of the at least two different activity patterns (strategy selection).
- **Plasticity:** structural and functional changes in response to environmental pressures, physiologic changes, and experiences.

1.3.2 Neural bases of BM

The preservation of neural resources is the main property of BM, which entails ongoing repair and replenishment of the brain in response to damage (Nyberg et al., 2012). Because BM is often defined as a relative lack of decline in one or more neural measures, the efficacy of it would depend both on the magnitude of the decline and the efficacy of repair (Cabeza et al., 2018).

The notion of BM is supported by diversity of MRI studies, which evidence that adults who display stable cognitive performance differ from those who decline, at the functional and structural level. As showed in previous sections, the interpretation of increased or decreased functional responses in aging remains unclear, with demonstrations that both associations with better as well as worse task performance coexist (see Grady, 2012 and Eyler et al., 2011). Still a dominating view is that frontal over-activation is a successful response to the challenges posed by declining neural structures and function (Park & Reuter-Lorenz, 2009), the longitudinal studies in the BM filed have suggested that increased brain activity may not necessarily be related to higher performance but rather, it may reflect cognitive decline. Nyberg et al., (2010) provided evidence that high performing elders, even expressing an over-recruitment observed with cross-sectional paradigms, can show age-related BOLD signal reductions when followed over time. In addition, longitudinal fMRI studies showed no significant functional effect in cognitively stable individuals (Pudas et al., 2018; Rieckmann et al., 2017), while cognitive decline was characterized by functional increases and hippocampal reductions (Pudas et al., 2018). More recent findings showed that episodic-memory stability in aging is related to maintain the regulation of PFC

encoding-retrieval according to task demands (Johansson et al., 2020). Thus, preserving a youthful functional signature in frontal areas seems to be a key aspect for a well-maintained cognition (Johansson et al., 2020; Nyberg et al., 2012; Pudas et al., 2013; Vidal-Piñeiro et al., 2019), rather than triggering compensatory mechanisms to counteract the damaged normal functioning (Morcom & Henson, 2018).

The structural findings corroborate this idea, suggesting that older adults with youthful cognitive abilities have youthful brains. In particular, the cingulate integrity has been related to higher cognitive level (Gefen et al., 2015; Sun et al., 2016), and postmortem investigations have further suggested that 'superagers' have a higher density of von Economo neurons (Allman et al., 2011; Butti et al., 2013) and least neurofibrillary degeneration in this area (Gefen et al., 2015). Relatedly, stronger functional connectivity between the anterior cingulate cortex and the right hippocampus was identified in older adults with excellent memory capacity (Lin et al., 2017b). Moreover, Sun et al., (2016) reported that the hippocampal volume of 'superagers' is comparable to that of younger adults, a finding supported by longitudinal studies where it has been evidenced that subjects displaying episodic memory stability show less hippocampal atrophy than individuals with cognitive decline (Gorbach et al., 2017; see also Persson et al., 2012). Notwithstanding, other researchers have been not able to identify such differential ratios of hippocampal atrophy (Dekhtyar et al., 2017; Pudas et al., 2018). As demonstrated by fMRI, it seems that maintaining the functional integrity of the hippocampus is an independent predictor of successful memory aging (Pudas et al., 2013; Düzel et al., 2011), additionally supported by resting state connectivity studies (Salami et al., 2014). Brain characteristics of successful cognition in aging can be found in cortical regions. For instance, minimal cortical Aβ deposition is likely a crucial factor not only in pathological processes but also in healthy elders (Farrell et al., 2017). Also, in 'superagers' have been detected higher GM thickness measures (Cook et al., 2017; Harrison et al., 2012; Gefen et al., 2015; Sun et al., 2016). Regarding WM, it has further been proved that fewer alterations in whole-brain WM microstructure were weakly linked to less negative trajectories in WMem performance, supporting the view that lower structural damage is associated with better performance (Charlton et al., 2010). To sum up, the specific mechanisms underlying 'superaging' and BM, remain unknown and in the case of BM could differ from those who start with high or low levels of a particular neural measure, but are likely to include both

neural components (i.e., neurogenesis) and other determinants such as vascular changes (Cabeza et al., 2018).

In the light of all this, new methodologies to apprehend and enhance the mechanisms related to optimal brain function in advancing ages become vital. As described in the following section, the combination of MRI and Non-Invasive Brain Stimulation (NIBS) might provide novel experimental data on the putative neurophysiological mechanisms underlying inter-individual differences in cognitive status among older adults, and also may help reformulating the theoretical models proposed within the cognitive neuroscience of aging literature. In addition, the NIBS procedures entail the capacity to modify the brain function in older adults, potentially leading to improvements in cognitive function (reviewed in Abellaneda-Pérez et al., 2019a).

1.4 Non-invasive brain stimulation techniques

Mounting evidence shows that the application of different NIBS techniques allow the modulation of human cognitive function (Dubljević et al., 2014). Originally, Merton & Morton (1980) proved that the application of brief electrical currents to the scalp could modify the underlying human cortex. From this on, nowadays, transcranial Electrical Stimulation (tES) techniques allow modifying brain activity (Dayan et al., 2013; Woods et al., 2016) by using electrodes that deliver electrical current to the scalp (Miniussi et al., 2013; see Figure 13). Amongst such methodologies, transcranial Direct Current Stimulation (tDCS) is the most widely used protocol (Polanía et al., 2018). Anodal tDCS increases cortical excitability in targeted brain areas by subthreshold alteration of neural resting membrane potentials (Giordano et al., 2017; Nitsche et al., 2003) in such a way that facilitates the communication between regions, while cathodal tDCS possesses capacity to induce inhibition (Stagg & Nitsche, 2011). In addition, tDCS exerts its effects not only at the stimulation site, but also modulates interconnected networks (e.g., Meinzer et al., 2012). Further, this technique can also induce Long Term Potentiation (LTP)- or Long Term Depression (LTD)-like plasticity mechanisms (Nitsche & Paulus, 2001; Nitsche et al., 2003).

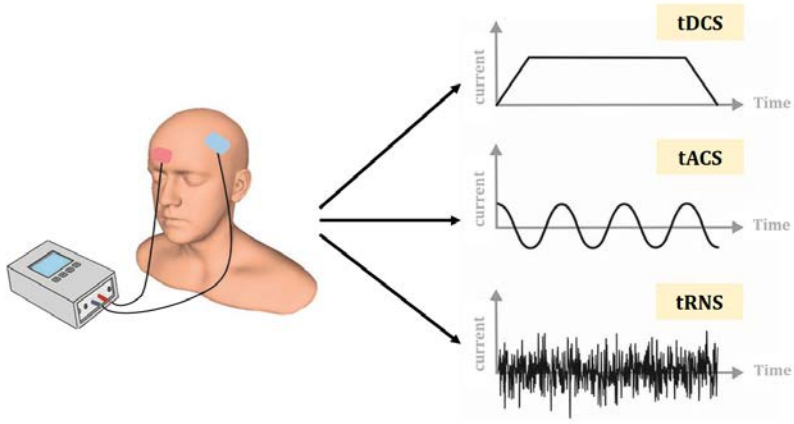

Figure 13. Bipolar electrode configuration with one electrode over left DLPFC and a reference electrode over the contralateral supraorbital region. The applied current in tES can be direct (tDCS), alternating (tACS), or random (tRNS). Beyond current shape, other stimulation parameters such as duration, frequency and phase can be adjusted independently. Adapted from Dayan et al., (2013) and Yavari et al., (2017). Abbreviations: DLPFC, Dorsolateral Prefrontal Cortex; tES, transcranial Electrical Stimulation; tDCS, transcranial Direct Current Stimulation; tACS, transcranial Alternating Current Stimulation; tRNS, transcranial Random Noise Stimulation.

1.4.1 Cognitive enhancement

A large body of evidence indicates that NIBS holds clear potential to transiently modulate cognitive functions in humans, including older adults, both resulting in transient enhancements or impairments, depending on the specific experimental settings (Tatti et al., 2016). There are results supporting that tDCS induces effects in cognition (i.e., Hill et al., 2017; Mancuso et al., 2016; Summers et al., 2016) and holds potential to maintain this cognitive modulation, days or even weeks after stimulation (Antonenko et al., 2018; Flöel et al., 2008, 2012; Sandrini et al., 2014, 2016). Furthermore, it had been demonstrated delayed 'offline' effect of anodal-tDCS impacting upon memory consolidation performance (Manenti et al., 2013; Reis et al., 2009; Sandrini et al., 2014, 2016). It is important to note, that WMem is a cognitive domain quite frequently used as a target for interventional NIBS strategies (Berryhill & Jones, 2012; Nilsson et al., 2017; Lukasik et al., 2018; Abellaneda-Pérez et al., 2020), demonstrating capability to modulate performance (specially reaction time, for a meta-analysis see Brunoni & Vanderhasselt, 2014) and its underlying neural underpinnings by targeting key hubs of this system, such as the DLPFC (Brunoni &

Vanderhasselt, 2014; Curtis & D'Esposito, 2003; Keeser et al., 2011; Peña-Gómez et al., 2012; Zaehle et al., 2011).

1.4.2 Variability in the stimulation induced effects

Nevertheless, the inconsistency of preceding findings in the field, the mixed results and even the negative outcomes reported in some studies (Boggio et al., 2010; Learmonth et al., 2015; Wiethoff et al., 2014), highlight the importance of elucidating neuronal underpinnings of tDCS-induced modulations, as well as, of determining individual predictors of responsiveness and its neurobiological correlates (Horvath et al., 2014; de Lara et al., 2017; Thair et al., 2017). Previous results supported the notion that the general variability in tDCS effects (Horvath et al., 2015) might be closely associated with anatomical differences between subjects (Kim et al., 2014). Even the baseline functional connectivity seems to successfully predict the efficiency of NIBS in terms of network connectivity in healthy individuals (Abellaneda-Pérez et al., 2019b; Antonenko et al., 2019; Polanía et al., 2012). Therefore, due to the increased inter-individual differences among elderly people (Tatti et al., 2016) this aspect is especially relevant in older cohorts (Antonenko et al., 2019).

In addition, mixed cognitive effects induced by tDCS coexist in the literature. Some studies reported improvements only for those subjects with high performance (Learmonth et al., 2015), others showed that high performance is related to a slight deterioration of accuracy induced by tDCS (Lukasik et al., 2018), while other studies have reported that low-performing participants would benefit more from tDCS while high-performers would not (Hsu et al., 2014, 2016; Tseng et al., 2012; Wu et al., 2016). As an example, Berryhill & Jones (2012) reported that only a subgroup of higher educated participants showed improvements of both visual and verbal WMem performance for both left and right DLPFC stimulation. In this case, since the authors did not employ neuroimaging techniques the interpretation of the present results is limited.

1.4.3 Combining NIBS with MRI

The combination of different innovative methodologies, such as NIBS with fMRI entails the capability to define the neural pathways whereby distinct NIBS-based

interventions improve cognition among elders, and at the same time provide valuable information about brain functioning (reviewed in Abellaneda-Pérez et al., 2019a).

Modulation of brain activity in aging

Promisingly, some authors had described that tDCS could be useful even to reverse age-related functional reorganizations in aging (Meinzer et al., 2013, 2014; Perceval et al., 2016) and holds potential to be considered a powerful cognitive intervention in elderly people (Antonenko et al., 2017; Cespón et al., 2018). During the last years the number of studies assessing the impact of tDCS on brain function during task-based fMRI has increased. In this light, it seems that age-related changes do not only affect the magnitude of tDCS-induced modulation but also the pattern of underlying functional reorganization (Antonenko et al., 2018; Fiori et al., 2017; Martin et al., 2017). From our knowledge, most of studies combining tDCS and task-based fMRI in elderly people have focused on language as cognitive domain of interest (Holland et al., 2011; Meinzer et al., 2013, 2014). Despite, nowadays, little is known about the explicit effects of tDCS on brain function in aging, the previous investigations conducted in this filed, suggested that anodal tDCS acts 'normalizing' task-related patterns through activity reductions (Martin et al., 2017; Meinzer et al., 2013, 2014; Holland et al., 2011).

1.5 Rationale

Thus far, previous research in the cognitive aging neuroscience field has stablished that achieving a successful cognitive level in aging may be possible through two not-mutually exclusive general mechanisms: 1) by means of compensation to counteract the age-related brain damage (i.e., CR; Stern et al., 2018a) or 2) associated with a relative lack of brain pathology due to neural processes of reparation and plasticity (i.e., BM; Nyberg et al., 2012). In this sense, research on CR has been traditionally focused on AD patients, emphasizing its compensatory view. However, their neuroprotective consequences may also be manifested in the healthy aging population or in initial stages of disease (Anthony & Lin, 2018; Arenaza-Urquijo et al., 2015). Years of education as the most used proxy variable of CR, has been robustly associated with greater cognitive function among middle-age and

older adults (Berggren et al., 2018; Gonzalez et al., 2013; Lenehan et al., 2015; Mungas et al., 2018; Zahodne et al., 2011). Nonetheless, the empirical evidence is not revealing a consistent association between CR estimates and longitudinal cognitive changes in healthy aging performance (e.g., Van Gerven et al., 2007; Vemuri et al., 2014; Wilson et al., 2009, 2019; Zahodne et al., 2011). Moreover, there is evidence that age may have a differential influence on brain structure regarding tissue type and topographic specificity (Fjell et al., 2014a). Thus, this observation may have an impact when considering the putative coexistence of neuroprotection (i.e., more preserved brain integrity) and compensatory mechanisms (i.e., capacity to counteract greater rates of atrophy) linked to CR (Stern et al., 2018a). A relevant aspect regarding the mechanisms predicated by the CR theory is the poor understanding of the associated biological pathways through which they may operate (Stern et al., 2018a), stressing the necessity of future investigations designed to bridging the gap between CR properties and their molecular mechanisms. In addition, within the fMRI framework, the age-related changes occurring at different cognitive demands and their neural substrates are not fully understood, in part due to the scarce number of longitudinal studies, which are those that allow investigating BM (Nyberg et al., 2012). In this regard, focusing on WMem domain, few works have explored the BM concept. However, the available evidences highlight that longitudinal activation stability seems to underlie a maintained performance, whereas over-activations are related to cognitive decline (Rieckmann et al., 2017). Thus, these latter results suggest that, as investigated longitudinally, increases in brain activity, instead of reflecting compensatory mechanisms as described in cross-sectional approaches (Cabeza, 2002; Cabeza et al., 2004; Mattay et al., 2006; Reuter-Lorenz & Park, 2014), might underlie cognitive decline (Nyberg et al., 2012). Furthermore, some studies have indicated that higher performance is associated with a lower risk of cognitive decline (Habib et al., 2007; Rosano et al., 2012; Yaffe et al., 2010) and it has been suggested that the maintenance mechanisms would differ depending on the baseline cognitive level (Cabeza et al., 2018), indicating a possible modulatory role of CR. Hence, the combined use of cross-sectional and longitudinal approaches seems crucial in order to apprehend the brain imaging features sustaining cognitive stability or decline in aging (Nyber et al., 2010). In addition, the application of other non-imaging techniques such as NIBS, suppose an innovative methodology, which allows interrogating and characterizing in a controlled manner the brain function and potentially inducing improvements in cognition (Abellaneda-Pérez et al., 2019a). Specifically, combine NIBS and

fMRI results in a powerful approach able to capture the brain activity modulations induced, which is useful in order to interpret the cognitive effects or the lack of thereof (Meinzer et al., 2013, 2014; Perceval et al., 2016). At the same time, this approach let to explore functional mechanisms sustaining different levels of cognition in aging and CR properties, such as plasticity (Abellaneda-Pérez et al., 2019b).

Overall, the divergences regarding structural and functional findings that seem to underlie healthy cognitive aging and the lack of clear mechanisms by which external factors such as CR may modulate these relationships, have motivated the different objectives of the present Doctoral Thesis that are summarized in the next chapter.

CHAPTER 2

Objectives and Hypotheses

Objectives

Main objective

The present Doctoral Thesis is contextualized in the study of a cognitively healthy aging population using distinct types of analyses. These include cross-sectional cognitive, MRI and genetic-molecular investigations, as well as longitudinal neuroimaging-based approaches. In addition, such observational reports were complemented with an interventional investigation applying NIBS combined with MRI. Overall, the overarching objective was to characterize the structural and functional brain substrates underlying healthy cognitive aging and to reveal how CR estimates may modulate the relationship between the measures studied.

Specific objectives

1. To explore how years of education, as the main proxy variable for CR, relates to the cognitive profiles amongst healthy elders.

2. To identify associations between CR estimations, GM and WM integrity MRI-based measures and to study if these are related to the impact of age on brain structure.

3. To describe the transcriptional genetic architecture of CR-related cortical regions using brain-wide gene expression data in combination with gene set enrichment analysis.

4. To investigate the brain properties underlying WMem stability and decline, using longitudinal functional and structural MRI.

5. To study whether combining NIBS and fMRI techniques could be a useful approach to better understand the functional mechanisms sustaining different cognitive trajectories in aging.

Hypotheses

1. Higher educational attainment, as a main proxy of CR, will be related to better cognitive performance but will not have a clear modulatory effect on the longitudinal progression.

2. Amongst cognitively preserved elders, CR estimates will be positively associated with measures of brain integrity in those areas relatively preserved in aging and at the same time, CR will be related to greater rates of atrophy in brain measures suffering more abrupt age-related disruption.

3. We further expected that the cortical areas showing positive associations between CR and integrity will exhibit a distinct gene expression profile characterized by the upregulation of gene sets related to neuroprotective molecular pathways.

4. With age, distinct brain characteristics will underlie stability and decline in WMem performance, with those subjects showing decline being more likely to engage unsuccessfully compensatory fMRI patterns and greater age-related cortical atrophy.

5. Among elderly people showing age-related stability in cognition, only those high performers will exhibit longitudinal brain features fitting with the BM hypothesis.

6. During a WMem task, tDCS will result in dissimilar modulations of brain activity in those subjects showing cognitive stability vs. decline, providing insight into the fMRI correlates of these two groups with different trajectories.

CHAPTER 3

Materials and Methods

The present Doctoral Thesis consists of a total of four studies. Below, a brief description of the principal methodological procedures for each study is provided. A summary of the settings and analyses for each one is given in Table 1 and Figure 14. Further details are specified along this chapter and in the corresponding manuscripts attached in Chapter 4.

3.1 Study sample

Study samples were mainly selected from a larger cohort of healthy elders recruited into a randomized controlled trial aimed at assessing the effects of walnuts on age-related diseases (Walnuts and Healthy Aging Study, WAHA; Rajaram et al., 2017; Sala-Vila et al., 2020). A total of 119 subjects from this cohort underwent brain MRI, of which only those with the MRI acquisitions and the main neuropsychological tests completed were included in the analyses involved in the present Thesis (see next paragraphs for more specific information about each subsample). The exclusion criteria for the WAHA study were illiteracy or inability to understand the protocol or undergo neuropsychological tests, morbid obesity [Body Mass Index (BMI) > 40 kg/m^2], uncontrolled diabetes [Hemoglobin A1c (HbA1c) > 8%], uncontrolled hypertension (on-treatment blood pressure ≥ 150/100 mmHg), prior cerebrovascular accident or major head trauma, any relevant psychiatric illness (including major depression), abnormal cognitive profile according to the normative scores (see next section), dementia, other neurodegenerative diseases (i.e., Parkinson's disease), and any chronic illness expected to shorten survival (i.e., heart failure, chronic liver disease, kidney failure, blood disease, cancer). All participants had normal cognitive profiles with MMSE scores > 25 (Mitchell, 2009) and performances no more than 1.5 SD below normative scores on any of the neuropsychological tests administered, i.e., they did not fulfill criteria for mild cognitive impairment (Petersen & Morris, 2005).

3.1.1 Study sample 1

A total of 100 subjects from the previously described sample were included in this study. The MRI acquisitions with excessive motion or artifacts were not considered, neither those subjects with missing information for any neuropsychological test of our interest.

3.1.2 Study sample 2

Study 2 included MRI data from study 1 that were analyzed together with an independent sample composed of 28 subjects of similar characteristics in order to increase the number of participants to 122. For the former subgroup, the inclusion criteria were having a normal cognitive profile with MMSE scores of >24 and normal cognitive function.

3.1.3 Study sample 3

This sample included longitudinal data (MRI images and neuropsychological tests at 2-years follow-up) of 47 control subjects (those who did not randomized to receive an enriched diet with walnuts in the WAHA project). Only the individuals exhibiting performance (i.e., hits at 3-back) above chance (50%) during the N-back task were included.

3.1.4 Study sample 4

A subsample from the main group was recruited for an independent study with tDCS by phone call, 4 years after the beginning of the WAHA study collection. As in study 3, only those subjects exhibiting a performance above the 50% in the highest load of the N-back task were included. In addition, in this case, the inclusion criteria considered that none of the participants reported NIBS contraindications (Antal et al., 2017; Rossi et al., 2009) in order to be able to safely apply tDCS. Finally, according to this criteria, 24 subjects were included.

Ethical Statements

The WAHA study was approved by the *Hospital Clínic de Barcelona* ethical committee. Study 4, which included tDCS intervention, was reviewed and approved by the University of Barcelona's Bioethics Commission. For all the studies, written informed consent was obtained from each participant prior to enrollment and the procedures have been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Study	Sample size	Age (years)	Design	MRI data	Analysis technique	Cognitive domain
1	N=100	68.35 ± 3.09	Cross-sectional	- T1w-structural - dMRI - FLAIR	- CTh - DTI (FA) - WMHV	- Speed of processing - Declarative memory
2	N=122	68.20 ± 3.51	Cross-sectional	- T1w-structural	- CTh (cortical gene expression profile)	- Declarative memory - Frontal lobe functions
3	N=47	68.40 ± 2.86 at baseline	Longitudinal (2-year FU)	- N-back fMRI - T1w-structural	- Task-related activity - CTh	- Working memory (N-back)
4	N=24	71.79 ± 2.67 at sham condition	Retrospective longitudinal (4-year FU) + Subject-blind and sham-controlled cross-over	- N-back fMRI during tDCS	- Task-related activity (tDCS effect)	- Working memory (N-back)

Table 1. Summary of main characteristics from each study. Abbreviation: FU, Follow-up; MRI, Magnetic Resonance Imaging; dMRI, diffusion Magnetic Resonance Imaging; fMRI, functional Magnetic Resonance Imaging; CTh, Cortical Thickness; DTI, Diffusion Tensor Imaging; FLAIR, Fluid-Attenuated Inversion Recovery; FA, Fractional Anisotropy; WMHV, White Matter Hyperintensity Volume; tDCS, transcranial Direct Current Stimulation.

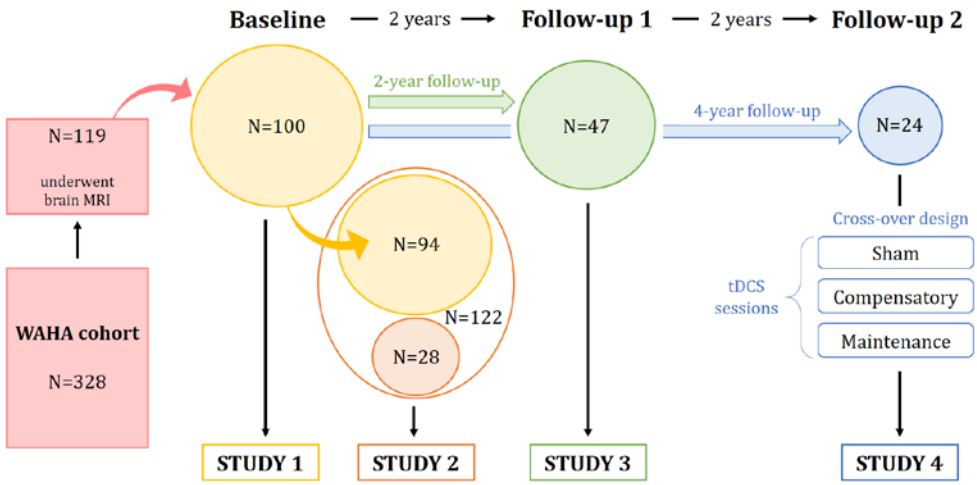

Figure 14. Samples diagram. All the subjects were originally from the WAHA cohort. Study 1 and 2 were cross-sectional and included N=100 and N=122, respectively. Study 3 included 2-year follow-up data from N=47 subjects. Study 4 combined two designs: 4-year follow-up data and a subject-blind sham-controlled cross-over approach using concomitant tDCS in the MR scan for N=24 participants. Abbreviations: MRI, Magnetic Resonance Imaging; tDCS, transcranial Direct Current Stimulation.

3.2 Neuropsychological assessment

In all study samples, a comprehensive battery of neuropsychological tests was administered (as described previously in Sala-Vila et al., 2020; Rajaram et al., 2017 and Vidal-Piñeiro et al., 2014). Should be noted that for all these tests, age and education-adjusted Spanish norms were applied (Peña-Casanova et al., 2009). The cognitive domains and their respective neuropsychological examinations are detailed here below. In all studies, principal components analysis (PCA) based on each specific dataset was used to create a composite scale representing the cognitive domains of interest.

3.2.1 Neuropsychological assessment study 1

We measured speed of processing and declarative memory. The following cognitive tests were included to calculate the speed of processing factor: Trail Making Test A (TMTA; Partington & Leiter, 1949), Symbol Digit Modalities Test (SDMT; Smith, 1973), and a computerized version of the Continuous Performance Test (CPT; Rosvold et al., 1956). The tests contributing to the declarative memory factor comprised Rey-Osterrieth Complex Figure recall (immediate: ROCF-3min and delayed: ROCF-30min; Rey, 1941), Rey Auditory Verbal Learning Test (Rey, 1964) total learning and delayed recall (RAVLT-total and RAVLT-delayed).

3.2.2 Neuropsychological assessment study 2

Declarative memory and 'frontal lobe function' were evaluated in study 2. The following cognitive tests were used to estimate the memory factor: RAVLT-total and RAVLT-delayed (Rey, 1964; N=94 cases) and Buschke total and delayed test (Buschke, 1984; N=28 cases). On the other hand, tests contributing to the 'frontal lobe function' domain included TMTB minus TMTA (TMTB-A; Partington & Leiter, 1949), SDMT (Smith, 1973), and phonemic (sum of letters F, A, S, 1min each, N=94; and letters P, M, R, N=28; Benton & Hamsher, 1976) and semantic (animals, 1min) fluency tests (Ramier & Hécaen, 1970). In this case, previously to the PCA, the total learning and delayed scores from both memory tests were transformed to a comparable metric by the percentage of maximum possible ('POMP') method (Moeller, 2015).

3.2.3 Neuropsychological assessment study 3 and 4

In a distinct way, the cognitive measure investigated in study 3 and 4 were obtained straight inside the scanner while the subjects performed the N-back task. The different loads of the task (Kirchner, 1958) were used to calculate a global score of this complex domain. For more details regarding the N-back scores see 'The fMRI paradigm: N-back task' section below.

3.3. Neuroimaging techniques

Briefly, the fundamental principle of MRI is based on the fact that the atomic nuclei of hydrogen (spin), which is abundant in the water molecules of the brain, acts like a tiny bar magnet. In the presence of a magnetic field, these hydrogen atoms showed a precession movement around the magnetic field axis at the Larmor frequency (unique for each type of nucleus). The scan has coils integrated that send radiofrequency excitatory pulses at the same Larmor frequency transferring energy to spins (high-energy state). After the excitation, while spins gradually return to their equilibrium state (low-energy), radio signal at the Larmor frequency is detected by the receiver coil. Importantly, the different properties of the tissue allow a longer or lesser recovery time of the spins and this aspect is what allows creating the final MRI images. In addition, this spin relaxation can occur in two different ways according to the orientation of the radiofrequency pulse applied, resulting in two MRI acquisition types: T1 (longitudinal recovery) and T2 (transverse component loss, dephasing) images (Currie et al., 2012; Martínez, 2018).

In the present Doctoral Thesis, structural, diffusion and functional MRI techniques were used. A detailed description of brain imaging types derived from different MRI modalities are presented in upcoming sections.

3.3.1 Structural MRI

In neuroimaging, the structural MRI acquisitions are typically used to investigate the brain anatomy and they are characterized by having a high contrast between GM and WM, and by providing a powerful spatial resolution. Here, we used two different structural MRI,

T1-weighted (T1w)-structural acquisitions and FLAIR acquisitions (for a review of structural MRI see Symms et al., 2004).

T1w-structural

The T1w-structural images are one of the basic pulse sequences in MRI and take advantage of the different properties of each tissue to return back to equilibrium after the radiofrequency pulse. Fat quickly realigns its longitudinal magnetization, and it, therefore, appears bright on this acquisition. Conversely, water has much slower longitudinal magnetization realignment after a pulse and therefore, it has low signal and appears dark. T1w-structural MRI is characterized by a high spatial resolution getting good CNR (contrast-to-noise ratio) and tissue discrimination (Symms et al., 2004). This is why these images are commonly used to outline the cerebral cortex, to measure subcortical volumes, and further may be the anatomical reference for the fMRI acquisitions (Jenkinson & Chappell, 2018).

FLAIR

The FLAIR sequence is an MRI acquisition with a long inversion time that allows removing CSF signal. Therefore, brain tissue appears similar to T2 weighted images with GM brighter than WM, but CSF is dark instead of bright (Saranathan et al., 2017). The FLAIR acquisitions highlight the WMH in the brain determined by the density of lipid protons within myelin (Barkhof & Scheltens, 2002). Thus, there is a brighter intensity signal for the WM lesions than for the surrounding matter (De Coene et al., 1992; DeCarli et al., 2005; Nordahl et al., 2006, 2005; Yoshita et al., 2006). WMH reflect various pathogenic mechanisms, including cerebral ischemia and degradation of myelin in adjacent fiber tracts (Kim et al., 2008), although lacking additional data, we can only indirectly infer which of these mechanisms contribute to specific WMHs based on their size and location (DeCarli et al., 2005).

3.3.2 Diffusion MRI

Diffusion MRI (dMRI; Assaf & Pasternak, 2008) is a non-invasive MRI approach that allows measuring and visualizing the anatomical connectivity of the brain, as well as to study the microstructural integrity of WM tissue (Lamar et al., 2014). Many reviews have described the technology underlying DTI data acquisition in detail (e.g., Bammer et al., 2009; Beaulieu, 2002; Chanraud et al., 2010; Mukherjee et al., 2008a, 2008b). The principle of this imaging technique consists on measuring the diffusion of water molecules in the brain. The random diffusion (i.e., Brownian motion) described by the water molecules can be altered in presence of barriers. The axonal fiber bundles have an important quantity of water in their composition, both inside and in the extracellular space. Consequently, in this environment, the molecules of water in WM tracts have a preferential directionality of diffusion in which the axons are oriented (i.e., anisotropic diffusion), whereas in brain tissues that lack this coherent organization (i.e., GM and CSF), water molecules can move similarly in all directions (Beaulieu, 2002; Le Bihan, 1991, 2003). Therefore, dMRI can be used to study the directionality of the WM axons. In this technique, it is necessary to realize an acquisition without diffusion directionality (called B0) and a set of diffusion-weighted images. Under the magnetic shift, we can obtain information about diffusion orientation of water molecules at every point (Alexander et al., 2008).

The dMRI images estimate fiber directionality by means of the eigenvectors and eigenvalues of the simplified diffusion matrix. Fractional Anisotropy (FA) measures the prevalence of the main direction over the perpendicular directions (see Figure 15). Low FA values represents isotropic diffusion (nearly equal motion in all directions), whereas high FA represents preferential diffusion in one direction but not in others. FA is highly sensitive to microstructural changes, but there are other diffusion tensor measures - Mean Diffusivity (MD), Radial Diffusivity (RD) and Axial Diffusivity (AxD) - that are used in order to maximize the specificity and to better characterize the WM tissue (see Figure 15). The MD is equivalent to the average of the eigenvalues and may help to better understand how the diffusion tensor is changing, the RD appears to be modulated by myelin, whereas the AxD is more specific to axonal degeneration (Bennett & Madden, 2014; Song et al., 2002).

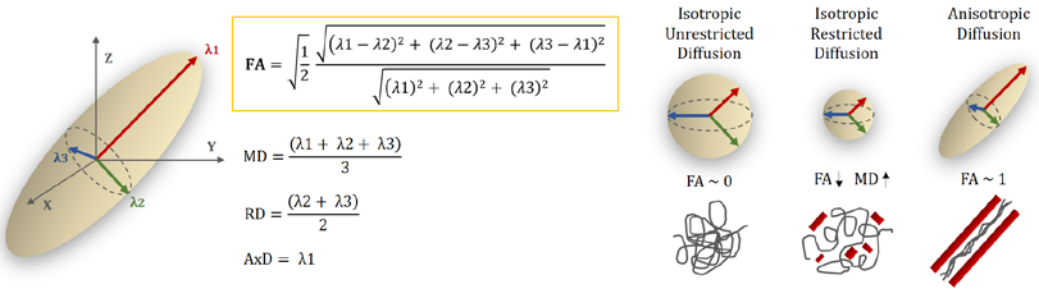

Figure 15. Mathematical description of the most commonly used measures derived from the diffusion tensor model (FA, MD, RD, AxD), *left*. Diffusion ellipsoids and tensors for isotropic unrestricted diffusion, isotropic restricted diffusion and anisotropic restricted diffusion, *right*. Adapted from Mukherjee et al., (2008). Abbreviations: FA, Fractional Anisotropy, MD, Mean Diffusivity; RD, Radial Diffusivity; AxD, Axial Diffusivity.

3.3.3 Functional MRI

Contrary to the previously described MRI acquisitions focusing on the anatomy of the brain, fMRI is characterized by a high temporal resolution, while having a low spatial quality. This type of MRI (for broader fMRI view see Matthews & Jezzard, 2004; Ramsey et al., 2002) is sensitive to dynamic changes in the blood (hemodynamics) in order to examine neuronal activity. More specifically, because the neuronal activity is a process that requires energy, which is supplied in the form of oxygen and glucose from the blood, the vessel system in the brain upsurges the blood flow to make sure that neurons are able to send action potential to one another. The fMRI is able to provide in vivo maps of changes in BOLD signal (Faro & Mohamed, 2006; Lee et al., 2013) in the brain across time because of a particularly useful property whereby oxygenated (diamagnetic) and deoxygenated (paramagnetic) forms of hemoglobin (Pauling & Coryell, 1936) interact with the magnetic fields of the scan generating inhomogeneities that result in detectable changes in the MRI signal (Buxton, 2009; Jenkinson & Chappell, 2018). The activation of neural tissues is accompanied by an increase of the oxyhemoglobin/deoxyhemoglobin ratio that leads to T2* signal increases (Ogawa, 1990; Logothetis et al., 2001). This blood response is characterized quantitatively by the hemodynamic response function (HRF), which is the brain response elicited by a stimulus that would be shown in ideal conditions (neglecting scan noise and other neuronal changes; see Figure 16). The HRF correlates strongly with the local field indicating that is related to the synaptic activity (including inhibitory and excitatory

activity) with a secondary and potentially more variable correlation with cellular action potentials (Arthurs & Boniface, 2002). Therefore, we are able to capture the delayed response due to the hemodynamic changes (i.e., on the order of seconds), but not the timing of neuronal spiking (i.e., on the order of miliseconds; Heeger & Ress, 2002).

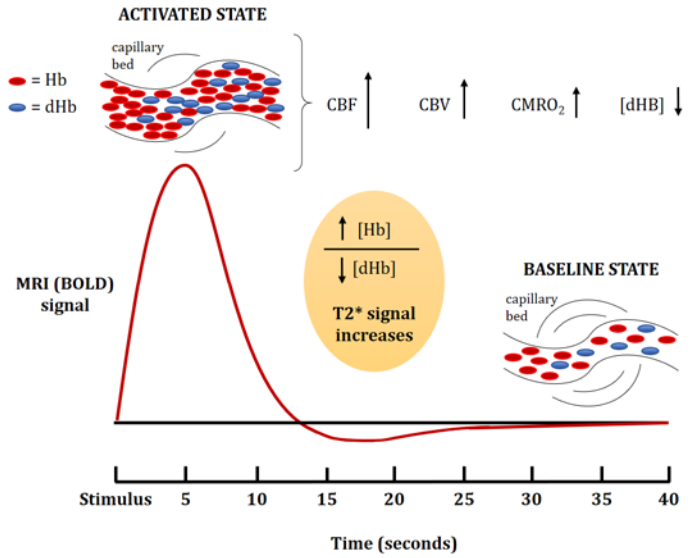

Figure 16. Illustration of the BOLD effect and the typical HRF for the adult human brain. It shows the change in MRI signal as a function of time, which takes around 6 s to peak and over 20 s to return to the baseline state. The activated state is characterized by large increases in CBF, CBV, $CMRO_2$, and in the amount of Hb by comparison with dHB, leading to an increase in MRI signal. Adapted from Jenkinson & Chappell (2018). Abbreviations: BOLD, Blood-Oxygen-Level Dependent; CBF, Cerebral Blood Flow; CBV, Cerebral Blood Volume; $CMRO_2$, Cerebral Metabolic Rate of Oxygen; Hb, oxygenated Hemoglobin; dHb, deoxygenated Hemoglobin.

In its more traditional application, fMRI has been used to identify areas of increased or decreased neuronal activity during the performance of a task (Logothetis et al., 2001, 2003; Raichle & Mintun, 2006).

Task-based fMRI

When fMRI is acquired while a subject is inside the scanner instructed to perform a goal-directed task, the differences in the BOLD signal level between states can be used to infer the spatial patterns of brain-activated regions under different task conditions. Task-

based fMRI requires exposing the subject to at least two different conditions or different cognitive demanding levels. Then, maps of brain activity can be obtained by subtracting the BOLD signal between states or conditions (Buchbinder, 2016). Task-related areas can be studied with fMRI by identifying patterns of brain regions that activate and deactivate in synchrony during the performance of a cognitive task. These activation changes are usually evaluated using different functional paradigms according to stimulus presentation strategies that can be block-designed or event-related tasks (Amaro & Barker, 2006; Jenkinson & Chappell, 2018). The fMRI task used in this Doctoral Thesis is block-designed because stimuli of the same category are presented subsequently along the duration of each concrete condition, maintaining cognitive engagement (Buchbinder, 2016). As a consequence of this, the BOLD response is actually composed of individual HRFs generated by each stimulus, producing a high signal magnitude.

One of the most studied domains in fMRI is human memory. In this regard, one of the most differentiable kinds of memory is WMem.

The fMRI paradigm: N-back task

A commonly used paradigm to study WMem with fMRI is the N-back task (for a meta-analysis see Yaple et al., 2019). It is a block-design task that consists on presenting a sequence of stimuli from which the subject is asked to indicate, by means of a response button, when some aspect of the currently showed stimulus is the same as that presented some defined number ('n') of trials previously. N-back tasks can involve a broad variety of representations (e.g., verbal, visual, auditory, spatial, etc.). Interestingly, task complexity can be controlled by changing the value of 'n' (i.e., the number of items to be remembered, that usually goes from n = 0 to n = 3 items). That is, 0-back refers to press the button when appears a specific target in the screen (e.g., the letter 'X'), 1-back asked to indicate each time the current stimulus matches the immediately preceding one, 2-back press the button if the current stimulus matches the stimulus two trials back, and 3-back means the same but three trials back. Amongst the N-back task loads there are common and specific processes that allow reproducing the complex cognitive function that the WMem is. Thus, the 0-back is typically considered a control condition that is related to identification and maintenance processes, while for the 1-back load an updating process is required in addition (i.e., as

every stimulus serves as the criterion for the subsequent trial). On the other hand, 2-back and 3-back, as the most difficult task levels, would draw upon identification, maintenance, updating, and inhibition of distractors because between every criterion and potential target, there is an additional stimulus that needs to be maintained but also inhibited if matched on the subsequent trial. It is important to highlight that a large number of contrasts can be derived from a comparison of the 4 levels mentioned above. Nevertheless, it seems that all the brain regions identified using task-based fMRI data have been reported to be consistently involved in WM tasks (Yaple et al., 2019).

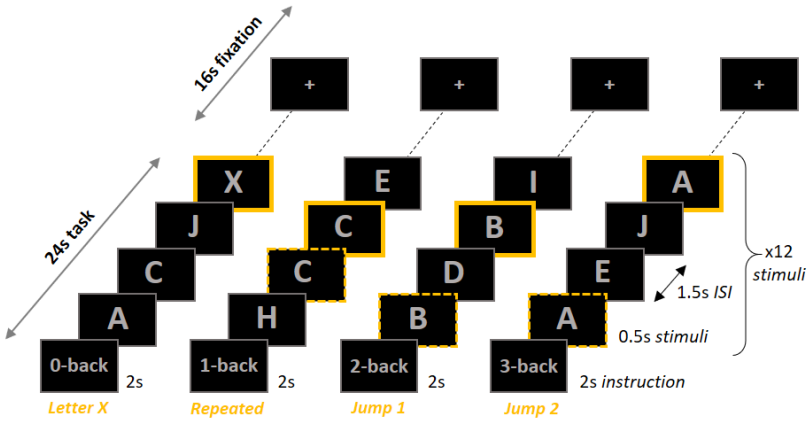

Figure 17. N-back task design. Four different loads are represented: 0-back, 1-back, 2-back, 3-back. Highlighted stimuli indicate the targets for each load. Abbreviations: ISI, Inter-Stimulus Interval. Extracted from Sala-Llonch et al., (2012).

In our case, participants from study 3 and study 4 performed a letter N-back task with different levels of memory load (from 0 to 3 letters to be retained) that were presented in a pseudo-random order inside the MR scan, as described (Sala-Llonch et al., 2012). Briefly, each N-back condition lasted 26s, followed by inter-block fixation periods of 13s. Before any N-back block, an instruction screen informed the subject of the upcoming block. Each stimulus (capital letter A-J) was shown in white in the center of a black screen during 500ms, with an inter-stimulus interval of 1500ms. Participants were instructed to press a button when the letter 'X' appeared (0-back) or when the letter shown matched the one seen one (1-back), two (2-back) or three (3-back) stimuli before (see Figure 17). In all studies included in the present Thesis using the N-back paradigm, the individual

performance was recorded and scores were calculated using the d' measure, which accounts for correct responses and false alarms, computed as: Z(hit rate) - Z(false alarm rate) where function Z(p), $p \in [0,1]$, as the inverse of the cumulative distribution function of the Gaussian distribution of the hits and false alarms rates.

- Among the variety of existing structural images, **T1w-structural** is the most common acquisition, used for getting good tissue contrast and high anatomical resolution.
- Diffusion of water assessed by **dMRI** provides indirect information about local WM microstructure and axonal fibers directions.
- The **fMRI** technique affords a surrogate measure of neuronal activity by using the BOLD signal, which is sensitive to blood oxygenation.
    - Task-based fMRI provides information about brain regions involved in the performance of a cognitive task.

3.4. Neuroimaging analyses

MRI was acquired in a 3T Siemens scanner (Magnetom Trio Tim syngo) with 32-channel head coil at the *Unitat d'Imatge per Ressonància Magnètica IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer)*, *Hospital Clínic de Barcelona*, Barcelona. All the images were inspected visually to ensure that they did not contain MRI artifacts or excessive movement before analysis. The acquisition parameters of all the MRI sequences are listed below:

- T1w-structural: magnetization-prepared rapid acquisition gradient-echo (MPRAGE) 3-dimensional protocol [repetition time (TR) = 2300 ms, echo time (TE) = 3 ms, inversion time = 900 ms, field of view (FOV) = 244 mm, 1-mm isotropic voxel, matrix size = 256 x 256].
- FLAIR: axial (TR = 9000 ms, TE = 96 ms, 40 slices, slice thickness = 3 mm, FOV = 240 mm, matrix size = 228 × 256).
- dMRI: were sensitized in 30 non-collinear directions with a b value of 1000 s/mm2 in an echo-planar imaging sequence (TR = 9300 ms, TE = 94 ms, section thickness = 2.0 mm, voxel size = 2.0 × 2.0 × 2.0 mm, FOV = 240 mm, and no gap).

* Task-based fMRI: interleaved acquisitions [T2*weighted Echo-Planar Imaging (EPI) scans, TR = 2000 ms, TE = 16 ms, 336 volumes, 40 slices, slice thickness = 3 mm, interslice gap = 25%, FOV = 220 mm, matrix size = 128 × 128] during the performance of the N-back task.

3.4.1 Structural MRI

Cortical Thickness

The Cortical Thickness (CTh) measurement is a powerful tool to study a wide variety of brain disorders since changes in the brain cortical sheet are usually manifested in normal aging, AD and other dementias (Fischl & Dale, 2000). The measurement of the thickness is enabled by a procedure for generating highly accurate models of both the gray/white and pial surfaces. The distance between these two surfaces then gives the thickness of the cortical GM at any point. The T1w-structural images were automatically processed with FreeSurfer (version 5.1; http://surfer.nmr.mgh.harvard.edu) in order to reconstruct the cortical surface and calculate the CTh. In summary, the procedures performed by the main FreeSurfer pipeline include removal of non-brain data, intensity normalization (Sled et al., 1998), tessellation of the GM/WM boundary, automated topology correction (Ségonne et al., 2007) and accurate surface deformation to identify tissue borders (Dale et al., 1999). CTh is then calculated as the distance between the white and gray matter surfaces at each vertex of the reconstructed cortical mantle (Fischl & Dale, 2000). The follow-up analysis in study 3, was carried out with the FreeSurfer longitudinal pipeline (Reuter et al., 2012) and the symmetrized percent change (spc) was selected as the measure of atrophy. In all cases, the individual results were inspected visually to ensure accuracy of registration, skull stripping, segmentation, and cortical surface reconstruction. Manual editing of the reconstructed surfaces was required in several cases. For group statistics, the CTh maps were smoothed using a 2D Gaussian kernel of 15 mm Full Width at Half Maximum (FWHM). The resulting vertex-wise statistical maps were considered significant at $p < 0.05$ level. Maps were further corrected for Family Wise-Error (FWE) using a Monte Carlo Null-Z simulation, with 10,000 repetitions and a cluster $p < 0.05$.

White matter hyperintensity volume (WMHV)

WMH volumes (WMHV) were estimated automatically through the use of the Lesion Segmentation Toolbox version 1.2.3 (http://www.neuro.uni-jena.de/software/lst/; Schmidt et al., 2012), an extension for the SPM software (http://www.fil.ion.ucl.ac.uk/spm). First, T1w-structural images were segmented into the three main tissue classes: GM, WM, and CSF. This information was then combined with the co-registered FLAIR intensities in order to calculate lesion belief maps. By thresholding these maps with a pre-chosen an initial threshold of 0.3, as recommended by default, an initial binary lesion map was obtained which was subsequently grown along the voxels that appeared hyperintense in the FLAIR image. The result was a lesion probability map.

3.4.2 Diffusion MRI

The dMRI images were analyzed with FMRIB's Diffusion Toolbox (FDT) software from FSL, (FMRIB's Software Library; http://fsl.fmrib.ox.ac.uk/fsl/; Jenkinson et al., 2012), which includes tools for data processing, local diffusion modeling, and tractography (Jbabdi et al., 2012). Prior to analysis, we applied eddy current, motion correction and brain extraction (Smith, 2002). Then, individual fractional anisotropy (FA) maps were obtained using a Diffusion Tensor Model fit (DTIFIT) and introduced to group analysis using the Tract-Based Spatial Statistics (TBSS) protocol (Smith et al., 2006). TBSS performs nonlinear registration (FNIRT) of FA images to the Montreal Neurological Institute (MNI) standard space and generates a mean FA skeleton that represents the center of all tracts common to the entire group. Then, the aligned FA image for each subject was projected onto the skeleton by filling the skeleton with FA values from the nearest relevant tract center. For group statistics, FA maps co-registered and projected to the group skeleton from TBSS were further analyzed using randomise from FSL. Images resulting from the group analyses were corrected for Family-Wise Error (FWE) using the threshold-free cluster enhancement (TFCE) method to define the clusters, the statistical threshold was set to corrected $p < 0.05$.

3.4.3 Task-based fMRI

The fMRI data were analyzed with the FEAT-FSL (FMRIB's Software Library version 5.0.6; http://fsl.fmrib.ox.ac.uk/fsl/; Jenkinson et al., 2012). We first performed a preprocessing of all individual fMRI scans, which included non-brain tissue removal, motion correction, spatial smoothing with a Gaussian kernel of 5 mm of FWHM, temporal filtering with a high pass filter of 160 s and a linear registration to a standard template. Further, the head motion parameters estimated by MCFLIRT (Jenkinson et al., 2002) were included as confounding explanatory variables in our model. Then, at the first level analysis (Woolrich et al., 2001), data were fit to a general a linear model (GLM) containing the task time-series with a gamma convolution of the hemodynamic response function. In this GLM, four regressors and their first temporal derivatives were modeled: 0-back, 1-back, 2-back and 3-back. By including the derivatives, we aimed to correct for shifting in the time series as well as for slice timing effects. We defined a single contrast of interest combining the previous four regressors as the difference of brain activity between the highest and lowest loads (3-back > 2-back > 1-back > 0-back), by using weights of 0.375, 0.125, -0.125 and -0.375. The results of the first level analyses were further fit into higher-level or group-level statistics, performed using the FMRIB's Local Analysis of Mixed Effects (FLAME), (Woolrich et al., 2004). All analyses were performed in the whole brain at a voxel-wise level, and a $Z > 2.3$ was used to define contiguous clusters of activity, then cluster significance levels were estimated and corrected using FWE correction. The significance threshold was set at a corrected $p < 0.05$.

3.5. Specific statistical methods

Throughout this thesis, besides the standard statistic pipelines implemented in the automated analysis tools (which are explained in detail within each paper in Chapter 4), other statistical analyses were implemented, namely the mediation model and the gene expression analyses. The main principles beyond these two methods are summarized below.

3.5.1 Mediation model

A mediation model suggests that the observed relationship between an independent variable (X) and a dependent variable (Y) can be explained by the effect of a third factor, known as mediator (M), (see the Web pages of Andrew Hayes, http://www.afhayes.com/). According to Baron & Kenny (1986), three prerequisites are necessary in order to establish mediation: (1) X should be a significant predictor of Y; (2) M should have a significant effect on the Y; and (3) the M should be a significant predictor of Y while controlling for the effect of X (see Figure 18). Thus, M is a mediator of the effect of X on Y if X carries its influence on Y at least partly influencing M, which then influences Y (Figgou & Pavlopoulos, 2015).

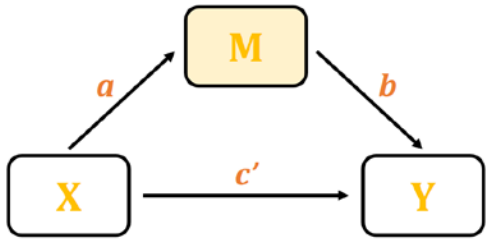

Figure 18. Mediation model. X significantly accounts for variability in M (path *a*), X significantly accounts for variability in Y (path *c'*), M significantly accounts for variability in Y when controlling for X, and the effect of X on Y decreases substantially when M is entered simultaneously with X as a predictor of Y.

In our analyses, we used bootstrapping to estimate the significance of the mediation effect (MacKinnon et al., 2007; Hayes, 2009; Preacher & Hayes, 2008). This is a non-parametric method that has become increasingly popular, which is recommended for small samples as it does not violate assumptions of normality (Preacher & Hayes, 2008). Each model is tested with 5000 iterations and a confidence interval (CI) level of 95 % and it is considered significant when the resulting CI of the mediating effect did not include the zero (i.e., no effect). Beyond statistical complexity, it should be underlined that mediation is primarily a conceptual issue, so the conclusions from a mediation analysis are valid only if the causal assumptions hold true (Judd & Kenny, 2010).

3.5.2 Molecular architecture of cortical regions

Cortical gene expression data

In study 2 the transcriptome data from the Allen Institute Human Brain Atlas (http://human.brain-map.org/; Hawrylycz et al., 2012, 2015) was used in order to characterize the cortical gene expression of a concrete MRI-based identified area (see Figure 19 for an exhaustive pipeline). In brief, complete microarray gene expression datasets from a total of 3,702 regional tissue samples from postmortem brains of six individuals were mapped to anatomical brain locations in corresponding structural MRI scans (these steps were performed by the Allen Institute; Shen et al., 2012). The produced raw expression values were then mean averaged across probes to obtain single expression values for each gene, resulting in 20,737 gene expression values per tissue sample. For each donor the individual tissue sample coordinates were spatially mapped to a cortical surface reconstruction obtained by processing the donors' MRI images in FreeSurfer (data available from Romero-Garcia et al., 2018).

Gene set enrichment analysis

Instead of focusing on one or a few predefined genes based on a priori hypotheses, we used a gene set enrichment analysis (GSEA; http://software.broadinstitute.org/gsea/index.jsp, software version 3.0; Subramanian et al., 2005) to more broadly explore differentially expressed functional pathways. The enrichment analysis helps to uncover general trends in differential gene expression in a more comprehensive manner, while extracting meaningful and interpretable information from the high-throughput microarray data. The method derives its power by focusing on gene sets, it means groups of genes that share common biological function, chromosomal location, or regulation. Given that statistical inference in GSEA is based on complete functional gene sets, not all of the genes included in a significant gene set are necessarily differentially expressed. A so-called leading-edge subset of genes can be identified that primarily accounts for a gene set's enrichment score and a leading-edge analysis then aims to find commonalities among the identified gene sets by clustering the respective leading-edge subsets (Subramanian et al., 2005), see Figure 20. To consider the existing redundancy

between the different gene sets and to facilitate interpretation of the enrichment results, functionally coherent gene sets were detected using a clusterization algorithm (Merico et al., 2010; Isserlin et al., 2014), and organized into a network layout (i.e., enrichment map). In addition, in our analyses, we included gene sets from multiple independent sources to increase the power and coverage of our analyses [Molecular Signatures Database (MSigDB) version 6.0; http://software.broadinstitute.org/gsea/msigdb/index.jsp; Liberzon et al., 2011, 2015].

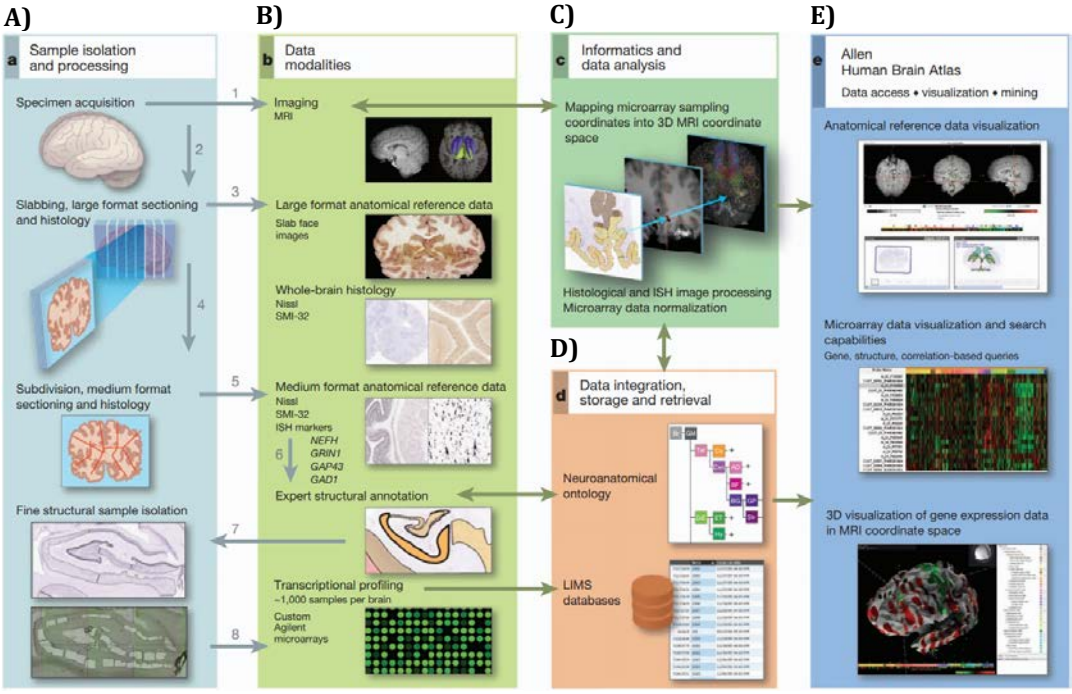

Figure 19. Transcriptome data from the Allen Institute Human Brain Atlas. A) Experimental strategy to subdivide intact brains and isolate precise anatomical samples. B) Anatomical reference data are collected at each stage, including whole-brain MRI, large format slab sectioning and histology, medium format Nissl histology and ISH markers, and images of dissections. Histology data are used to identify structures, which are assembled into a database using a formal neuroanatomical ontology (D panel), and to guide laser microdissection of samples (A, lower panel). Isolated RNA is used for microarray profiling of ~900 samples per brain (B, lower panel). C) Microarray data are normalized, and sample coordinates mapped to native 3D MRI coordinates. E) Data visualization and mining tools underlie the online public data resource. Extracted from Hawrylycz et al., (2012). Abbreviations: ISH, In Situ Hybridization; MRI, Magnetic Resonance Imaging; MNI, Montreal Neurological Institute; RNA, Ribonucleic Acid.

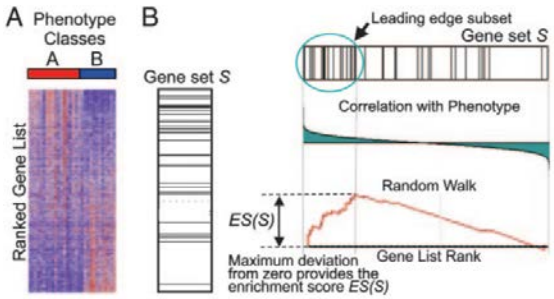

Figure 20. GSEA overview. A) An expression data set sorted by correlation with phenotype, the corresponding heat map, and the 'gene tags' (i.e., location of genes from a set S within the sorted list). B) Plot of the running sum for S in the data set, including the location of the maximum enrichment score and the leading-edge subset. Extracted from Subramanian et al., (2005). Abbreviations: GSEA, Gene Set Enrichment Analysis.

3.6. Multifocal tDCS

Classically, in tDCS, which aim to modulate particular cortical regions, a single anode accompanied by its corresponding cathode has been used. More recently, novel multifocal tDCS protocols have been developed in order to target multiple brain areas simultaneously (Ruffini et al., 2014). In multifocal tDCS, multiple electrodes are employed potentially resulting in higher modulatory efficacy than classic tDCS (Fischer et al., 2017).

In study 4, we designed two multifocal tDCS montages (Ruffini et al., 2014) in collaboration with Neuroelectrics® (Barcelona, Spain) to obtain distinct electric field distribution patterns over the brain cortical surface. Stimulation was delivered via an MRI-compatible StarStim Neuroelectrics® system in order to evaluate the online tDCS effects using concomitant MRI data. Briefly, 8 cm^2 electrodes were placed inside a sponge and into the holes of a neoprene cap corresponding to the 10/10 international system for electrode placement, with the central Cz position aligned to the vertex of the in every subject to ensure an accurate placement. Electrodes' sponges were soaked with saline solution and a thin layer of Ten20 conductive paste to ensure good conductivity and stability throughout the MRI acquisition. The stimulator was situated outside the MRI room and the current was delivered to each electrode with a wireless neurostimulator connected to a computer via Bluetooth using Neuroelectrics Instrument Controller (NIC) engine software.

Chapter 4

Results

Study 1

Vaqué-Alcázar, L., Sala-Llonch, R., Valls-Pedret, C., Vidal-Piñeiro, D., Fernández-Cabello, S., Bargalló, N., Ros, E. & Bartrés-Faz, D. (2017). Differential age-related gray and white matter impact mediates educational influence on elders' cognition. *Brain Imaging and Behavior, 11*(2), 318–332.

Brain Imaging and Behavior
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SI: RESILIENCE/RESERVE IN AD

Differential age-related gray and white matter impact mediates educational influence on elders' cognition

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Abstract High education, as a proxy of cognitive reserve (CR), has been associated with cognitive advantage amongst old adults and may operate through neuroprotective and/or compensation mechanisms. In neuromaging studies, indirect evidences of neuroprotection can be inferred from positive relationships between CR and brain integrity measures. In contrast, compensation allows high CR elders to sustain greater brain damage. We included 100 cognitively normal old-adults and investigated the associations and interactions between education, speed of processing (SP), memory and two brain integrity measures: cortical thickness (CTh) of gray matter (GM) and fractional anisotropy (FA) in the white matter (WM). High education was associated with better cognitive performance, enlarged CTh in frontal lobe areas and reduced measures of FA in several areas. Better SP performance in higher educated subjects was related to more preserved GM and WM, while memory status amongst high educated elders was better explained by a putative compensatory mechanism and independently from cerebrovascular risk indicators. Moreover, we analyzed the direct effect of age on measures of brain integrity and found a stronger negative effect on WM than in CTh, which was accentuated amongst the high CR sample. Our study suggests that the cognitive advantage associated to high education among healthy aging is related to the coexistence of both neuroprotective and compensatory mechanisms. In particular, high educated elders seem to have greater capacity to counteract a more abrupt age impact on WM integrity.

Keywords Aging · Education · Cognitive reserve · Neuroprotection · Compensation · Structural changes · Speed of processing · Memory

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Introduction

Healthy aging is associated with a general decline in several cognitive domains. In particular, reductions in episodic memory (Nyberg et al. 2003; Rönnlund et al. 2005) and speed of processing (SP) (Park and Reuter-Lorenz 2009; Salthouse 1996) are widely documented. However, cognition in senescence is characterized by inter-individual differences (Nyberg et al. 2012), subtended by both genetic and environmental origins, as well as by their interactions (Staff et al. 2012; Valenzuela and Sachdev 2006; Brooks-Wilson 2013). In this line, the cognitive reserve (CR) model stresses the individual differences in tolerating age-related brain changes and

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pathology (Stern 2012). Exposure to particular lifestyles, as well as education, leisure and physical activities, are commonly used as proxies of CR (Stern 2002; Barnes and Yaffe 2011). According to this view, subjects with higher CR will exhibit a greater capacity to withstand brain burden and thus minimize its impact on cognitive and/or clinical manifestations (Stern 2009, 2012).

Neuropsychological and neuroimaging studies have been widely used to investigate how some lifestyle factors, usually coincident with proxies of CR, impact age-related brain changes. These studies include structural Magnetic Resonance Imaging (MRI) to investigate gray matter (GM) integrity, as well as Diffusion Tensor Imaging (DTI) to estimate white matter (WM) connectivity. In general, it has been confirmed that CR estimates such as education are consistently related to better cognition (for a recent meta-analysis, see Opdebeeck et al. 2016), and to preserved whole brain or regional GM (Solé-Padullés et al. 2009; Bartrés-Faz et al. 2009; Foubert-Samier et al. 2012; Liu et al. 2012; Arenaza-Urquijo et al. 2013a). These findings may therefore suggest a direct neuroprotective effect of reserve (Arenaza-Urquijo et al. 2015). In contrast, in clinical populations, such as demented patients, CR proxies have been typically related to greater indicators of brain pathology that do not correspond with the clinical/cognitive status of patients. This has been observed as reduced brain metabolism, increased amyloid (Aβ) deposition (e.g. Perneczky et al. 2006; Kemppainen et al. 2008), or higher atrophy rates (Solé-Padullés et al. 2009). The findings here suggest a compensatory view of reserve, reflecting an increased capacity to tolerate brain damage (Stern 2009, 2012). Finally, a recent theoretical account of reserve (Arenaza-Urquijo et al. 2015) interestingly proposes that neuroprotective and compensatory mechanisms may operate in an overlapping manner all along the continuum between health and disease. Based on AD pathological changes, this model suggests that lifestyle factors may exert their effects mainly through neuroprotective mechanisms in early stages of the disease (for example by slowing Aβ deposition rates, Landau et al. 2012; Wirth et al. 2014) and compensatory mechanisms latter in disease progression (for example supported by evidences showing that cognitively normal Aβ-positive subjects had glucose hypomethabolism in AD-vulnerable regions, Ewers et al. 2013).

A common methodological approach to investigate the impact of CR on brain structure and cognitive function is to first equate cognitive or clinical status amongst subjects, and then to perform correlations between CR estimates and brain measures. In these procedures, age is usually included as a covariate, because it is known to alter brain anatomy more than any typical biomarker of preclinical conditions (Fjell et al. 2013). Moreover, there is evidence that age may have a differential influence regarding tissue type and topographic specificity. For example, DTI studies indicate that WM integrity follows an inverted "U" during the lifespan, with a period of relative stability during adulthood followed by a prominent degeneration (Westlye et al. 2010; Sexton et al. 2014). This trend differs from the overall trajectory of the GM in the cerebral cortex, which declines almost linearly at macroscopic level from the age of 20 years onwards (Fjell et al. 2014a, b).

These latter observations may have an impact when considering the putative coexistence of neuroprotection and compensatory mechanisms linked to CR amongst healthy old adults. On the one hand neuroprotective evidences would fit with findings demonstrating more preserved brain integrity parameters with age (that is less age-related decline or less age-related differences) amongst high CR. However it should be noted, that 'neuroprotection', is relatively general term and likely reflects a potential number of diverse mechanisms. Consequently, it has been evoked to account for the positive impact of CR to slow the progression of disease biomarkers as stated above, but also to exemplify the positive impact of cognitive training on brain structures (Arenaza-Urquijo et al. 2015). In this broader context, neuroprotection may also refer to the positive effects of lifestyles related to CR on measures of brain integrity, on optimal developmental processes during youth (Staff et al. 2012). Here, positive associations between CR estimates and measures of brain integrity should be detectable throughout lifespan, albeit not necessarily restricted to regions suffering late-life age-associated atrophy. In contrast to neuroprotection, support for compensation could be concluded if high CR elders exhibited greater rates of atrophy or loss integrity in those regions, while preserving good cognitive function. This interpretation would be conceptually similar to the 'compensation' evidences reported above amongst patients, but now it would be mainly associated to the capacity to counteract the effects of atrophy related to advancing age, rather than to pathology.

In the present study we aimed to explore if distinct associations between education, a common surrogate measure of CR, and GM and WM integrity mediate the cognitive status of healthy elders. We first hypothesized that higher levels of education would be related to better cognitive performance. We subsequently investigated associations between education and cortical thickness (CTh) and WM integrity measures, as well as its interactions with cognitive performance. According to the statements above, we predicted that neuroprotective mechanisms would be revealed by positive associations between education and measures of brain integrity in those areas suffering reduced or nil impact of advancing age. In contrast, cognitive advantage in high CR elders will be *simultaneously* related to greater rates of atrophy in brain measures supporting greater age-associated changes, hence favouring compensatory interpretation. Finally, when evidences of compensation were observed, we investigated whether it could be related to indicators suggesting that the individuals under study may be in an 'at risk' or potential 'preclinical' condition for brain disease.

Methods

Subjects

One hundred participants aged 63–76 years (see Table 1 below) were included in the study. They were normal functioning community elders recruited from the *Fundació Institut Català de l'Envelliment (FICE)* and were free of major neuropsychiatric diseases. Exclusion criteria were illiteracy or inability to understand the protocol or undergo neuropsychological tests, morbid obesity ($BMI^3 > 40$ kg/m^2), uncontrolled diabetes (HbA1c > 8 %), uncontrolled hypertension (on-treatment blood pressure ≥ 150/100 mmHg), prior cerebrovascular accident, any relevant psychiatric illness (including major depression), dementia, other neurodegenerative diseases (i.e., Parkinson's disease) and any chronic illness expected to shorten survival (heart failure, chronic liver disease, kidney failure, blood disease, cancer). Following previous reports (Foubert-Samier et al. 2012), we recorded the total years of formal education completed, classified in three levels: primary school (< 8 years), secondary school (9–14 years) and university level (≥15 years). We further stratified our sample into high or low educated elders, using a cutoff point of 15 years of education to create the groups. Forty-five individuals were classified as high-educated (tertiary, i.e. having reached university level) and 55 as low-educated (non-university level: see Table 1). The study was approved by the institutional ethics committee and was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from each participant prior to enrollment in the study. All subjects underwent an extensive neuropsychological assessment and a MRI scanning protocol.

Neuropsychological assessment

A set of neuropsychological tests was administered to assess the main cognitive functions as described in our previous reports (Vidal-Piñeiro et al. 2014). All participants had a normal cognitive profile with MMSE scores >25 and performances not more than 1.5 SD below normative scores adjusted for age, gender and years of education on any of the neuropsychological tests administered (i.e., they did not fulfill cognitive criteria for mild cognitive impairment, Petersen and Morris 2005). For the current study, our aim was to investigate correlations with declarative memory and speed of processing, cognitive domains, which are commonly affected with age (Park and Reuter-Lorenz 2009). Therefore, applying the approach used in previous studies (Vidal-Piñeiro et al.

Table 1 Demographics and neuropsychological data

	All subjects $n = 100$	High Educated $n = 45$	Low Educated $n = 55$	p-value
Age	68.35 ± 3.09	68.25 ± 3.09	68.79 ± 3.14	0.224[a]
Gender (M/F)	33/67	24/21	9/46	0.000[b]*
APOE status (C/NC)	19/81	6/39	13/42	0.191[b]
Education	11.53 ± 4.03	15.31 ± 1.43	8.44 ± 2.54	0.000[a]*
MMSE	29.35 ± 1.01	29.51 ± 0.87	29.22 ± 1.1	0.158[a]
Memory scores				0.014[c]*
ROCF-3'	20.25 ± 6.36	21.64 ± 5.72	19.11 ± 6.69	0.543[c]
ROCF-30'	20.11 ± 6.12	21.87 ± 5.32	18.67 ± 6.40	0.221[c]
RAVLT-total	48.67 ± 7.59	51.00 ± 6.89	46.76 ± 7.67	<0.001[c]*
RAVLT-delayed	10.44 ± 2.52	10.93 ± 2.50	10.04 ± 2.49	0.012[c]*
SP scores				0.001[c]*
TMTA	40.46 ± 18.36	34.67 ± 10.05	45.2 ± 22.02	0.007 [c]*
SDMT	40.71 ± 11.78	46.02 ± 8.29	36.36 ± 12.49	<0.001[c]*
CPT	60.40 ± 10.38	59.28 ± 10.85	61.32 ± 9.99	0.688[c]

Data are presented as mean ± standard deviation

M/F male/female

C/NC ε4 carriers/ ε4 non-carriers

ROCF-3' and ROCF-30' (Rey-Osterrieth Complex Figure recall at 3 and 30 min), RAVLT-total and RAVLT-delayed (Rey Auditory Verbal Learning Test), TMTA (Trail Making Test A), SDMT (Symbol Digit Modalities Test) and CPT (computerized version of the Continuous Performance Test)

TMTA and CPT are time measures; the higher scores the worse performance

[a] p-values were obtained by two sample t-test (parametric data)

[b] p-values were obtained by χ^2 test (categorical data)

[c] p-values were obtained by ANOVA test (gender as covariate)

*Significant differences were found in education, gender, memory and SP scores between the two groups

2014) we used Principal Components Analysis (PCA) based on the data set of the current study to create a composite scale which represented the declarative memory and speed processing functional domains separately. The following cognitive tests were used to calculate the memory factor: the Rey-Osterrieth Complex Figure recall (ROCF-3′ and ROCF-30′), Rey Auditory Verbal Learning Test total learning and delayed recall (RAVLT-total and RAVLT-delayed), while the tests contributing to the speed of processing factor included Trail Making Test A (TMTA), Symbol Digit Modalities Test (SDMT), and a computerized version of the Continuous Performance Test (CPT-RT). Importantly, both factors were found to be 'positive', i.e., higher scores on the factor indicated higher scores on memory or lower times on the SP-related tests.

MRI acquisition

All participants were scanned with a Siemens Magnetom Trio Tim syngo 3-T system at the *Unitat d'Imatge per Ressonància Magnètica IDIBAPS (Hospital Clínic)*, Barcelona. A high-resolution T1-weighted structural image was obtained for each subject with a magnetization-prepared rapid acquisition gradient-echo (MPRAGE) 3-dimensional protocol (repetition time [TR] = 2300 milliseconds, echo time [TE] = 3 milliseconds, inversion time = 900 milliseconds, field of view [FOV] = 244 mm, and 1-mmisotropic voxel). Diffusion-weighted images were sensitized in 30 non-collinear directions with a b value of 1000 s/mm2 in an echo-planar imaging sequence (TR = 9300 milliseconds; TE = 94 milliseconds; section thickness = 2.0 mm; voxel size = 2.0 × 2.0 × 2.0 mm; FOV = 240 mm; and no gap). In addition, we acquired a FLAIR sequence (Axial; TR = 9000 ms, TE = 96 ms, 40 slices, slice thickness = 3 mm, FOV = 240 mm, matrix size = 228 × 256). All the images were inspected visually to ensure that they did not contain MRI artifacts or excessive movement before analysis.

Cortical thickness analysis

Cortical surface reconstruction and calculation of CTh from the structural T1-weighted images were carried out by FreeSurfer software package (v5.1; http://surfer.nmr.mgh.harvard.edu). In summary, the procedures performed by the main FreeSurfer pipeline include removal of non-brain data, intensity normalization (Sled et al. 1998), tessellation of the GM/WM boundary, automated topology correction (Ségonne et al. 2007) and accurate surface deformation to identify tissue borders (Dale et al. 1999). CTh is then calculated as the distance between the white and gray matter surfaces at each vertex of the reconstructed cortical mantle (Fischl and Dale 2000). Individual results were inspected visually to ensure accuracy of registration, skull stripping, segmentation, and cortical surface reconstruction. Manual editing of the reconstructed surfaces was required in seven subjects, the threshold used for brain extraction was changed in 27 other subjects, and four subjects were excluded due to poor reconstruction caused by excessive motion.

White matter hyperintensity volumes (WMHV)

White matter hyperintensity volumes (WMHV) were estimated automatically through the use of the Lesion Segmentation Toolbox version 1.2.3 (http://www.neuro.uni-jena.de/software/lst/; Schmidt et al. 2012), an extension for the SPM software (http://www.fil.ion.ucl.ac.uk/spm). First, T1 images were segmented into the three main tissue classes: GM, WM and cerebrospinal fluid. This information was then combined with the co-registered FLAIR intensities in order to calculate lesion belief maps. By thresholding these maps with a pre-chosen initial threshold of 0.3, as recommended by default, an initial binary lesion map was obtained which was subsequently grown along the voxels that appeared hyperintense in the FLAIR image. The result was a lesion probability map.

DTI processing

Diffusion MRI images were analyzed with FMRIB's Diffusion Toolbox (FDT) software from FSL, (http://www.fmrib.ox.ac.uk/fsl), which includes tools for data processing, local diffusion modeling and tractography (Jbabdi et al. 2012). Prior to analysis, we applied eddy current and motion correction and brain extraction (Smith 2002). Then, individual fractional anisotropy (FA) maps were obtained using a Diffusion Tensor Model fit (DTIFIT) and introduced to group analysis using the Tract-Based Spatial Statistics (TBSS) protocol (Smith et al. 2006). TBSS performs nonlinear registration (FNIRT) of FA images to the Montreal Neurological Institute (MNI) standard space and generates a mean FA skeleton that represents the center of all tracts common to the entire group. Then, the aligned FA image for each subject was projected onto the skeleton by filling the skeleton with FA values from the nearest relevant tract center.

Statistics

All non-imaging data analyses were performed using SPSS v20.0 (Statistical Package for Social Sciences, Chicago, UU, USA. IBM Corp. Released 2011. Armonk, NY). Demographic, cognitive, WMHV and Body Mass Index (BMI) data were described as mean ± standard deviation (Tables 1 and 3). To evaluate differences in cognition, demographics and vascular risk factors between the two educational groups, we used the two-sample t-test for the parametric data

(or ANOVA when needed to adjust for covariates) and the χ^2 test for the categorical data.

Cortical thickness

Analyses of CTh maps were carried out using voxel-wise General Lineal Models (GLM) as implemented in FreeSurfer. Previous to statistical analysis, CTh maps were smoothed using a Gaussian kernel of 15 mm full-width at half maximum (FWHM). We then evaluated the different GLM matrices in order to study: (Almeida et al. 2015) group differences in CTh between high and low educated subjects; (Arenaza-Urquijo et al. 2015) correlations between CTh and cognitive performance; and (Arenaza-Urquijo et al. 2013a) interactions between group (high educated vs low educated) and cognitive performance. All analyses were adjusted by age and gender. Images were corrected for Family wise-error (FWE) using a Monte Carlo Null-Z simulation, with 10,000 repetitions. Both the initial vertex thresholding and the cluster thresholding were set to $p < 0.05$. Then, mean CTh within significant clusters in the analysis of group differences was extracted for each subject in order to obtain summary statistics (*CThcluster*).

Diffusion tensor imaging

FA maps co-registered and projected to the group skeleton from TBSS were further analyzed using *randomise* from FSL. We used the same GLM designs described above (see Cortical Thickness section) to explore group differences and correlations. Images were corrected for FWE using the threshold-free cluster enhancement (TFCE) method to define the clusters, the statistical threshold was set to corrected $p < 0.05$. As in the CTh analysis, here we obtained mean FA within the areas that differed between groups (*FAmask*).

Quantitative multimodal MRI analyses

With the numerical values extracted from each imaging modality (*FAmask* and *CThcluster*), we confirmed the group differences using two sample t-tests and we performed Pearson's correlations with cognition and age. We also performed the same analysis with global measures, obtained as an average of the CTh in the cortex and FA within the skeleton. We conducted all the correlation analyses considering the whole group or the high and low educated groups separately. These correlations were carried out using SPSS and all the results were considered significant at the level of $p < 0.05$.

In addition, we normalized the scores of FA and CTh in order to compare their slopes in the age-correlation using factorial ANOVA. For this purpose, all values were divided by their corresponding maxima. In all analyses conducted to investigate the effects of CR on cognition, brain structure or their interactions age and gender were included as covariates.

Mediation analyses

As a result of the relationships identified between cognition, FA and education (see below), we tested a mediation analysis (Preacher and Hayes 2008). Because these relationships could have different interpretations, we tested two different models (Salthouse 2011). First, the brain variable (measure of FA) can act as a mediator of the effect of education on cognition, that is, the effect of education on cognition would the result of a direct effect of education and an indirect effect through FA. In another possible model, education can mediate the effect of FA on cognition, in the sense that the correlation between FA and cognition would be explained by an indirect effect of education. We hypothesized that both models could fit within CR based assumptions. On the one hand, the effects of CR on cognition can be influenced by the effect of education on the brain and therefore FA will mediate this association (i.e., first model). Complimentarily, the cognitive advantage of education would counteract the negative effect of brain atrophy on cognition (second model). Given that memory and SP are different cognitive domains, with putative distinct anatomical substrates as regards WM and associations with educational background, we tested the two models separately for each domain, with the hypothesis that they are not necessarily supported by the same mechanism. We used bootstrapping to estimate the significance of the mediation effect (MacKinnon et al. 2007; Hayes 2009; Preacher and Hayes 2008). Each model was tested with 5000 iterations and a confidence interval (CI) level of 95 %. A model was considered significant when the resulting CI of the mediating effect did not include the zero (i.e., no effect).

Results

Education: demographic and cognitive associations

The main demographic and cognitive characteristics of the sample, separated according to high and low education groups, are detailed in the Table 1. Specifically, there were no differences between groups regarding age, although there were more women in the low educated group. APOE status did not differ between groups. The higher educated subjects achieved significantly better scores on RAVLT (total and delayed), TMTA and SDMT (Table 1), and the results remained when age was introduced as a covariate. No differences were identified in memory and SP performance between men and women.

Effects of education on cerebral structure

Group differences in CTh

The vertex-wise analysis of CTh identified higher values in high educated elders compared with their low educated peers when age and gender were introduced as covariates. Specifically, we identified a significant cluster within the right frontal lobe, largely spanning the medial prefrontal cortex and comprising regions form the anterior cingulate and medial orbitofrontal areas as well as the frontal pole, superior frontal, rostral and caudal (cluster size: 2974.22mm^2, cluster-wise $p = 0.0003$), (Fig. 1a, b).

Group-differences in FA

In contrast to CTh, we found lower FA values in the higher educated group in the majority of WM tracts when age and gender were introduced as covariates (Fig. 2a, b). Significant regions included bilateral associative tracts such as parts of the inferior longitudinal, the superior longitudinal, the inferior fronto-occipital fasciculus, the superior longitudinal temporal, the cingulum, the cingulum hippocampal, the uncinate and the anterior thalamic radiation, as well as commissural tracts such as the forceps major and forceps minor and projection fibers such as the corticospinal tract (Fig. 2a).

Relationship between brain structure, education and cognition

Next, we examined how the differences identified in CTh and DTI analyses affected cognitive performance. We found a significant interaction between group and SP in CTh. Specifically, we identified a cluster in the right pre-frontal region (cluster size: 1685.88mm^2, cluster-wise $p = 0.0115$; Fig. 3a) where higher educated subjects exhibited a positive correlation between CTh and SP scores ($r = 0.447$, $p = 0.004$), whereas their lower educated peers showed a non-significant negative effect on SP ($r = -0.234$, $p = 0.102$; Fig. 3b). In this cluster, we found a significant difference between the slopes of high- vs low- educated subjects ($F = 4.961$, $p = 0.009$; see Fig. 3b). No results were observed in the relationship between CTh and memory.

The same analysis with the FA maps (i.e., voxel-wise analysis of interactions defined with GLM) did not identify any region as significant. However, in order to evaluate other possible interactions, we used the mean FA within the regions with significant differences between high and low educated subjects (named *FAmask*, blue tracts in Fig. 1a). In these regions, FA correlated negatively with memory ($r = -0.254$, $p = 0.012$) when age and gender were introduced as covariates in the whole group (Table 2). That is, subjects with lower FA performed better in the memory tests; however, when years of education were included in the analysis the association was no longer significant (Table 2), suggesting a possible mediation effect of education. In contrast, we found that the *FAmask* correlated positively with SP with age, gender and years of education as covariates ($r = 0.226$, $p = 0.026$, Table 2) meaning that subjects with higher FA were more likely to respond faster in the related tests.

The relationships observed between memory and SP with *FAmask* and years of education suggested a mediation effect between these variables (Table 2). We tested the two different scenarios for the mediation model reported above for memory and SP domains. Testing this model, we found that education mediated the effect of FA on memory (Indirect effect, CI: −6.792 [−12.3503, −1.2333]; Fig. 4a) and that FA was the mediator for the relationship between education and SP (Indirect effect, CI: −0.018 [−0.0508, −0.0055]; Fig. 4b).

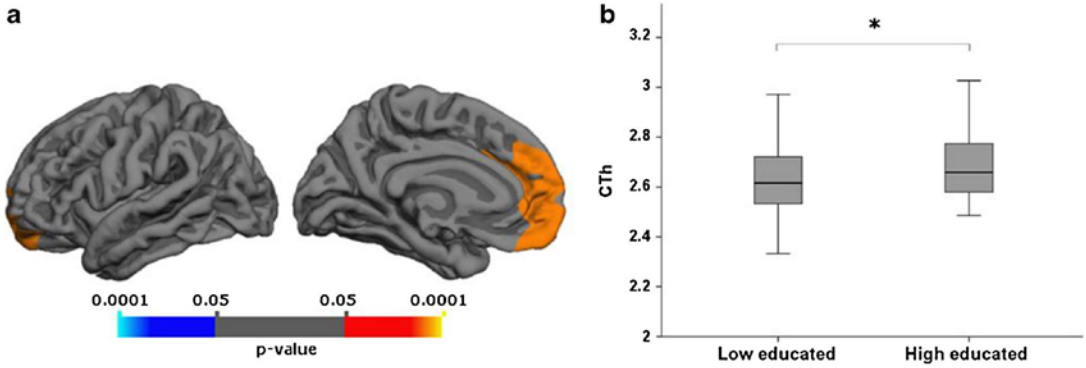

Fig. 1 High-educated subjects exhibited thicker cortical thickness. **a)** Map of CTh in high educated and low educated subjects. Only clusters surviving FWE multiple comparison correction with a final cluster-wise $p < 0.05$ are considered (*CThcluster*). **b)** Box diagram showing that the high educated group exhibited higher values of CTh in the total surface (* $F = 12.954$, $p = 0.001$)

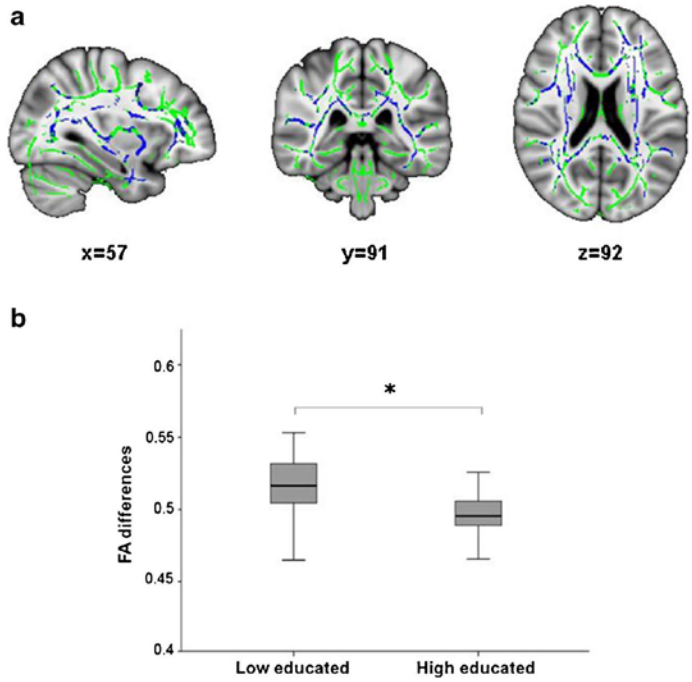

Fig. 2 High-educated subjects exhibited lower FA ratings. **a)** Spatial map of areas with significant differences between high and low educated subjects (*FAmask*). Thresholded at $p < 0.05$. Results are shown in *blue* on the standard MNI FA map and the skeleton FA mask in *green*. **b)** The graph displays statistical differences between educational groups when mean FA values from the WM tracks reported above were extracted (* $F = 40.735$, $p < 0.001$)

Differential age-related effects on GM and WM measures

Age had a negative impact in global measures of both GM and WM although the effect was only significant for the second type of tissue (GM, $r = -0.042$, $p = 0.684$; WM, $r = -0.344$, $p = 0.001$). We next tested the hypothesis that the associations observed between education, cognition and brain integrity measures might be related to a differential

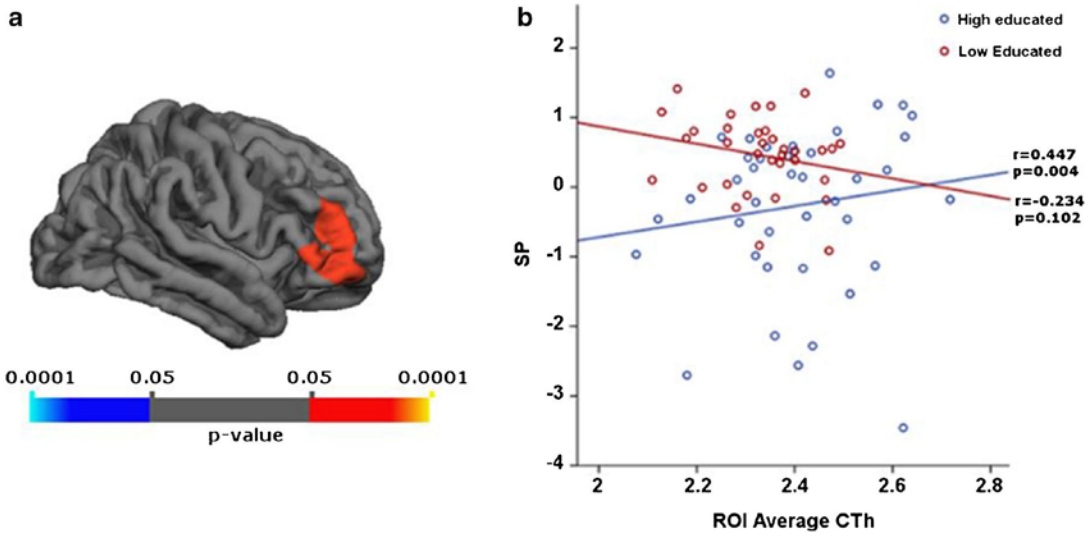

Fig. 3 CTh map of education interaction in SP. **a)** Map of significant group-interaction cluster in the correlation between CTh and SP. Only clusters surviving FWE multiple comparison correction with a final cluster-wise $p < 0.05$ are considered. **b)** Scatterplot showing SP factor and average CTh at the region of interest (ROI). Low educated subjects are shown in red and high educated are shown in *blue*

Table 2 Correlations with FA, education, memory and SP

	FAmask					
	All subjects		High educated		Low educated	
Education	$r = -0.479$		$r = -0.150$		$r = -0.048$	
	$p = 0.000$		$p = 0.336$		$p = 0.734$	
Memory	$r = -0.254$	$r = 0.113^a$	$r = -0.174$	$r = -0.148^a$	$r = -0.137$	$r = -0.159^a$
	$p = 0.012$*	$p = 0.269$	$p = 0.266$	$p = 0.350$	$p = 0.327$	$p = 0.363$
SP	$r = -0.068$	$r = 0.226^a$	$r = -0.071$	$r = -0.043^a$	$r = 0.159$	$r = 0.210^a$
	$p = 0.506$	$p = 0.026$*	$p = 0.653$	$p = 0.789$	$p = 0.257$	$p = 0.134$

[a]Age, gender and years of education as covariates

*Significant correlation at the level of $p < 0.05$

tissue-specific impact of chronological age on brain integrity. We observed a strong negative correlation between the *FAmask* and age ($r = -0.332$, p = 0.001), while the correlation between *CThcluster* and age was not significant ($r = 0.113$, $p = 0.273$; Fig. 5a). When we compared the age-related slopes of the two measures (i.e., WM and GM), we observed a highly differential impact (F = 56.020, $p < 0.001$). We further investigated whether the effects of advancing age on brain integrity measures for our group differed between high and low education groups. First, both groups exhibited a significant negative association between age and WM integrity measures (high educated elders, $r = -0.403$, $p = 0.007$; low educated elders, $r = -0.444$, p = 0.001) but not for GM (high educated, $r = 0.233$, $p = 0.132$; low educated, $r = 0.058$, $p = 0.678$; Fig. 5b). Further, the difference in the slope between GM and WM remained when the two groups were considered separately (high educated, F = 8.493, p < 0.001; low educated F = 71.235, p < 0.001; Fig. 5b). Moreover, when we compared the slopes within each tissue type for education groups we observed differences for both CTh and FA (*CThcluster*: F = 4.795, $p = 0.010$ and *FAmask*: F = 25.406, p < 0.001). These results indicate that the negative associations observed between age and loss of WM integrity and the positive associations observed for CTh were of greater magnitude in the highly educated group.

Vascular risk factors comparison

The results of the mediation analysis obtained for the associations between education, memory and WM integrity indicate that high CR elders in our sample may be able to tolerate a greater loss of white matter integrity and still score high on memory performance. Therefore, we investigated whether these observations, which suggest that CR may operate through a compensatory mechanism, might be related to a greater proportion of 'at risk' individuals amongst our highly educated cognitive sample. As the main anatomical substrate of interest accounting for such results was WM integrity, we focused on the comparison of classic 'cerebrovascular risk' measures and the rates of white matter hyperintensity between educational groups. As can be seen in Table 3, the highly educated group did not present higher scores for, type 2 diabetes, dyslipidemia or BMI, and did not differ in terms of WM burden. For hypertension we observed higher proportion of this diagnosis amongst highly educated old adults, albeit the difference was not statistically significant.

Discussion

In the present study we used MRI-derived CTh and DTI analyses in a group of elders to investigate their associations with

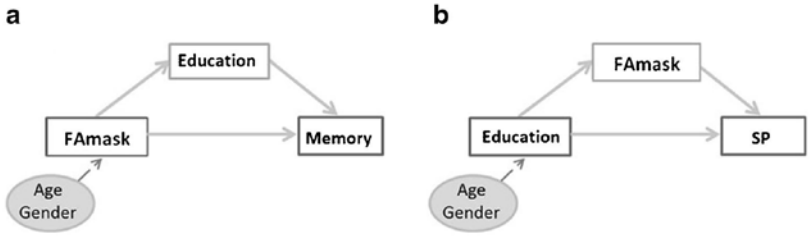

Fig. 4 Valid models after the bootstrapping analysis representation, for both mediation models. We introduced age and gender as covariates. **a**) The memory model indicates that education mediates the effect of FA on memory. **b**) This model indicates that FA mediates the effect of education on SP

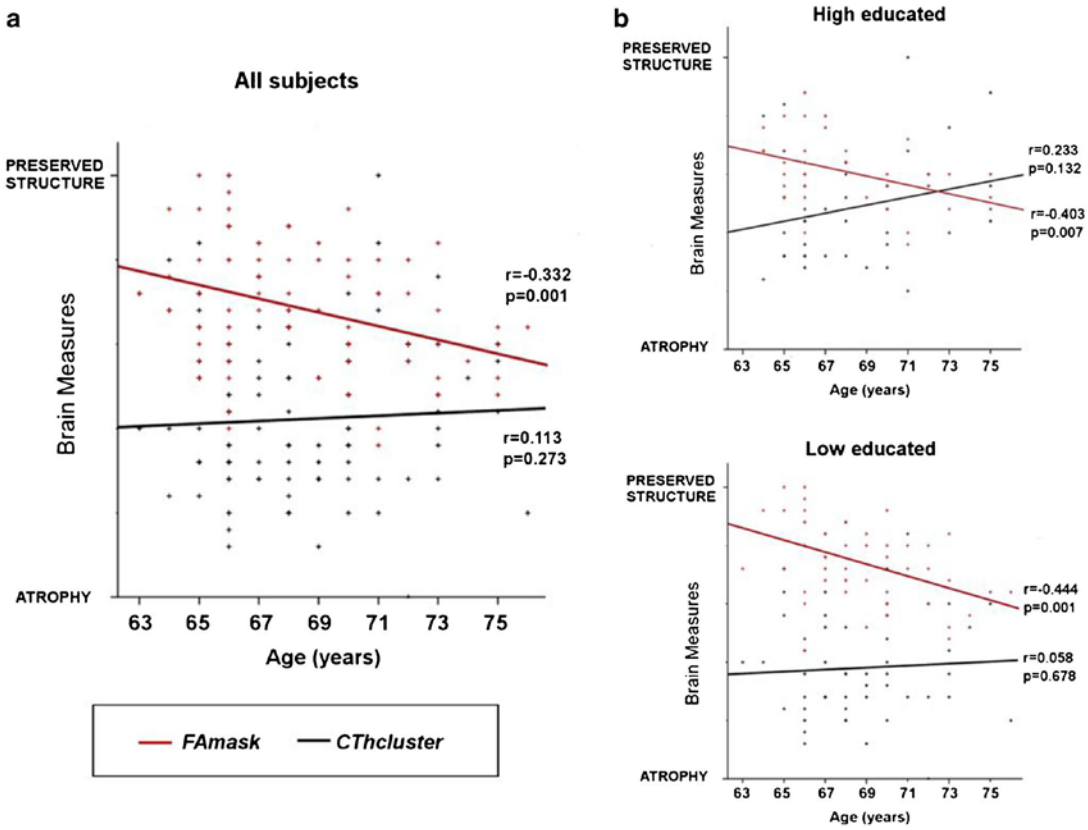

Fig. 5 Effects of advancing age on GM and WM measures. **a)** Scatterplots showing the cluster measures impacted by age. Scaling factors were calculated for *FAmask* (shown in *red*) and *CThcluster* (shown in *black*). **b)** Scatterplots taking into account high and low educated groups separately

declarative memory, SP, and years of education. High education was strongly associated with better neuropsychological performance and with thicker cortex in frontal lobe areas. With DTI, we found reduced FA values in high-educated subjects compared with their low educated peers. Further, there was a negative correlation between FA and memory. The mediation models suggested that education accounted for the negative impact of FA on memory performance, while FA mediated the effect between education and SP. Advancing age had an impact on WM integrity but not on CTh ratings. Furthermore, CR impacted the age x GM and WM correlation slopes in the sense that stronger positive and negative associations respectively were observed for the high educated group. Finally, we observed that our results could not be accounted

Table 3 WMHV and vascular risk factors

	All subjects $n = 100$	High Educated $n = 45$	Low Educated $n = 55$	p-value
WMHV	9.26 ± 9.09	10.64 ± 9.72	8.15 ± 8.45	0.183
Hypertension (Y/N)	52/48	28/17	24/31	0.064[a]
Type 2 Diabetes (Y/N)	5/95	4/41	1/54	0.107[a]
Dyslipidemia (Y/N)	44/76	20/25	24/31	0.236[a]
BMI	26.58 ± 3.79	26.25 ± 3.81	26.86 ± 3.79	0.427

Data are presented as mean ± standard deviation

WMHV (White Matter Hyperintensity Volumes)

BMI (Body Mass Index)

[a] p-values were obtained by χ^2 test (categorical data)

for by significantly increased ratings of variables that typically reflect cerebrovascular risk factors in this group.

The positive associations between education and memory/SP performance observed in our study corroborate the prediction of a cognitive advantage conferred by CR amongst healthy populations (Stern 2002) and the empirical evidence from previous reports in the CR field. A recent meta-analysis by Opdebeeck et al. (2016), concluded that educational level, used either alone or in combination with other CR proxies, showed a smaller variation in its relation with cognitive abilities than occupational status or complex cognitive activities. Further, a recent study examined which kind of information derived from educational measures was most closely related to a lower risk of dementia, and concluded that the stratification of subjects as high vs low educated elders (i.e. tertiary vs non tertiary education) had the strongest associations (Then et al. 2016), providing support to the similar approach undertaken in our study.

As regards the impact of education on brain integrity measures, we observed that more educated subjects exhibit greater regional CTh, particularly in frontal lobe-related regions. This observation is in agreement with previous studies in cognitively intact individuals (reviewed in Bartrés-Faz and Arenaza-Urquijo 2011; Liu et al. 2012; Kim et al. 2015), which have included findings of specific CR x frontal lobe measure associations (Bartrés-Faz et al. 2009; Arenaza-Urquijo et al. 2013a). It should be noted, however, that one study (Chételat et al. 2010) found higher memory performance and enlarged temporal regional GM volumes including the hippocampus in higher Pittsburgh Compound-B (PIB) vs low-PIB healthy elders, indicating that enlarged regional brain outcomes obtained by MRI could reflect higher brain reserve, particularly amongst preclinical AD cases. Nevertheless, in that study the findings were not related to subjects' educational status. In fact, subsequent reports testing for the associations between CR proxies (such as education) and brain measures tended to report higher than expected cortical atrophy (Arenaza-Urquijo et al. 2013b) or reduced metabolism (Ewers et al. 2013) amongst preclinical AD cases with high CR. Conversely, in a recent study of cognitive normal elders in which the presence of pathological amyloid deposition was explicitly excluded, positive associations between education and GM volumes were observed including medial frontal areas such as the anterior cingulate (Arenaza-Urquijo et al. 2013a). Consequently, the directionality and the topographical distribution of our observations regarding associations between education and CTh measures are in accordance with the patterns observed in samples of cognitively and 'biologically' normal elders. Earlier studies reported associations between CR estimates and other regions such as the hippocampus (Valenzuela et al. 2008), which were not observed here. However, more recent reports indicate that occupational activity (Suo et al. 2012), parental socioeconomic status (Staff et al. 2012; Persson et al. 2016) or physical activity rates (Okonkwo et al. 2014) may reflect the type of reserve proxies that are most closely associated with volumetric preservation of the medial temporal lobe region. Further longitudinal studies are necessary to confirm whether different proxies of cognitive reserve are differentially related to the structural maintenance of particular brain areas in normal aging.

In contrast to the GM findings, our DTI analyses revealed a clear negative association between years of education and FA ratings in widespread tracts, affecting not only regions of the frontal lobe but also more posterior areas, in a pattern reminiscent of age-related effects on WM integrity (Giorgio et al. 2010; Bennett and Madden 2014). Early observations suggested that high educated people exhibited greater tolerance against WM changes (Dufouil et al. 2003; Nebes et al. 2006). In contrast, a previous study investigating the correlates of years of education reported relatively small but positive associations with WM volumes (Foubert-Samier et al. 2012), but findings from our group in a sample of cognitively preserved elders and using FA indicated that higher CR estimates were related to lower WM integrity in the anterior corpus callosum (Arenaza-Urquijo et al. 2011). Finally, a recent investigation using quantitative tractography amongst cognitively normal old adults, reported that higher rates of CR attenuate the associations between reduced fiber bundle length (a parameter thought to reflect axonal loss, demyelination and gliosis and associated with advancing age) in specific association tracts and cognitive performance (Baker et al. 2016). The findings of the present study, as regards the directionality of the associations between CR estimates and WM integrity, are aligned with these latter studies studying WM integrity based on DTI parameters.

According to recent conceptualizations of the reserve theory, the presence of neuropathological markers, even at preclinical stages, would predict the emergence of compensatory mechanisms (Bartrés-Faz and Arenaza-Urquijo 2011; Arenaza-Urquijo et al. 2015). Hence, a possible explanation for our findings concerning the negative correlations between reserve estimates and FA could be that a larger proportion of individuals amongst our highly educated elders are at 'risk stages' for potential clinical conditions affecting WM integrity. A first candidate could be the positivity for Aβ deposition, which has been shown to compromise WM, particularly in individuals showing WMH (Chao et al. 2013). However, since the principal effect of amyloid is still expected to occur in GM areas, the positive associations reported above between CR and CTh measures in the highly educated group make this alternative unlikely. A second possibility, arguably more plausible with regards to WM, is that our educational groups differ in terms of other typical risk factors, particularly those related to cerebrovascular conditions. First, it should be noted that when evaluated according to population parameters, the considered values for hypertension, diabetes, dyslipidemia and

obesity in both groups concurred with those described for populations with similar demographic characteristics (Marrugat et al. 2003; Grau et al. 2011). Hence, despite none of our study groups can seem to represent a particularly 'high vascular risk group', it should be noted that higher educated sample showed a trend towards higher rates of hypertension, and hence the effect of this variable in interaction with CR and WM integrity should be investigated in further studies.

In our study, it cannot be completely ruled out that lower FA values in high educated, high performing subjects may reflect reorganizations of some sort, such as increases in axon diameter or the development of crossed fibers reflecting rearrangements (Johansen-Berg et al. 2012) linked to a more enriched lifetime experience, rather than to age-related losses of integrity. In this regard a recent study found that greater individual gains in memory performance across 2 years were related to linear reductions in FA (Bender et al. 2015). However, due to the cross-sectional nature of our data, these assumptions should be considered as speculative at this stage, and further studies including younger samples and longitudinal designs are warranted to clarify these issues.

We found that higher educated elders performed better both on memory and SP composite cognitive scores. However, the effects seem to be subtended by distinct mechanisms in view of the associated MRI correlates and the impact of education. In memory performance, we did not find any direct correlation with CTh measures. Although we do not have a definitive explanation for this lack of association, it is likely that the relative narrow age-range of our sample and/or the low variability in memory performance (the main inclusion criterion being that all participants should present normal memory functioning) accounted for this lack of effect. Other imaging parameters, in particular functional measures, might have revealed more clear associations. Previously, we have reported (Bartrés-Faz et al. 2009; Solé-Padullés et al. 2009; Bosch et al. 2010) that fMRI allows the investigation of concepts such as neural efficiency imbedded within the CR theory (Barulli and Stern 2013), indicating high sensitivity of functional based measures to reveal reorganization of functional structures linked to CR. Hence, future studies should address how fMRI information can contribute to our better understanding regards the coexistence of compensatory and neuroprotective mechanisms highlighted in this study.

In contrast to memory, the cognitive advantage of high education in SP was directly related to greater CTh in prefrontal areas, whereas no significant association was observed for the lower educated group. We also observed direct positive correlations with FA, underlining the importance of WM integrity for tasks involving speed of processing (Kerchner et al. 2012; Sasson et al. 2013; Salthouse 2000; Madden et al. 2004). Specifically, some of the tracts identified here, including the anterior thalamic radiation, the inferior longitudinal fasciculus, have been recently reported to show a positive covariation with SP performance across adulthood (Gazes et al. 2016). Furthermore, our mediation analyses confirmed FA as a mediator of the association between education and SP. This result indicates that preservation of WM microstructure plays an important role in explaining the high SP performance among highly educated elders. Altogether, then, we found that SP in highly educated elders is sustained by positive associations with frontal lobe CTh and with FA.

Our quantitative multimodal analysis revealed a stronger age-related decline in FA compared with CTh. WM alterations have been extensively reported (Laukka et al. 2013; Salat 2011; Bennett et al. 2010) and the fast FA decline in this age range studied is in accordance with prior findings (Westlye et al. 2010; Sala et al. 2012; Sexton et al. 2014). The lack of a CTh reduction is supported by other authors (Storsve et al. 2014; Hogstrom et al. 2013) who propose that the CTh progression in the life span shows a dynamic relationship with surface area changes. Therefore, the regional specificity in changes associated with aging (Fjell et al. 2014a), in which CTh describes linear and non-linear degeneration and damage repair process, may even result in age-related regional increases (Fjell et al. 2014b) may have prevented to see clear age x GM associations in our study as CTh was derived from a whole-brain measure.

To date, few studies in the field of cognitive reserve in adult samples have explicitly explored whether advancing age moderates the impact of CR estimates on cognitive and brain measures. Studies of the associations between education (Almeida et al. 2015) and physical activity (Okonkwo et al. 2014) have indicated that these CR-related measures attenuate the impact of some AD related-biomarker measures, particularly in older individuals. However, other studies have found that the effect of education in measures such as brain metabolism is similar when study groups are separated into 'younger' and 'older' counterparts (Arenaza-Urquijo et al. 2015). Furthermore, recent longitudinal data indicate that the effects of lifestyle enrichment variables (such as education/occupation) is minimal on the age-related rate of change of typical neurodegenerative markers, or may be seen only for particular subgroups and measures (i.e., APOE ε4 allele carriers for Aβ and glucose metabolism, Vemuri et al. 2016). Despite the cross-sectional nature of our study, our results indicate that within the studied age-range, the slopes between advancing chronological age and indicators of grain integrity were modulated by CR estimates. Hence, though further longitudinal studies are required, our results amongst cognitive preserve elders suggest the existence of a putative neuroprotective effect of education on frontal-lobe GM integrity in parallel to a compensatory role in the maintenance of memory performance in the context of a greater age impact on WM integrity. Finally, it should be noted that we have used the term 'neuroprotection' to account for evidences of thicker cortices in the high educated group as well as to observations of significant age-related slopes of this

measure between low and high educated samples. However, in our study we did not find an overall effect of advancing chronological age being significantly associated with increasing cortical thinning. This is reminiscent with the abovementioned study by Arenaza-Urquijo et al. (2013a) were positive associations between education and gray matter volume in the anterior cingulate cortex were observed, and as highlighted in that report, this is a structure where no aging effects or even positive aging effects had been reported in the literature. Therefore and within this context, our use of the 'neuroprotection' term as regards GM findings, does not refer that CR protects against atrophy in a region particularly affected by age, but rather to the fact that high CR elders have more preserved volumes of this structure, related to their optimal cognitive performance.

The main limitations of our study are to do with the sample and the study design. First, our sample was relatively narrow as regards the age-range included, and it was also homogeneous as regards cognitive performance. Second, using a cross-sectional design we cannot fully determine the causality of the associations between education and GM and WM as regards putative evidences of neuroproteciton or compensation, as well that of our results indicating that CR measures impact differently the trajectories of age-related structural brain changes. The true intra-individual trajectories allowing responding some critical questions suggested above (i.e. when associations between education and brain measures first appeared? and how did they change during the lifespan of our individuals?) can only be revealed using longitudinal data. Furthermore, despite we did not observe a significant effect of the APOE polymorphism in our results (data not shown) further studies should be undertaken with larger samples of individuals to have a representative group of APOE ε4 carriers for statistical analyses. In addition, we cannot completely rule out the possibility that the negative associations between WM metrics and education are due to the fact that some of the subjects were 'at risk' for disease or in preclinical conditions, even though the direction of the positive findings for the CTh analysis in the same sample makes this rather unlikely. For instance, CR may increase «positive brain features» (i.e. CTh) and at simultaneously reduce pathological biomarkers (i.e. Aβ). These might postpone the development of symptoms associated with brain diseases. So, our results are probably highly determined by the balance in our particular sample between «true healthy older adults» vs «compensating healthy older adults». The fact that neurodegenerative diseases are often characterized by a long asymptomatic phase makes this even more complex. Finally, we investigated the associations between education, as a unique proxy of reserve, and cortical thickness measures. While the association between gray matter volumes and education has been reported in a number of studies of the previous literature (reviewed in Bartrés-Faz and Arenaza-Urquijo 2011), specific associations between education and gray matter thickness have been less frequently addressed (but see Liu et al. 2012 and Kim et al. 2015 reported above). Notably, there is now direct evidence in middle aged persons, of mapping onto distinct cortical areas of gray thickness when different proxies of reserve including education, estimations of premorbid IQ and composite compounds of CR (comprising rates of social, dietary, leisure and physical activity related lifestyles) are considered separately (Ferreira et al. 2016). Hence, further research is needed to test if proxies other than education map to distinct associations of reserve in cortical thickness amongst healthy elders samples.

Despite these limitations, our study offers novel and relevant results within the field of CR in older populations. First, we provide empirical evidence to support previous suggestions (Arenaza-Urquijo et al. 2013a) that both neuroprotective and compensatory mechanisms linked to reserve are simultaneously at work amongst cognitively normal older adults. Second, our data indicate that a greater capacity to tolerate brain changes while maintaining cognitive performance (i.e., the compensatory mechanism) is not only characteristic of patient populations or even of groups of individuals representing 'at risk' or preclinical conditions, but may instead be observed in healthy aging in response to a direct but differential impact of chronological age on distinct anatomical substrates. Finally, we found evidence suggesting that the positive and compensatory effects associated with high reserve ratings reported in the present study may be accentuated as the age advances in the study groups.

Compliance with ethical standards

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Conflict of interest Lídia Vaqué-Alcázar declares that she has no conflict of interest. Roser Sala-Llonch declares that she has no conflict of interest. Cinta Valls declares that she has no conflict of interest. Dídac Vidal-Piñeiro declares that he has no conflict of interest. Sara Fernández-Cabello declares that she has no conflict of interest. Núria Bargalló declares that she has no conflict of interest. Emilio Ros declares that he has no conflict of interest. David Bartrés-Faz declares that he has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

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Study 2

Bartrés-Faz, D., González-Escamilla, G., **Vaqué-Alcázar, L.**, Abellaneda-Pérez, K., Valls-Pedret, C., Ros, E. & Grothe, M. J. (2019). Characterizing the molecular architecture of cortical regions associated with high educational attainment in older individuals. *The Journal of Neuroscience, 39*(23), 4566–4575.

Behavioral/Cognitive

Characterizing the Molecular Architecture of Cortical Regions Associated with High Educational Attainment in Older Individuals

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Neuroimaging investigations have revealed interindividual variations in anatomy, metabolism, activity, and connectivity of specific cortical association areas through which years of education (YoE), as a common proxy of cognitive reserve, may operate in the face of age- or pathology-associated brain changes. However, the associated molecular properties of YoE-related brain regions and the biological pathways involved remain poorly understood. In the present study we first identified brain areas that showed an association between cortical thickness and YoE among 122 cognitively healthy older human individuals (87 female). We subsequently characterized molecular properties of these regions by studying brain-wide microarray measurements of regional gene expression. In accordance with previous studies, we observed that YoE were associated with higher cortical thickness in medial prefrontal, anterior cingulate, and orbitofrontal areas. Compared with the rest of the cortex, these regions exhibited a distinct gene expression profile characterized by relative upregulation of gene sets implicated in ionotropic and metabotropic neurotransmission as well as activation of immune response. Our genome-wide expression profile analysis of YoE-related brain regions points to distinct molecular pathways that may underlie a higher capacity for plastic changes in response to lifetime intellectual enrichment and potentially also a higher resilience to age-related pathologic brain changes.

Key words: cognitive reserve; cortical thickness; gene expression; immune response; synaptic transmission

Significance Statement

We combined a neuroimaging-based analysis with a transcriptome-wide gene expression approach to investigate the molecular-functional properties of cortical regions associated with educational attainment, as a commonly used proxy for cognitive reserve, in older individuals. The strongest association with education was observed in specific areas of the medial prefrontal cortex, and these areas exhibited a distinct gene expression profile characterized by relative upregulation of gene sets implicated in neurotransmission and immune responses. These findings complement previous neuroimaging studies in the field and point to novel biological pathways that may mediate the beneficial effects of high educational attainment on adaptability to cope with, or prevent, age-related brain changes. The identified genes and pathways now warrant further exploration in mechanistic studies.

Introduction

The concept of cognitive reserve (CR), most commonly estimated through the use of "proxies" such as years of education (YoE; Nucci et al., 2012), addresses interindividual differences in the adaptability and susceptibility of cognitive abilities or day-to-

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day function to brain aging, pathology, or insult (Stern et al., 2018b). Neuroimaging studies of cognitively normal older individuals have revealed positive associations between CR proxies and measures of brain integrity (i.e., regional volumes or cortical thickness; for review, see Bartrés-Faz and Arenaza-Urquijo, 2011). These associations are primarily observed in limbic, paralimbic, and heteromodal cortical association areas, particularly within the prefrontal cortex, and have typically been interpreted as reflecting a higher capacity for plastic change, which may increase the adaptability of higher-educated individuals to age- or disease-related brain changes (Valenzuela et al., 2008; Foubert-Samier et al., 2012; Arenaza-Urquijo et al., 2013, 2017; Lee et al., 2016; Vaqué-Alcázar et al., 2017). This interpretation is corroborated by functional imaging studies that have demonstrated differential patterns of brain activation in these latter regions that are suggestive of greater neural efficiency and capacity in higher-educated individuals (Bartrés-Faz et al., 2009; Solé-Padullés et al., 2009; Fernández-Cabello et al., 2016; Stern et al., 2018a) and may reflect greater capacity for plastic change associated to the CR concept (Bartrés-Faz and Arenaza-Urquijo, 2011).

The involvement of distinct brain areas in mediating the positive effects of CR is likely to be related to their specific anatomo-functional properties. For example, although the prefrontal cortex is highly affected by the aging process in terms of brain atrophy (Hedden and Gabrieli, 2004; Fjell et al., 2014), the functional reorganization capacities of both its lateral and medial sections have been related to maintenance of cognitive function (Cabeza et al., 2002; Davis et al., 2008). Also, parts of the lateral prefrontal cortex exert a regulatory role on the functional organization of other networks strongly involved in cognitive processes (Chen et al., 2013; Spreng et al., 2013; Franzmeier et al., 2017, 2018). Within the medial parts of the frontal lobe, the anterior cingulate cortex (ACC) has been one of the most frequently involved brain areas in previous CR studies (see references in the previous paragraph), and this paralimbic region is known to exert an integrative role between limbic and associative cortices, promoting goal-directed behaviors and executive cognitive processes (Devinsky et al., 1995; Carter et al., 1999; Ridderinkhof et al., 2004). At the cellular level, both the ACC region and the dorsolateral prefrontal cortex (Fajardo et al., 2008) contain a characteristic type of large spindle-shaped neuron called von Economo neurons (Allman et al., 2011; Butti et al., 2013) that have been linked to superior memory capacity in old age (Gefen et al., 2015).

Although previous neuroimaging investigations identified heteromodal and paralimbic brain regions and associated networks as likely substrates of the "neural implementation" of CR, our understanding of the associated biological pathways through which they may operate to sustain the mechanisms predicated by the CR theory (i.e., efficiency, capacity, flexibility, and compensation; Stern et al., 2018b) are poorly understood. Former investigations have shown that differential gene expression profiles across brain regions are tightly associated with differences in morphometric (Romero-Garcia et al., 2018b; Seidlitz et al., 2018; Shin et al., 2018) and functional (Hawrylycz et al., 2015; Richiardi et al., 2015; Wang et al., 2015) brain tissue characteristics, as well as differential vulnerability to disease (Romme et al., 2017; Grothe et al., 2018; Romero-Garcia et al., 2018a). Here we aimed to further delineate molecular-functional properties of selective cortical regions associated to YoE among cognitively healthy older individuals. First, through an magnetic resonance image (MRI)-based cortical thickness analysis, we identified brain regions associated to high versus low educational attainment. Subsequently we investigated the transcriptional architecture of these regions using brain-wide regional gene expression data (Hawrylycz et al., 2012) in combination with gene set enrichment analysis (Subramanian et al., 2005; Grothe et al., 2018).

Materials and Methods

Participants. One hundred and twenty-two normal functioning older human participants (87 female, 35 male, mean age 68.2 years) were enrolled from the *Fundació Institut Català de l'Envelliment*. All volunteers had normal cognitive function, with Mini-Mental State Examination test (MMSE) scores ≥25 and performances ≥1.5 SD according to normative scores in a neuropsychological assessment (i.e., they did not fulfill cognitive criteria for mild cognitive impairment; Petersen and Morris, 2005). The study was approved by the local ethics committee and was conducted in accordance with the Helsinki Declaration. Written informed consent was obtained from each participant before enrollment in the study.

Experimental design. For each subject we recorded the total years of completed formal education and stratified the sample according to high or low education using a cutoff point of 15 years of education as in our previous report (Vaqué-Alcázar et al., 2017). We used this cutoff on the basis of a recent study examining which kind of information derived from educational measures was most closely related to a lower risk of dementia and concluding that the stratification of subjects into high versus low educational groups (i.e., tertiary vs non-tertiary education) showed the strongest associations (Then et al., 2016). A further reason to use this dichotomous stratification of our sample rather than a continuous approach was based on the frequency distribution of educational years. Hence, in accordance with the National Spanish education stages at the time our older participants completed the education, educational years were bimodally distributed, with a concentration of cases ~8 years of completed education and a second major grouping at 15 years. Using the dichotomous classification with a cutoff point of 15 years, 48 participants were classified as high-educated and 74 as low-educated (see group comparisons in Results).

Neuropsychological assessment. Applying a similar approach used in previous studies (Vidal-Piñeiro et al., 2014; Vaqué-Alcázar et al., 2017) we used principal components analysis (PCA) based on the dataset of the current study to create a composite scale representing separate declarative memory and "frontal lobe function" domains. The following cognitive tests were used to estimate the memory factor: Rey Auditory Verbal Learning Test total learning and delayed recall (RAVLT-total and RAVLT-delayed, $N = 94$ cases) and Buschke test (total and delayed scores, $N = 28$ cases). Previously, the total learning and delayed scores from both memory tests were transformed to a comparable metric by the percentage of maximum possible ("POMP") method (Moeller, 2015). On the other hand, the tests contributing to the frontal lobe function domain included the Trail Making Tests (TMT B-A), the Symbol Digit Modalities Test, and the phonemic (sum of letters F, A, S, 1 min each, $N = 94$; and letters P, M, R, $N = 28$) and semantic (animals, 1 min) fluency tests. Both factors were calculated in a way that higher scores on the factor indicated better cognitive performances.

MRI acquisition. MRI for each participant were acquired in a Siemens Magnetom Trio Tim syngo 3-T system. High-resolution T1-weighted structural images were obtained with a magnetization-prepared rapid acquisition gradient echo 3-dimensional protocol (repetition time = 2300 ms, echo time = 3 ms, inversion time = 900 ms, field-of-view = 244 mm, and 1 mm isotropic voxel).

Cortical thickness analyses. Cortical surface reconstruction and calculation of cortical thickness (CTh) from the structural T1-weighted images were performed using FreeSurfer v5.1 software package (http://surfer.nmr.mgh.harvard.edu). In summary, the procedures performed

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by the main FreeSurfer pipeline include removal of non-brain data, intensity normalization (Sled et al., 1998), tessellation of the gray matter/white matter boundary, automated topology correction (Ségonne et al., 2007), and accurate surface deformation to identify tissue borders (Dale et al., 1999). CTh is then calculated as the distance between the white and gray matter surfaces at each vertex of the reconstructed cortical mantle (Fischl and Dale, 2000). Individual results were inspected visually to ensure accuracy of registration, skull stripping, segmentation, and cortical surface reconstruction. Manual editing of the reconstructed surfaces was required in 15 subjects and the threshold used for brain extraction was changed in 31 other subjects. The CTh maps were smoothed using a Gaussian kernel of 15 mm full-width at half-maximum. We then evaluated the statistical analysis using voxelwise general linear models (GLMs) to study group differences in CTh between high and low YoE subjects, adjusted by age and gender (Vaqué-Alcázar et al., 2017). Image was corrected for familywise error using a Monte Carlo null-Z simulation, with 10,000 repetitions. Both the initial vertex and the cluster thresholds were set to $p < 0.05$.

Statistical analysis of group differences in sociodemographic and cognitive characteristics. Data analyses were performed using SPSS v24 (IBM). Demographic and cognitive data were described as mean ± SD. For quantitative data, we evaluated differences between groups using two-sample t tests. For categorical variables, we assessed χ^2 tests. In addition, when the normal distribution assumptions were not met as indicated by the Shapiro–Wilk test, the Mann–Whitney's U test was used. Finally, because age and gender showed differences between the YoE groups (see results section) the MMSE scores and the final cognitive factors obtained from the PCA analyses (see Neuropsychological assessment) were regressed by age and gender. Because there were differences in the type of memory and verbal fluency tests administered between individuals, this "group test" variable was also introduced as a regressor to obtain the final memory and frontal lobe domain values. For all the analyses results were considered statistically significant at $p < 0.05$.

Cortical gene expression data. The transcriptome data from the Allen Institute Human Brain Atlas (http://human.brain-map.org/; Hawrylycz et al., 2012, 2015) was used in the present study. In brief, complete microarray gene expression datasets from a total of 3702 regional tissue samples from postmortem brain tissue of six individuals were mapped to anatomical brain locations in corresponding structural MRI scans; these steps were performed by the Allen Institute (Shen et al., 2012). The produced raw expression values were then mean averaged across probes to obtain single expression values for each gene, resulting in 20,737 gene expression values per tissue sample. From the 3702 brain samples available in the atlas, we first discarded samples not belonging to the cortex according to their anatomical annotation ($N = 1752$). Further, because of the lack of right hemisphere gene expression data for most of the donors (Hawrylycz et al., 2012), we only focused on the left hemisphere in further analyses, leaving a total of 1452 cortical tissue samples. Next, for each donor the individual tissue sample coordinates were spatially mapped to a cortical surface reconstruction obtained by processing the donors' MRI images in FreeSurfer (data available from Romero-Garcia et al., 2018b). The YoE-related region-of-interest (ROI) together with a reference ROI covering the rest of the cortex were then mapped from the FreeSurfer template space used for cortical thickness analysis to the individual surface reconstruction of each donor's brain. The surface-based ROIs of each donor's brain were then transformed into volumetric parcellations and extended 2 mm into the subjacent white matter to account for possible registration misalignments. Individual tissue samples were then mapped to their nearest point on the cortex, resulting in a total of 44 tissue samples being mapped to the YoE-related ROI and 1356 samples to the cortical reference region. Fifty-two of the 1452 initial cortical samples (3.6%) were discarded because their distance to the nearest cortical voxel was larger than 2 mm. Finally, for each ROI the expression values were averaged across all tissue samples to obtain median expression values for each gene (French and Paus, 2015; Romero-Garcia et al., 2018b).

Gene expression profile of YoE-related brain regions and gene set enrichment analysis. After identification of brain regions with higher cortical thickness in the higher-education group (YoE-related areas; see Results), we quantified the difference in gene expression between these areas and the background expression levels in the rest of the cortex, calculating a delta-score for each gene (Freer et al., 2016):

$$\Delta_g = \bar{E}'_{g,YoE} - \bar{E}'_{g,rest},$$

here, for each gene (g) the median gene expression within the YoE-related brain area ($E'_{g,\mathrm{YoE}}$) was contrasted to the median expression in the rest of the cortex ($E'_{g,\mathrm{rest}}$). In an additional sensitivity analysis we calculated delta scores using an alternative reference region that excluded all cortical regions with positive (i.e., high > low YoE) β estimates in the GLM assessing cortical thickness differences between high and low YoE groups, regardless of their statistical significance. This spatially reduced reference region covered a total of 814 tissue samples with gene expression data (compared with the 1354 samples in the original reference region covering the entire rest of the cortex). The rationale behind this sensitivity analysis is that a reference region based on the entire rest of the cortex may include gene expression signal from some regions that show marginal, subthreshold associations with YoE, thus possibly blurring gene expression differences of YoE-related areas with cortical background levels.

The resulting delta scores were then ranked in descending order, where the top (positive values) and bottom parts (negative values) of the ranked list contain the genes with relative overexpression or underexpression, respectively, in YoE-related areas compared with the rest of the cortex. Instead of examining single genes at the extremes of the ranked list, we then used gene set enrichment analysis (GSEA; http://software.broadinstitute.org/gsea/index.jsp, software version 3.0; Subramanian et al., 2005) to more broadly explore differentially expressed functional pathways. Because to our knowledge this represents the first study using the present approach in this specific field, we used the GSEA approach to characterize molecular properties of the YoE-related brain regions in an exploratory manner. Instead of focusing on one or a few predefined genes based on a priori hypotheses, the enrichment analysis helps to uncover general trends in differential gene expression in a more comprehensive manner, while extracting meaningful and interpretable information from the high-throughput microarray data. To identify functional gene sets that are differentially expressed in YoE-related regions in reference to cortical background levels, GSEA determines whether the genes of prespecified functional gene sets, derived from curated gene set databases (see next paragraph), cluster toward one of the extremes (top or bottom) of the ranked list. Using this location information an enrichment score (ES) is calculated for each gene set, reflecting the degree of clustering of the gene set's genes toward the top (positively enriched/overexpressed) or bottom (negatively enriched/underexpressed) of the ranked list. A gene set nominal p value is then created by comparing the ES with a null-distribution obtained after permuting 1000 times the gene set's gene positions in the list and recomputing a new ES at each permutation. To adjust the estimated significance level and account for the independent testing of multiple gene sets, the ES are normalized by the size of the set, obtaining a normalized ES (NES). We then controlled the proportion of false-positives by calculating the false discovery rate (FDR) for every NES (Subramanian et al., 2005).

To increase the power and coverage of our analyses, we included gene sets from multiple independent sources. The gene sets are defined by the common implication of genes in particular biological states or processes, and are retrieved from a reviewed, curated, and annotated repository [the Molecular Signatures Database (MSigDB) v6.0; http://software.broadinstitute.org/gsea/msigdb/index.jsp; Liberzon et al., 2011, 2015]. In the present study, we explored a total of 5429 gene sets, including 497 curated and peer-reviewed gene sets of functional pathways derived from the Reactome database (http://reactome.org/), 177 from the Kyoto Encyclopedia of Genes and Genomes (https://www.genome.jp/kegg/), 50 from the MSigDB hallmark collection (Liberzon et al., 2015), 147 from the BioCarta repository (http://cgap.nci.nih.gov/Pathways/BioCarta_Pathways), and 4558 gene sets that group genes annotated by the same gene ontology term (http://www.geneontology.org/).

Given that statistical inference in GSEA is based on complete functional gene sets, not all of the genes included in a significant gene set are

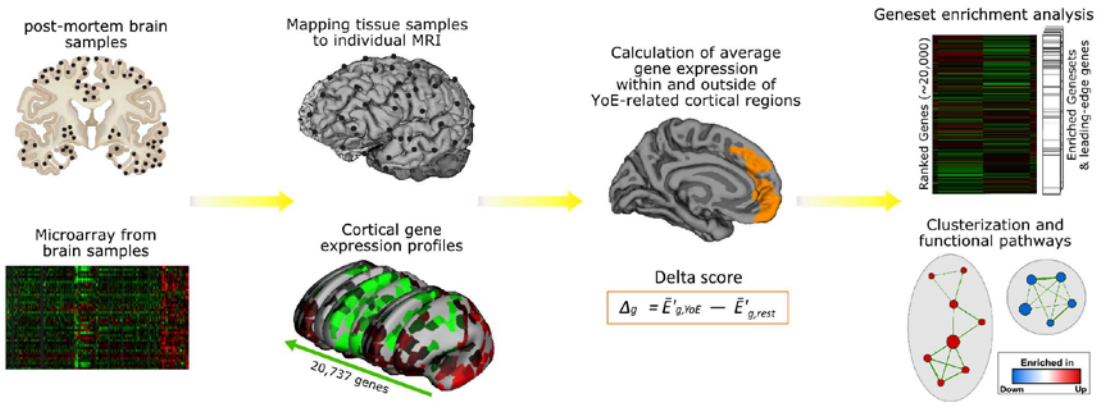

Figure 1. Enrichment analysis workflow. Outline of the processing of gene expression data from regional probe level to enrichment map for the identification of functionally coherent clusters of gene sets. First, regions associated with YoE in healthy older adults are identified. Then, individual tissue sample coordinates are assigned to the nearest point of the reconstructed MRI brain surface, and for each gene the median expression levels within and outside YoE-related cortical areas are calculated and subtracted (delta score). Finally, genes are ranked according to their delta score (differential expression within YoE-related areas), and the ranked list is submitted to GSEA to search for significantly over- or under-represented gene sets in YoE-related areas. Identified gene sets are then clustered and organized into a network layout to identify groupings of functionally related gene sets into overarching functional pathways.

necessarily differentially expressed. A so called leading-edge subset of genes can be identified that primarily accounts for a gene set's enrichment score, and a leading-edge analysis then aims to find commonalities among the identified gene sets by clustering the respective leading-edge subsets (Subramanian et al., 2005). To account for the existing redundancy between the different gene sets and to facilitate interpretation of the enrichment results, functionally coherent gene sets were detected using a clusterization algorithm (Merico et al., 2010; Isserlin et al., 2014), and organized into a network layout ("enrichment map"). In this network, nodes represent gene sets, and their edges or connections represent the genetic overlap (i.e., number of overlapping genes) between pairs of nodes. Each resulting cluster in the network indicates functionally related gene sets implicated in the same overarching biological pathway. The network was created with Enrichment Map v2.0.1 (http://www.baderlab.org/Software/EnrichmentMap) using the GSEA leading-edge subsets as input (FDR $p < 0.05$; combined Jaccard and overlap coefficients cutoff = 0.5; Fig. 1).

Results

Sociodemographic and cognitive differences related to YoE

Table 1 depicts the comparison of high and low YoE groups in terms of sociodemographic and cognitive characteristics. Between-group comparisons revealed that the low-educated individuals were significantly older and there were more low-educated women. There were no age and sex-adjusted MMSE differences between groups, although high YoE older adults outperformed those with low YoE in the composite scores for memory and "frontal lobe" domains. When comparisons within the specific cognitive domains were retested adding the MMSE as a covariate, differences were still observed indicating that the cognitive advantage of higher-educated elders in memory and frontal lobe function is not explained by global cognitive difference.

Identification of YoE-related cortical areas

The vertex-wise CTh analysis identified a large cluster where healthy older adults in the high YoE group exhibited greater CTh than those in the low YoE group (left hemisphere only). The cluster was restricted to areas within the frontal lobe, including parts of the dorsomedial prefrontal cortex (medial parts of the superior frontal gyrus) overlapping with Brodmann areas (BAs) 8, 9, and 32, the ACC (BA 24), frontal pole (BA 10), and orbitofrontal cortex (BA 11; Fig. 2). No brain areas reached significant differences in the opposite direction (i.e., greater thickness in low compared with high YoE groups).

Table 1. Demographic and cognitive differences between groups

	High YoE ($N = 48$)	Low YoE ($N = 74$)	Group differences
Age, y	67.2 ± 3.2	68.9 ± 3.6	$U = 2.850$ $p = 0.005^{a*}$
Gender, F/M	24/24	63/11	$\chi^2 = 17.568$ $p < 0.001^{b*}$
YoE	15.7 ± 1.7	9.0 ± 2.6	$U = 9.480$ $p < 0.001^{a*}$
MMSE	29.6 ± 0.7	29.4 ± 1	$U = 0.656$ $p = 0.512^{a}$
Memory domain	0.39 ± 0.94	−0.25 ± 0.96	$t = 3.122$ $p = 0.002^{c*}$
Frontal lobe domain	0.55 ± 0.66	−0.30 ± 1.02	$t = 4.427$ $p < 0.001^{c*}$

Data are presented as mean ± SD. F/M, Female/male. MMSE represent direct values, Memory and Frontal lobe scores were composite factors calculated using PCA. Statistical comparisons reflect the results after these variables were further adjusted by age, gender, and for the test group variable (see Materials and Methods).[1]

[a]p values were obtained by Mann–Whitney's U test pairwise comparisons (nonparametric data).

[b]p values were obtained by χ^2 test (categorical data).

[c]p values were obtained by two-sample t test.

*Significant differences.

A distinct gene expression profile characteristic of YoE-related areas

GSEA analysis identified 11 gene sets that were positively enriched (FDR < 0.05) in YoE-related areas compared with cortical background levels (Table 2), and the respective enrichment signals were driven by a total of 130 unique leading-edge genes (Table 3).

No significantly negatively enriched gene sets were identified. Network organization of the gene sets clustered by their leading-edge subsets (enrichment map) evidenced strongly overlapping leading-edge genes among some of the gene sets, resulting in a total of six different gene sets/clusters (Fig. 3). The largest cluster included four gene sets implicated in biological events related to

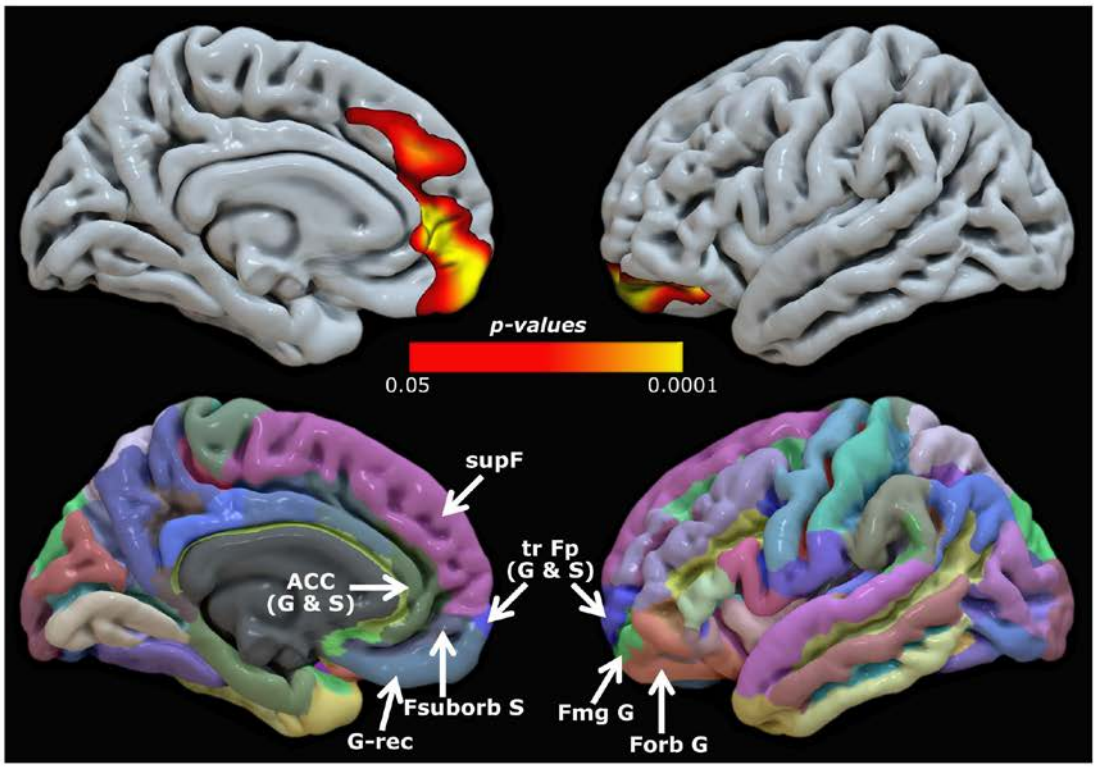

Figure 2. Top, Regions showing greater cortical thickness in healthy older adults with high YoE compared with low YoE individuals. For anatomical reference, overlapping anatomical regions as delineated in the Desikan–Killiany atlas are shown (bottom). G, Gyrus; S, sulcus; trFp, transverse frontopolar; Fmg, fronto-marginal; Forb, frontal orbital (including H-shaped); G-rec, gyrus rectus; Fsuborb, frontal suborbital; supF, superior frontal.

Table 2. GSEA

Gene set	NES	FDR q-val	Size	GSEA ID	URL
1. Ionotropic neurotransmission					
GO_EXTRACELLULAR_LIGAND_GATED_ION_CHANNEL_ACTIVITY	2.159	0.012	25	M18911	http://amigo.geneontology.org/amigo/term/GO:0005230
GO_EXCITATORY_EXTRACELLULAR_LIGAND_GATED_ION_CHANNEL_ACTIVITY	2.204	0.011	28	M18473	http://amigo.geneontology.org/amigo/term/GO:0005231
GO_TRANSMITTER_GATED_CHANNEL_ACTIVITY	1.999	0.048	29	M17983	http://amigo.geneontology.org/amigo/term/GO:0022835
GO_NEUROTRANSMITTER_RECEPTOR_ACTIVITY	2.049	0.031	79	M18363	http://amigo.geneontology.org/amigo/term/GO:0030594
2. NEUROPEPTIDE SIGNALING					
GO_NEUROPEPTIDE_HORMONE_ACTIVITY	2.057	0.033	68	M18214	http://amigo.geneontology.org/amigo/term/GO:0005184
GO_NEUROPEPTIDE_RECEPTOR_BINDING	2.120	0.012	51	M18007	http://amigo.geneontology.org/amigo/term/GO:0071855
3. Transmembrane signaling					
GO_RESPONSE_TO_AMMONIUM_ION	2.037	0.033	26	M10335	http://amigo.geneontology.org/amigo/term/GO:0060359
4. Purinergic signaling					
GO_PURINERGIC_RECEPTOR_SIGNALING_PATHWAY	1.991	0.049	28	M16435	http://amigo.geneontology.org/amigo/term/GO:0035587
5. Immune response					
GO_MYELOID_DENDRITIC_CELL_ACTIVATION	2.124	0.014	55	M16283	http://amigo.geneontology.org/amigo/term/GO:0001773
GO_MACROPHAGE_ACTIVATION	2.133	0.016	75	M13410	http://amigo.geneontology.org/amigo/term/GO:0042116
6. Vesicle trafficking					
GO_CLATHRIN_COATED_VESICLE_MEMBRANE	2.013	0.043	31	M17518	http://amigo.geneontology.org/amigo/term/GO:0030665

GSEA results identifying 11 gene sets belonging to 6 larger functional clusters.

ionotropic neurotransmission, and the large majority of the up-regulated leading-edge genes corresponded to genes coding for components of several different ligand-gated ion channels, including those receptive for glutamate (kainate, AMPA, and NMDA receptors), GABA ($GABA_A$), glycine, acetylcholine (nicotinic receptors), and serotonin ($5\text{-}HT_3$). Other clusters related to neurotransmission included gene sets implicated in neuropeptide (2 gene sets) and G-protein-coupled transmembrane signaling (1 gene set), as well as purinergic signaling (1 gene set). A different category of overexpressed gene sets was represented by a cluster related to immune response (2 gene sets), including several leading-edge genes related to pathogen recognition (e.g.,

Table 3. Leading-edge genes of positively enriched gene sets in education-related cortical areas

Gene	Rank in gene list	Delta-score	Functional group
CHRNA1	2073	0.055	Ionotropic neurotransmission
GABRB3	4013	0.034	Ionotropic neurotransmission
GABRB1	1966	0.057	Ionotropic neurotransmission
CHRNA2	383	0.112	Ionotropic neurotransmission
CHRNA4	3822	0.035	Ionotropic neurotransmission
GRIK5	3748	0.036	Ionotropic neurotransmission
CHRNA6	30	0.228	Ionotropic neurotransmission
GRIK3	2188	0.053	Ionotropic neurotransmission
GRIK4	1311	0.069	Ionotropic neurotransmission
GRIK1	725	0.090	Ionotropic neurotransmission
GRIK2	3892	0.035	Ionotropic neurotransmission
CHRND	2309	0.051	Ionotropic neurotransmission
GLRA2	2534	0.049	Ionotropic neurotransmission
GLRA3	193	0.139	Ionotropic neurotransmission
PTK2B	1126	0.074	Ionotropic neurotransmission
GABRD	3157	0.042	Ionotropic neurotransmission
GRIA4	3367	0.040	Ionotropic neurotransmission
GABRQ	1422	0.067	Ionotropic neurotransmission
CHRNB2	3933	0.035	Ionotropic neurotransmission
GRID2	3549	0.038	Ionotropic neurotransmission
CHRNB3	172	0.145	Ionotropic neurotransmission
GABRA5	2148	0.054	Ionotropic neurotransmission
GABRA3	2268	0.052	Ionotropic neurotransmission
HTR3A	1185	0.072	Ionotropic neurotransmission
HTR3B	107	0.166	Ionotropic neurotransmission
GRIN2C	886	0.083	Ionotropic neurotransmission
GRIN2B	1247	0.071	Ionotropic neurotransmission
GRIN2D	3196	0.041	Ionotropic neurotransmission
GRIN3A	293	0.122	Ionotropic neurotransmission
GRIA3	4508	0.030	Ionotropic neurotransmission
CHRM3	1533	0.064	Ionotropic neurotransmission
CHRM1	695	0.092	Ionotropic neurotransmission
CHRM5	841	0.084	Ionotropic neurotransmission
HRH3	1832	0.059	Ionotropic neurotransmission
DRD4	679	0.092	Ionotropic neurotransmission
UCN	2726	0.046	Neuropeptide signaling
GRP	61	0.194	Neuropeptide signaling
CCK	327	0.118	Neuropeptide signaling
TRH	1531	0.064	Neuropeptide signaling
ADCYAP1	723	0.090	Neuropeptide signaling
CORT	2852	0.045	Neuropeptide signaling
GAL	13	0.300	Neuropeptide signaling
NPY	1125	0.074	Neuropeptide signaling
PNOC	98	0.170	Neuropeptide signaling
NPPA	1561	0.064	Neuropeptide signaling
CRH	308	0.120	Neuropeptide signaling
VIP	1573	0.064	Neuropeptide signaling
HCRT	2355	0.051	Neuropeptide signaling
EDN1	1281	0.070	Neuropeptide signaling
CCKBR	2641	0.048	Neuropeptide signaling
GHRH	847	0.084	Neuropeptide signaling
NMU	27	0.235	Neuropeptide signaling
SHANK1	746	0.089	Neuropeptide signaling
KCNC2	1181	0.072	Transmembrane signaling
SLC34A1	187	0.140	Transmembrane signaling
OPRM1	255	0.127	Transmembrane signaling
ASS1	1852	0.058	Transmembrane signaling
PPP1R9B	3268	0.041	Transmembrane signaling
CCNA2	976	0.079	Transmembrane signaling
CRHBP	1198	0.072	Transmembrane signaling
HRH1	674	0.092	Transmembrane signaling
GNA15	3229	0.041	Transmembrane signaling
GNAQ	2989	0.043	Transmembrane signaling
GNB1	1626	0.062	Transmembrane signaling
RGS10	910	0.082	Transmembrane signaling
RGS8	1677	0.061	Transmembrane signaling
CDK5R1	2769	0.046	Transmembrane signaling
P2RY12	95	0.172	Purinergic signaling
P2RX7	1776	0.060	Purinergic signaling
P2RY13	33	0.224	Purinergic signaling
P2RX6	263	0.126	Purinergic signaling
P2RY6	309	0.120	Purinergic signaling
P2RX5	1255	0.071	Purinergic signaling
P2RY11	1570	0.064	Purinergic signaling
GPR34	271	0.124	Purinergic signaling
ADORA3	155	0.148	Purinergic signaling
PTAFR	961	0.080	Purinergic signaling
GNAI2	1428	0.067	Purinergic signaling
PYCARD	517	0.101	Immune response
BATF3	985	0.079	Immune response
TGFB1	564	0.098	Immune response
PYDC1	505	0.102	Immune response
DOCK2	461	0.105	Immune response
DHRS2	122	0.160	Immune response
TM7SF4	253	0.127	Immune response
CX3CR1	269	0.125	Immune response
ZAP70	545	0.099	Immune response
TYROBP	653	0.093	Immune response
SYK	295	0.121	Immune response
CD93	777	0.088	Immune response
SLC11A1	950	0.080	Immune response
TLR7	1188	0.072	Immune response
AIF1	96	0.171	Immune response
CLU	1748	0.060	Immune response
TLR3	994	0.078	Immune response
CX3CL1	1772	0.060	Immune response
SNCA	1060	0.076	Immune response
RAB3A	2097	0.055	Vesicle trafficking
TYRP1	328	0.118	Vesicle trafficking
CLTA	3693	0.037	Vesicle trafficking
AP2A1	1624	0.062	Vesicle trafficking
KIAA1199	3293	0.041	Vesicle trafficking
AP2A2	2058	0.055	Vesicle trafficking
NCALD	511	0.101	Vesicle trafficking
NRGN	229	0.132	Vesicle trafficking
CLTCL1	693	0.092	Vesicle trafficking
AP2S1	666	0.093	Vesicle trafficking
SLC17A7	2179	0.053	Vesicle trafficking
FCGR1A	1250	0.071	Vesicle trafficking
FCGR1B	257	0.127	Vesicle trafficking
HLA-DQA2	2184	0.053	Vesicle trafficking
LDLR	3312	0.040	Vesicle trafficking
AP1M2	1516	0.065	Vesicle trafficking
AP2M1	2364	0.051	Vesicle trafficking
HLA-DQA1	4017	0.034	Vesicle trafficking
AP1M1	2776	0.046	Vesicle trafficking
HLA-DPA1	4290	0.032	Vesicle trafficking
CD74	708	0.091	Vesicle trafficking
HLA-DRB5	3488	0.039	Vesicle trafficking
HLA-DRB4	8	0.312	Vesicle trafficking
DBNL	764	0.088	Vesicle trafficking
FZD5	4176	0.033	Vesicle trafficking
SLC32A1	4411	0.031	Vesicle trafficking
WNT5A	4491	0.031	Vesicle trafficking
AP1B1	3964	0.035	Vesicle trafficking
AP3B2	3468	0.039	Vesicle trafficking
HLA-DPB1	2465	0.050	Vesicle trafficking
HLA-DRA	62	0.193	Vesicle trafficking
CD9	3327	0.040	Vesicle trafficking
HLA-DQB1	239	0.130	Vesicle trafficking

(*Table continues.*)

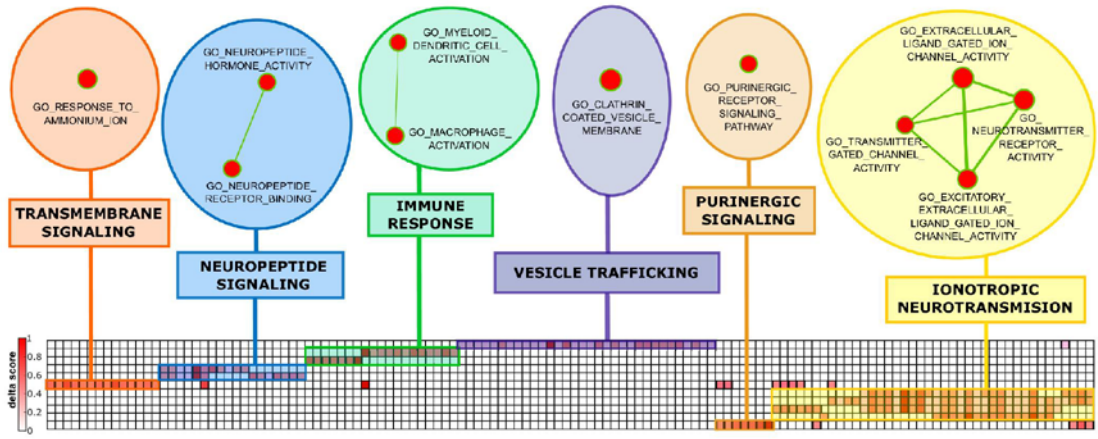

Figure 3. Heat map of the clustered leading edge genes of the positively enriched gene sets. Rows correspond to the gene sets listed in Table 2 and columns correspond to their respective leading-edge gene as detailed in Table 3. Color coding from white to dark red reflects degree of relative upregulation (delta score). The upper part shows the enrichment map of the clustered gene sets, containing six different clusters representing the overarching functional pathways. Node size represents the gene set size and line thickness shows the degree of overlap (shared genes) between the two gene sets it connects.

TLR family), cytokine signaling, and immune activation (e.g., TGFB1, AIF1, PYCARD), as well as a closely related gene set implicated in vesicle trafficking, which mainly contained leading-edge genes coding for major histocompatibility complex (MHC) molecules and adaptor complex proteins (AP1–3). Complementary GSEA analysis of delta scores calculated with the alternative reference region revealed identical results.

Discussion

The observed association between YoE and higher cortical thickness in medial prefrontal, ACC, and orbitofrontal cortices provides further converging evidence to other neuroimaging studies conducted within the CR model (Arenaza-Urquijo et al., 2013; Kim et al., 2015; Lee et al., 2016; Boller et al., 2017; Vaqué-Alcázar et al., 2017). The relevance of distinct frontal lobe areas as mediators of CR mechanisms is probably related to their key role in cognitive processes such as working memory (Owen et al., 2005; Barbey et al., 2011), cognitive conflict monitoring (Jahn et al., 2016), or decision-making (Shenhav et al., 2013). We further observed that these cortical areas exhibited a distinct gene expression profile characterized by relative upregulation of gene sets implicated in neurotransmission and immune response.

Our pathway analyses identified several gene sets involved in cell communication and neurotransmission processes. The majority of the upregulated leading-edge genes encode for major ionotropic glutamate receptors (i.e., *GRIK*, *GRIA*, and *GRIN* gene families), nicotinic acetylcholine receptors (*CHRN* gene family), as well as $GABA_A$ receptor subunits (*GABR* gene family), suggesting an important role for these brain areas in fast excitatory and inhibitory signaling. This molecular characteristic corresponds to the well described role of these medial prefrontal areas as highly active, functionally connected network hubs subserving transmodal information integration (Buckner et al., 2009; Sepulcre et al., 2012; Braga et al., 2013). In addition, neurotransmitter signaling through calcium-permeable ion channels, particularly glutamatergic AMPA and NMDA receptors as well as nicotinic acetylcholine receptors, is considered to be one of the most important molecular features of postsynaptic plasticity (Cull-Candy et al., 2006; Anggono and Huganir, 2012; Pankratov and Lalo, 2014).

In addition to ionotropic neurotransmission, we also identified several other upregulated gene set families involved in G-protein-coupled transmembrane signaling through neuropeptide and purinergic receptor binding. Beyond their role in homeostatic and neuroendocrine processes, neuropeptide/G-coupled receptor signaling is increasingly recognized as a mediator of activity-dependent refinement of local brain circuits and plasticity (McClard and Arenkiel, 2018), including important roles for learning and memory (Gøtzsche and Woldbye, 2016). Postmortem autopsy studies (Riudavets et al., 2007; Iacono et al., 2009), revealed that subjects with considerable AD pathology but preserved cognition exhibited neuronal hypertrophy in specific brain areas including the ACC. Such changes were interpreted as evidence of morphological plasticity in response to the incipient neuropathological changes of AD, suggesting that modulation of synaptic transmission and associated plasticity may reflect a neurobiological mechanism of resilience to cope with pathology and maintain cognition, which is the core concept of the CR theory.

A second category of gene sets that were found to be upregulated in the examined YoE-related areas was related to immune processes. This may reflect a particular sensitivity of the identified cortical regions for microglial activation (e.g., MHC-II molecules are frequently used as markers of microglial activation; Hopperton et al., 2018). In this sense, imaging-based PET studies have demonstrated region-specific activation of microglia in normal aging, including the frontal lobe and ACC regions (Schuitemaker et al., 2012). Although excessive microglial hyperactivation seems to contribute to the susceptibility of cognitive deficits (Kohman, 2012), transiently activated microglia also play an important role in the clearance and degradation of misfolded protein aggregates associated with neurodegenerative disease, such as the extracellular Aβ fibrils characteristic for Alzheimer's disease (Cai et al., 2014; Cho et al., 2014). Recent PET imaging studies have indicated that high education and lifelong intellectual enrichment may result in attenuation of age-related Aβ aggregation in medial

prefrontal cortical regions such as the ACC (Landau et al., 2012) and orbitofrontal areas (Arenaza-Urquijo et al., 2017), particularly in those individuals at risk for AD (Wirth et al., 2014). Thus, together with these previous findings our present results could indicate that such education-related Aβ suppression effects may be primarily observed in these brain areas partially because they are characterized by a high expression of microglial activity genes.

Regarding the characteristic overexpression of both microglial activation and neurotransmission pathways, it is interesting to note that purinergic signaling has been implicated in excitatory neurotransmission as well as in neuronal–glial communication, inflammation regulation, and phagocytotic activity of microglia (North and Verkhratsky, 2006), including clearance of Aβ fibrils (Kim et al., 2012; Erb et al., 2018).

In summary, our results suggest that the gene expression profile and enriched biological pathways of the prefrontal regions identified here may entail fundamental mechanisms to account for why these brain areas represent key regions mediating the positive physiological and cognitive effects of high educational attainment. One possibility is that because of their particular molecular characteristics, lifetime exposure to CR proxies such as YoE may lead to specific changes in the anatomy and activity of these regions over other cortical areas, possibly explaining the frequently reported increases of gray matter or cortical thickness measures in these areas in elders with high CR ratings. On the other hand, enrichment for biological pathways implicated in immune response and microglial activity would align with evidence for a protective effect of lifetime exposure to enriched cognitive activity against pathology accumulation in these regions (Landau et al., 2012; Arenaza-Urquijo et al., 2017).

The present study is not without limitations. A first constraint refers to the lack of biomarker information to characterize our sample of older adults. According to population-based studies (Jansen et al., 2015; Toledo et al., 2015) ~20% of individuals with normal cognition and a mean age comparable to our older adult individuals are likely to harbor significant brain pathology (i.e., amyloid-β or tau deposition). Furthermore, because our main inclusion criterion was normal cognitive function, these percentages may be expected to be even higher among highly educated elders, as preserved cognition in the face of brain pathology is likely to be more frequent among high CR elders. Therefore, our current approach does not allow discerning the degree to which the detected associations between education and cortical thickness may be specific to normal aging or confounded by coexisting brain pathology.

A further restriction of our study is that the cross-sectional structural MRI analysis does not provide specific information regarding the possible mechanisms underlying the observed education/cortical thickness relationship. Thus, these relations could either reflect a higher capacity for plastic brain changes related to efficiency or compensation mechanisms of CR (for review, see Bartrés-Faz and Arenaza-Urquijo, 2011), or they could reflect brain maintenance associated to less age-related cortical thickness loss (Nyberg et al., 2012). Although CR and brain maintenance are two interrelated and not mutually exclusive concepts (Stern et al., 2018b), both being putatively related to brain plasticity mechanisms (Bartrés-Faz and Arenaza-Urquijo, 2011; Cabeza et al., 2018), disentangling their relative contributions would typically require a longitudinal study design (or comparison with young brains' characteristics; Nyberg et al., 2012; Cabeza et al., 2018). The detailed characterization of neuronal systems underlying CR and maintenance effects is an ongoing and highly active area of research (Stern et al., 2018a). In future studies the inclusion of other CR proxies or lifestyle-based measures (IQ, occupation, leisure-time, physical activity), cognitively impaired individuals (e.g., patients with AD or MCI), and functional imaging modalities, together using distinct approaches such as the residual (van Loenhoud et al., 2017) or those based on physiological age estimations (Steffener et al., 2016) will help to further characterize detailed CR-related brain networks, possibly also disentangling regionally diverging implications in compensation/efficiency and maintenance mechanisms.

Finally, our study is limited by the available data for estimating region-specific cortical gene expression profiles. Although the used gene expression data from the Allen Brain Atlas provides the anatomically most comprehensive transcriptome data for the human brain available to date, the data were derived from only six brain donors. Furthermore, this gene expression dataset was obtained from postmortem specimens of relatively young subjects (mean age 42.5 at death; Hawrylycz et al., 2012, 2015; Shen et al., 2012) with no educational background information provided. We acknowledge that these aspects may pose a bias when interpreting the findings based on brain regions identified from older participants, given that cortical gene expression values are known to change across the lifespan (Naumova et al. 2013).

In conclusion, to our knowledge, this study represents the first attempt to characterize the transcriptome-wide gene expression profile of YoE-related brain areas. Our results point to a distinctive enrichment with certain biologic pathways that may equip these regions with a higher capacity for plastic change in response to lifetime intellectual enrichment and potentially also a higher resilience to age-related pathologic brain changes. Further research is needed to advance our mechanistic understanding of the biological pathways through which cognitive and brain reserve operate to preserve cognition and day-to-day functionality among older adults.

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Study 3

Vaqué-Alcázar, L., Sala-Llonch, R., Abellaneda-Pérez, K., Coll-Padrós, N., Valls-Pedret, C., Bargalló, N., Ros, E. & Bartrés-Faz, D. (2020). Functional and structural correlates of working memory performance and stability in healthy older adults. *Brain Structure & Function, 225*(1), 375–386.

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ORIGINAL ARTICLE

Functional and structural correlates of working memory performance and stability in healthy older adults

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Abstract
Despite the well-described deleterious effects of aging on cognition, some individuals are able to show stability. Here, we aimed to describe the functional and structural brain characteristics of older individuals, particularly focusing on those with stable working memory (WM) performance, as measured with a verbal N-back task across a 2-year follow-up interval. Forty-seven subjects were categorized as stables or decliners based on their WM change. Stables were further subdivided into high performers (SHP) and low performers (SLP), based on their baseline scores. At both time points, magnetic resonance imaging (MRI) data were acquired, including task-based functional MRI (fMRI) and structural T1-MRI. Although there was no significant interaction between overall stables and decliners as regards fMRI patterns, decliners exhibited over-activation in the right superior parietal lobule at follow-up as compared to baseline, while SHP showed reduced the activity in this region. Further, at follow-up, decliners exhibited more activity than SHP but in left temporo-parietal cortex and posterior cingulate (i.e., non-task-related areas). Also, at the cross-sectional level, SLP showed lower activity than SHP at both time points and less activity than decliners at follow-up. Concerning brain structure, a generalized significant cortical thinning over time was identified for the whole sample. Notwithstanding, the decliners evidenced a greater rate of atrophy comprising the posterior middle and inferior temporal gyrus as compared to the stable group. Overall, fMRI data suggest unsuccessful compensation in the case of decliners, shown as increases in functional recruitment during the task in the context of a loss in WM performance and brain atrophy. On the other hand, among older individuals with WM cognitive stability, differences in baseline performance might determine dissimilar fMRI trajectories. In this vein, the findings in the SHP subgroup support the brain maintenance hypothesis, suggesting that stable and high WM performance in aging is sustained by functional efficiency and maintained brain structure rather than compensatory changes.

Keywords Aging · Brain stability · Cortical thickness (CTh) · Functional magnetic resonance imaging (fMRI) · Working memory

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Abbreviations

BL	Baseline
FU	Follow-up
SHP	Stables high performers
SLP	Stables low performers
WM	Working memory
WMf	Working memory factor

Introduction

Working memory (WM) performance results from the interaction between attention, short-term retention and manipulation of information, carried out by the coordinate activation of many brain regions (Eriksson et al. 2015). The WM capacity is central for daily life activities and is predictive for a wide range of higher level cognitive measures (Unsworth et al. 2014). The aging process is associated with a broad impact on cognition, being impairments in WM a well-known disabling phenomenon in advanced age (Park and Reuter-Lorenz 2009; Salthouse 2010). However, previous studies in elderly individuals have shown high inter-individual variability in the WM cognitive profile and a great heterogeneity in WM-related cognitive trajectories over time (Wilson et al. 2002; Habib et al. 2007), as well as in the associations between cognition and brain functioning (Persson et al. 2006; Nagel et al. 2009). Hence, it appears that cognitive dysfunction is not a universal phenomenon in aging (Nyberg and Pudas 2019). In this regard, Nyberg et al. (2012) introduced the concept of brain maintenance to reflect that some cognitively stable older adults achieve successful cognitive aging presumably because they exhibit little or no age-related neurochemical, functional and/or structural changes.

Regarding neuroimaging studies, cross-sectional findings in healthy aging using functional magnetic resonance imaging (fMRI) have reported distinct associations between brain activity patterns and its correlates with successful aging, including both increased and decreased activity, which normally coexist (reviewed in Grady 2012; Li et al. 2015; Rajah and D'Esposito 2005; Spreng et al. 2010). These functional adjustments have been explained by way of distinct cognitive hypotheses (Eyler et al. 2011). In general, increased blood-oxygen level-dependent (BOLD) activity has been interpreted as a compensatory mechanism to counteract the lack of functionality of the typical brain resources (Cabeza et al. 2002, 2018; Reuter-Lorenz and Park 2014). Furthermore, compensation stands as a main functional mechanism imbedded within the concept of cognitive reserve (CR), which predicts that high-CR individuals are more able to cope with age-related or disease-associated brain changes (Stern 2002, 2009). On the other hand, these functional brain changes are not invariably linked to a successful cognitive profile. In these cases, increased activations have been conceived as attempted compensatory mechanisms (Cabeza and Dennis 2013) or dedifferentiation processes (Park et al. 2004; Carp et al. 2011).

Studies entailing fMRI longitudinal observations overcome some limitations of cross-sectional research, notably as the longitudinal approach is more sensitive in identifying continuous ongoing changes in aging (Fjell et al. 2014a). In this line, Nyberg et al. (2010) provided evidence that elders, even expressing an over-recruitment observed with cross-sectional paradigms, can show age-related BOLD signal reductions when followed over time. In addition, and closely associated with the brain maintenance concept, a more recent longitudinal fMRI study showed functional increases in declarative memory decliners, while no significant effects were observed in cognitively stable individuals (Pudas et al. 2017). These results highlight that when measured across time, increased brain activity may not necessarily be related to higher performance but rather, it may reflect ongoing cognitive decline.

Besides functional brain changes, aging is also associated with widespread modifications in structural integrity (Salat 2004, 2011; Fjell et al. 2014a, b), and recent efforts have been conducted to characterize the structure underlying preserved cognition in successful aging. Previous studies focused on the hippocampal volumes highlight that there are no clear evidences that different ratios of atrophy occur in stables and decliners (Dekhtyar et al. 2017; Pudas et al. 2017). However, some investigations have identified higher cortical thickness (CTh) measures for those older adults exhibiting high (Bartrés-Faz et al. 2019), or above-average to superior cognitive performance, these latter individuals being so-called 'superagers' (Harrison et al. 2012; Gefen et al. 2015; Sun et al. 2016; Cook et al. 2017). Interestingly, some studies have used a multimodal approach to explore the interaction of cognition, brain function, and structure, demonstrating a significant relationship between cognitive performance and structural integrity in aged samples (Burzynska et al. 2013; Steffener et al. 2012). Also, Burianová et al. (2015) evidenced that a more preserved structure facilitated the recruitment of functional compensatory mechanisms, thereby enabling better WM accuracy.

Age-related functional changes occurring during cognitive demands and their neural substrates are not fully understood, in part due to the scarce number of longitudinal studies. Focusing on WM, only one previous work has explored the maintenance concept (Rieckmann et al. 2017) and showed that a stable left lateral activation of the prefrontal cortex area over a 4-year follow-up underlies a maintained WM performance. Therefore, in the present investigation, through a 2-year longitudinal design, we aimed to characterize functional brain patterns during a common WM task paradigm (N-back task), as well as, the

associated underlying structural integrity changes. Based on their longitudinal WM performance, elders showing age-related cognitive decline (decliners) were compared with those showing stability on WM (stables). Further, since some studies have indicated that higher performance is associated with a lower risk of cognitive decline (Habib et al. 2007; Yaffe et al. 2010; Rosano et al. 2012) and because it has been suggested that the maintenance mechanisms would differ depending on the baseline cognitive level (Cabeza et al. 2018), we subdivided the stable group by their performance at the starting point. We hypothesized that, with age, the functional patterns underlying WM would differ between stables and decliners, with the decliners likely to engage non-task-related areas, thus evidencing an unsuccessful compensatory process. In addition, we aimed to characterize the structural progression of these groups and we hypothesized that the age-related cortical atrophy after 2-year follow-up will be also related to task performance and/or stability. Furthermore, within the stable group, only those achieving higher performance scores will probably exhibit a brain profile fitting with the 'maintenance' concept (at functional and structural level).

Materials and methods

Subjects

Forty-seven subjects aged 68.40 ± 2.86 years (mean ± standard deviation, SD) at baseline and 70.47 ± 2.97 years at 2-year follow-up, with cognitive and magnetic resonance imaging (MRI) data at both time points, were selected from the control group of a larger cohort of fit community elders recruited to a randomized controlled trial aimed at assessing the effects of walnuts on age-related diseases (Rajaram et al. 2017). Exclusion criteria were illiteracy or inability to understand the protocol or undergo neuropsychological tests, morbid obesity ($BMI^3 > 40\ kg/m^2$), uncontrolled diabetes (HbA1c > 8%), uncontrolled hypertension (on-treatment blood pressure ≥ 150/100 mm Hg), prior cerebrovascular accident or major head trauma, any relevant psychiatric illness (including major depression), abnormal cognitive profile according to the normative scores (see next section), dementia, other neurodegenerative diseases (i.e., Parkinson's disease), and any chronic illness expected to shorten survival (i.e., heart failure, chronic liver disease, kidney failure, blood disease, cancer). The study was approved by the Hospital Clínic de Barcelona ethical committee and has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from each participant prior to enrollment.

Neuropsychological assessment

A comprehensive battery of neuropsychological tests was administered to assess cognitive functioning, as described previously (Rajaram et al. 2017; Vidal-Piñeiro et al. 2014). All participants had normal cognitive profiles with mini-mental state examination (MMSE) scores > 25 (Mitchell 2009) and performances no more than 1.5 SD below normative scores on any of the neuropsychological tests administered, i.e., they did not fulfill criteria for mild cognitive impairment (Petersen and Morris 2005).

MRI acquisition

MRI was acquired in a 3 T Siemens scanner (Magnetom Trio Tim syngo) with 32-channel head coil at the *Unitat d'Imatge per Ressonància Magnètica IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer)* at Hospital Clínic de Barcelona, Barcelona, at baseline and at a 2-year follow-up evaluation. All participants underwent fMRI interleaved acquisitions [T2*weighted EPI scans, repetition time (TR) = 2000 ms, echo time (TE) = 16 ms, 336 volumes, 40 slices, slice thickness = 3 mm, inter-slice gap = 25%, field of view (FOV) = 220 mm, matrix size = 128 × 128] during the performance of the N-back task. In addition, a high-resolution T1-weighted structural image was obtained for each subject with a magnetization-prepared rapid acquisition gradient-echo (MPRAGE) three-dimensional protocol (TR = 2300 ms, TE = 3 ms, inversion time = 900 ms, FOV = 244 mm, 1-mm isotropic voxel, matrix size = 256 × 256). For all participants, MRI images were examined by a senior neuroradiologist (N.B.) for any clinically significant pathology (none found). Then, all the acquisitions were visually inspected before analysis by the first author (L.V.-A.) to ensure that they did not contain MRI artifacts or excessive motion. At this level, no images were excluded.

The time average between both MRI visits was 23.17 ± 1.22 months. The average time between the neuropsychological assessment and the MRI acquisition was 27.40 ± 31.29 days at baseline and 12.89 ± 12.49 days at follow-up.

N-back task

Participants performed a letter N-back task with different levels of memory load (from 0 to 3 letters to be retained) inside the MR scan, as described (Sala-Llonch et al. 2012). Briefly, we used a block-designed task where each N-back condition lasted 26 s, followed by inter-block fixation periods of 13 s. Before any N-back block, an instruction screen informed the subject of the upcoming block. Each stimulus (capital letters A–J) was shown in white in the center of a

black screen during 500 ms, with an inter-stimulus interval of 1500 ms. Participants were instructed to press a button when the letter "X" appeared (0-back) or when the letter shown matched the one seen one (1-back), two (2-back) or three (3-back) stimuli before. The individual performance was recorded and scores were calculated using the *d prime* (d') measure, which accounts for correct responses and false alarms, computed as Z(hit rate) − Z(false alarm rate), where function $Z(p)$, $p \in [0,1]$, as the inverse of the cumulative distribution function of the Gaussian distribution of the hits and false alarm rates.

Working memory factor (WMf) calculation and group classification

We used principal component analysis (PCA) to create a composite scale which represented the WM factor (WMf), calculated using the d' scores from the 0-back, 1-back, 2-back and 3-back conditions during the N-back fMRI task. Subsequently, we calculated the change in WMf as the difference between follow-up and baseline and we classified subjects as stables ($N = 23$) or decliners ($N = 24$), according to the change in WMf (above/below zero). Stables were further subdivided into high and low performers according to the WMf at baseline using the median as threshold. As a result, 11 out of 23 subjects were classified as stables high performers (SHP) and 12 out of 23 as stables low performers (SLP).

The main demographic and cognitive characteristics are shown in the Supplementary Material (Supplementary Table 1). It should be noted that differences in years of education and gender were detected when the sample was divided into three groups ($F = 7.723$, $p < 0.001$ and $\chi^2 = 8.313$, $p = 0.016$; respectively). The SLP group disclosed a lower educational level than the SHP ($t = 3.824$, $p = 0.001$) and decliners ($t = 2.876$, $p = 0.007$). Moreover, there were differences regarding gender due to the small representation of females in the SHP compared to SLP ($p = 0.039$) and decliners ($p = 0.011$).

Neuroimaging analyses

Functional MRI (N-back task)

Data were analyzed with the FEAT-FSL software (FMRIB's Software Library version 5.0.10; https://fsl.fmrib.ox.ac.uk/fsl/; Jenkinson et al. 2012). We first performed a preprocessing of all individual fMRI scans, which included non-brain tissue removal, motion correction, spatial smoothing with a Gaussian kernel of 5 mm of full width at half maximum (FWHM), temporal filtering with a high-pass filter of 160 s and a two-step linear registration to a standard template. Further, the head motion parameters estimated by MCFLIRT (Jenkinson et al. 2002) were included as confounding explanatory variables in our model. Then, at the first-level analysis (Woolrich et al. 2001), data were fit to a general a linear model (GLM) containing the task time series with a gamma convolution of the hemodynamic response function. In this GLM, four regressors and their first temporal derivatives were modeled: 0-back, 1-back, 2-back and 3-back. By including the derivatives, we aimed to correct for shifting in the time series as well as for slice timing effects. We defined a single contrast of interest combining the previous four regressors as the difference of brain activity between the highest and lowest loads (3-back > 2-back > 1-back > 0-back), using weights of 0.375, 0.125, − 0.125 and − 0.375. The results of the first level analyses were further fit into higher level or group-level statistics, performed using the FMRIB's Local Analysis of Mixed Effects (FLAME), (Woolrich et al. 2004). We first calculated the group-mean activity maps of the task contrast and the difference between time points for all subjects. Then, we created group GLM designs to evaluate: (1) time–group interactions, (2) patterns of time-related change for each group and (3) differences between groups (stables vs. decliners; SHP vs. SLP; SHP vs. decliners; SLP vs. decliners) at both time points. Due to the gender differences when the sample was split into three groups, this variable was included as a regressor in the higher level analyses concerning SHP, SLP and decliners. All analyses were performed in the whole brain at a voxel-wise level, and a $z > 2.3$ was used to define contiguous clusters of activity, then cluster significance levels were estimated and corrected using family-wise error (FWE) correction. The significance threshold was set at a corrected $p < 0.05$. Finally, to obtain summary statistics of functional imaging data, we computed individual mean BOLD signal values within the significant region of interest (ROI) derived from the functional analyses.

Cortical thickness (CTh)

Structural T1-weighted images were automatically processed with Freesurfer (version 5.1; https://surfer.nmr.mgh.harvard.edu) using its longitudinal pipeline (Reuter et al. 2012). First, the two time points were processed individually, and the results were inspected visually to ensure the accuracy of registration, skull stripping, segmentation, and cortical surface reconstruction. The first author (L.V.-A.) did the manual edition to correct the brain extraction step that was needed in eight subjects at baseline and ten at follow-up. There were no differences between groups regarding the number of corrections required at both time points. One participant (from the SLP group) was excluded due to the pial and white matter surface mismatches caused by the excessive motion. Then, within-subject template volumes (Reuter and Fischl 2011) and longitudinal files were created for each

subject and time point through the longitudinal stream. The CTh maps were first smoothed using a 2D Gaussian kernel of 15 mm FWHM. The symmetrized percent change (spc) was used as the longitudinal measure of CTh. We performed vertex-wise statistics to study the CTh loss after the 2 years (1) for the whole sample, (2) related to group differences, and (3) for each group. As reported in the N-back task section, all the CTh analyses considering the three groups were adjusted by gender. The resulting vertex-wise statistical maps were considered significant at $p < 0.05$ level. Maps were further corrected for family-wise error (FWE) using a Monte Carlo Null-Z simulation, with 10,000 repetitions and a cluster $p < 0.05$. In addition, the global CTh values for each subject were calculated to obtain summary statistics.

Additional statistical analyses

Statistical analyses for non-imaging data were performed using IBM SPSS Statistics (Statistical Package for Social Sciences. Version 24.0. Armonk, NY: IBM Corporation). Demographic and cognitive data were described as mean ± SD (Supplementary Table 1). For categorical data, differences between groups were evaluated using the chi-squared (χ^2) test, while Fisher's exact test was used to compare data regarding the subgroups. For quantitative data, we evaluated differences at each time point between groups (decliners and stables) using independent-sample t tests and between the three groups (decliners, SHP, and SLP) using a one-factorial analysis of variance (ANOVA). Following this ANOVA, if there were significant interactions, independent-sample t tests were conducted to compare groups by pairs at each time point. Furthermore, the differences among group trajectories were investigated using repeated measures ANOVAs. As post hoc pairwise analyses, differences between baseline and follow-up for each group were analyzed using paired-samples t tests. Wilcoxon signed-rank test was used to explore the differences across time in the whole sample. The statistically significant difference for all the analyses was considered at $p < 0.05$. The graphical representations were performed using GraphPad Prism (version 6.00, GraphPad Software, La Jolla, CA, USA).

Results

Functional imaging analyses

Age-related over-activation at follow-up

We evaluated BOLD activity associated with our contrast of interest in the WM task (3-back > 2-back > 1-back > 0-back) at both time points independently. In all the subjects, we observed task-related activity in brain areas including the bilateral frontal region, paracingulate, anterior cingulate, supramarginal and angular gyrus, precuneus and lateral occipital cortex, and the right insular cortex, among others (Fig. 1a, b). As compared to baseline, follow-up analyses revealed increased task-related activity on right areas

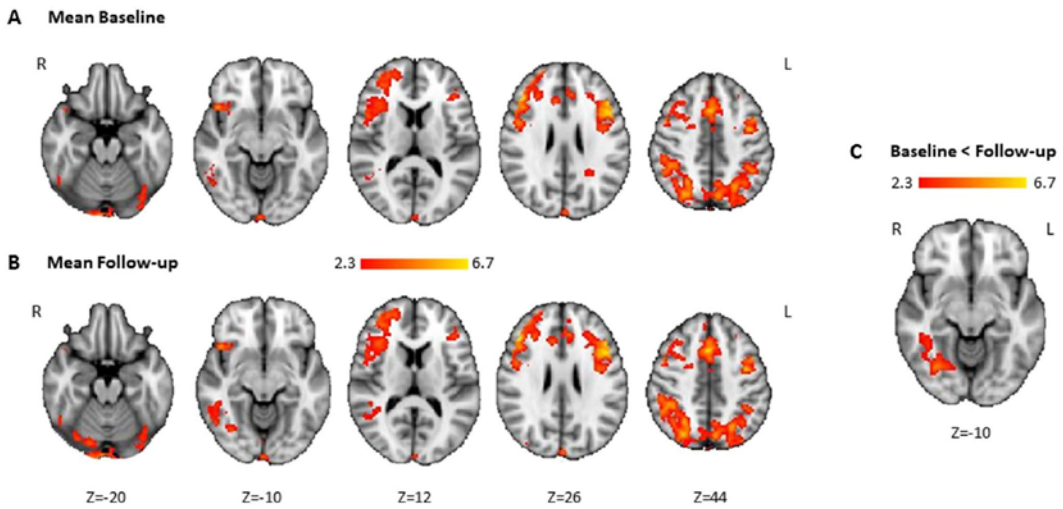

Fig. 1 Significant fMRI results (corrected $p < 0.05$ and $z > 2.3$) shown on the standard MNI map. Group mean activity maps representing the WM pattern for the contrast of interest are shown in red. **a** Mean baseline; **b** mean follow-up; **c** differences between baseline and follow-up are shown in red

comprising the inferior division of the lateral occipital cortex, the superior temporal gyrus and the occipital fusiform gyrus for the whole sample (Fig. 1c).

Functional changes underlying performance stability and decline

There was no significant time–group interaction between stables and decliners. The functional progression for each group and their cross-sectional differences are detailed in Supplementary Material (Supplementary Figs. 4 and 5). Nevertheless, when the stable group was subdivided into SHP and SLP, we identified a time–group interaction between SHP and decliners (Fig. 2). The SHP group showed a reduction of activity at 2-year follow-up, while the decliners exhibited increased activation in a cluster encompassing the right postcentral and supramarginal gyrus and the superior parietal lobule. Furthermore, the longitudinal progressions for each group were additionally investigated. Pairwise analysis for the decliners showed increased activation (see Supplementary Fig. 4, in green). Nevertheless, no significant differences were found as regards the SHP subgroup.

Cross-sectional differences considering the three groups (SHP, SLP and decliners)

When assessing the two time points separately, SHP showed more activity than SLP at baseline in the right frontal pole (Fig. 3). After 2 years, the SHP kept showing more activity than the SLP group, but at this time point, in the thalamus bilaterally (Fig. 4, in blue). Also, at follow-up, the decliners showed more activity than both stable subgroups. The difference between decliners and SLP comprised the right basal ganglia (Fig. 4, in green) and compared to SHP, the decliners exhibited more activity mainly comprising the left temporo-parietal cortex and the bilateral posterior cingulate and lingual gyrus (Fig. 4, in red).

Cortical thickness results

We observed widespread CTh atrophy in the entire sample between baseline and the 2-year follow-up (see Supplementary Material, Fig. 6). When the sample was divided into the two main groups, as compared to stables, decliners exhibited more cortical thinning in a cluster comprising the left posterior middle and inferior temporal gyrus and the lateral occipital area (Fig. 5a). Then we calculated the specific maps of cortical atrophy for each group. The stables showed significant atrophy across time over the left caudal middle frontal and ventral precentral gyrus, and right middle temporal and inferior parietal cortex (Fig. 5b). On the other hand, the decliners exhibited a more extended pattern of atrophy in both hemispheres, with three clusters in the left hemisphere over the temporal and lateral occipital areas, rostral middle frontal, and the entorhinal areas, along with two clusters in the right hemisphere partially including the superior temporal and banks of superior temporal sulcus region (Fig. 5c). No significantly different atrophy patterns

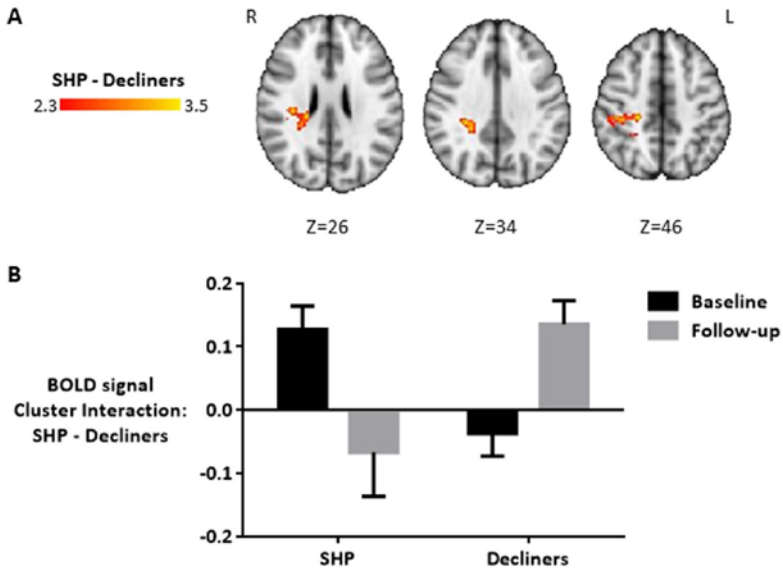

Fig. 2 Comparisons in fMRI between SHP and decliners. **a** Time–group interaction, showing decreases in SHP compared with decliners. **b** Plot of mean BOLD signal values at the ROIs in the two groups separated by baseline and follow-up measures. Error bars: ± 1 standard error of the mean (SEM). Abbreviations: SHP, stables high performers

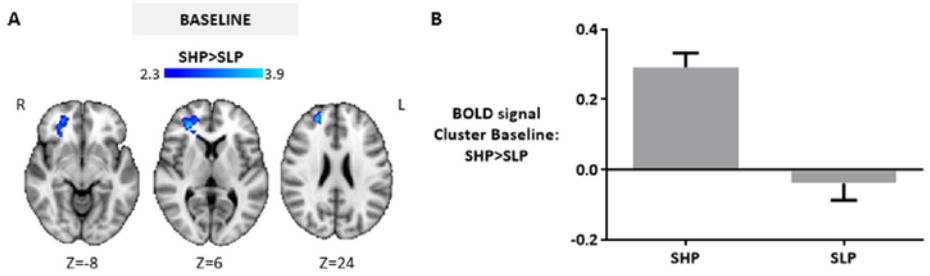

Fig. 3 The fMRI differences between groups. **a** Significant activity maps for the difference between SHP and SLP (in blue) at baseline. **b** Plot of baseline mean BOLD signal values at the ROI separated by group through pairwise comparison. Error bars: ± 1 SEM. *SHP* stables high performers, *SLP* stables low performers

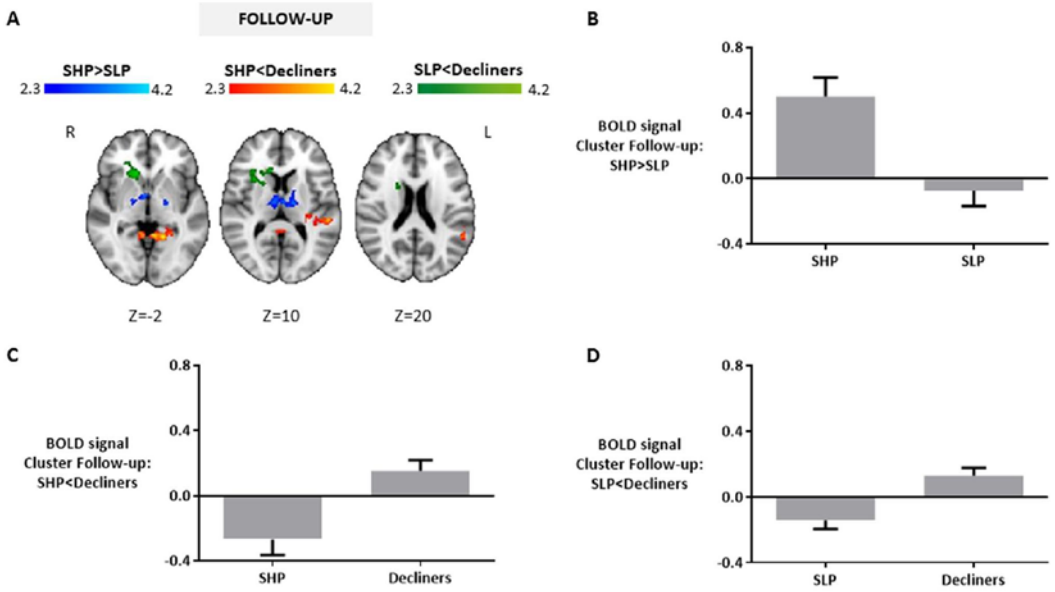

Fig. 4 The fMRI differences between groups. **a** Significant activity maps for the difference between SHP and SLP (in blue), SHP and decliners (in red) and SLP and decliners (in green) at follow-up. Plot of mean BOLD signal values after 2 years at the ROI separated by group through pairwise comparison between **b** SHP vs. SLP, **c** SHP vs. decliners, and **d** SLP vs. decliners. Error bars: ± 1 SEM. *SHP* stables high performers, *SLP* stables low performers

were observed comparing the SHP and SLP subgroups with the decliner group.

Lastly, it should be noted that we did not identify any significant correlation between the measures derived from the task-fMRI analyses and the CTh values.

Discussion

In this study, we investigated brain activity changes in healthy elders who underwent an fMRI acquisition during a WM task at baseline and at 2 years of follow-up.

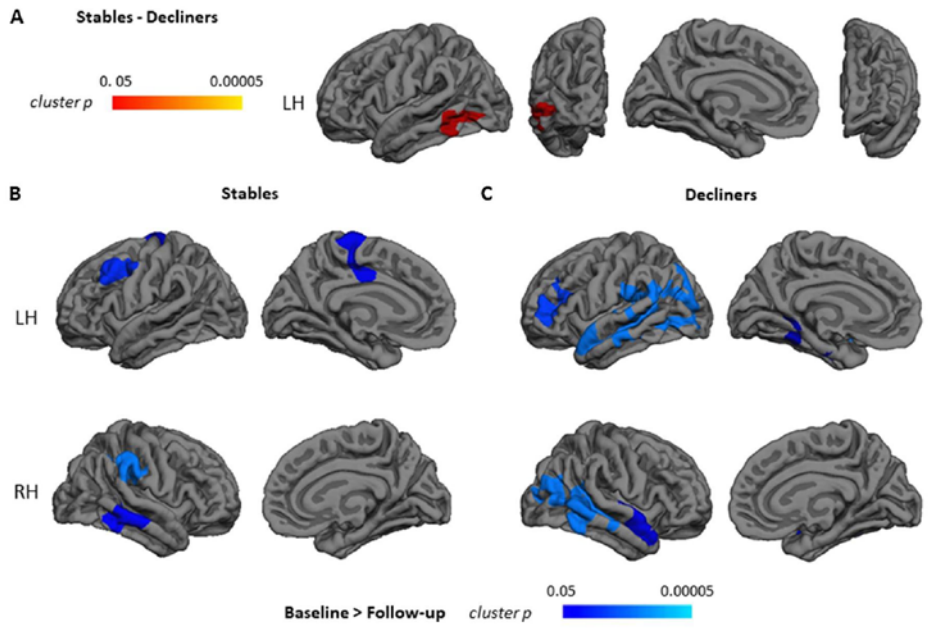

Fig. 5 Vertex-wise symmetrized percent change (spc) maps of significant clusters surviving FWE multiple comparison correction with a final cluster-wise $p < 0.05$. **a** Differences between stable and decliners. Atrophy maps at 2-year follow-up for **b** stables and **c** decliners. *LH* left hemisphere, *RH* right hemisphere

Participants were classified as stables or decliners based on their longitudinal measures of WM performance. Further, the stable group was subdivided (i.e., SHP and SLP) on the basis of their WM performance at baseline. Interestingly, a significant interaction between SHP and decliners emerged: decliners exhibited over-activation at follow-up compared to baseline, while SHP reduced the activity. As stated below, these observations may be reflecting dedifferentiation vs. neural efficiency processes. Albeit, due to the heterogeneity in the stable group, as well as to the resulting reduced sample sizes investigated, the interaction considering the whole stable group and the decliners was non-significant. Moreover, cross-sectional analyses showed differences between groups in fMRI patterns. At baseline, less activity in the SLP compared to the SHP was observed. At follow-up, this difference remained, while additionally the decliner group showed more activity as compared to both stable subgroups. At the structural level, we observed significant group differences across time as regards atrophy rates over a left temporo-occipital area, where the decliners showed a more noticeable pattern of change than subjects exhibiting WM performance stability, indicating differences in the temporal evolution of brain structure between these groups.

Differences in working memory performance

Initially, the sample was split into stables and decliners according to cognitive scores, as done in prior studies in the field (Josefsson et al. 2012; Persson et al. 2012; Pudas et al. 2017; Rieckmann et al. 2017). After subdividing the stables by baseline performance, differences regarding years of education and gender emerged between the three groups. The SHP had a larger proportion of males, probably because there was a bias towards highly educated male participants reflecting a generational effect. Remarkably, the SHP and decliners had more years of education compared to SLP. Hence, according to the classification we used, a higher educational level is associated with higher initial WM performance, whereas cognitive stability can occur amongst both high- and low-educated elders (i.e., SHP and SLP differed in years of education and in performance at both time points). These results concur with those of larger longitudinal studies suggesting that education mainly contributes to higher starting cognitive performance, rather than being associated with a slower rate of decline (Vemuri et al. 2014; Wilson et al. 2019).

Distinctive fMRI trajectories underlying stability and decline in WM function

In the whole sample, we found patterns of increased fMRI brain activity (Rieckmann et al. 2017) in a cluster comprising regions outside the WM-related areas which comprised the right lateral occipital cortex (Grady et al. 2006; Jamadar et al. 2013; Archer et al. 2018). When the sample was split into stables and decliners, no significant functional trajectory differences were identified. However, this lack of results could be explained in part since stables are a heterogeneous group, as the following findings suggested. In this vein, the subdivision of the stables based on their performance at baseline allowed the identification of a significant interaction between the SHP subgroup and decliners in a cluster entailing the right superior parietal lobule, indicating an expansion of the typical WM-related areas for the decliners (Rieckmann et al. 2017; Pudas et al. 2017). Although the decliner group exhibited increased brain activity at follow-up compared to baseline, this was not accompanied with stable cognition. Thus, this over-recruitment phenomenon, far to be reflecting a compensatory mechanism (Cabeza et al. 2002; Grady et al. 2006; Reuter-Lorenz and Cappell 2008), should be interpreted as progressive neural dedifferentiation because this additional neural recruitment was unsuccessful at cognitive level (Logan et al. 2002; Park et al. 2004; Carp et al. 2011). On the other hand, the previously mentioned interaction revealed an activity reduction for the SHP, which could not be confirmed studying the specific group functional trajectory, probably due to the small sample size. Therefore, this finding suggested a more efficient fMRI pattern and preservation of neural resources for the SHP group across time (Nyberg et al. 2010). Concurring with the 'brain maintenance' concept (Nyberg et al. 2012), the observed activity reduction, instead of relating to a loss in cognitive performance, was associated with the maintenance of WM. In sum, our data support the notion that over-activation is a common trait in aging, but which might be mainly driven by decliner subjects. Further, our data emphasized that optimal cognitive function in older adults depends on the successful brain maintenance rather than triggering compensatory mechanisms to counteract the damaged normal functioning (Morcom and Henson 2018).

Cross-sectional fMRI findings

Although the groups' stratification is based on the longitudinal change, we take advantage of the two approaches used (longitudinal and cross-sectional). Even if these results should be considered exploratory given the small sample size, they contribute to understanding the differences between 'hyperactivation' and 'over-activation'. Specifically, at baseline, we found increased activity in SHP compared to SLP ('hyperactivation'), which can be interpreted as a distinctive feature of the high functioning subjects and not as a typical compensatory mechanism ('over-activation'), (Nyberg et al. 2010). These results suggested that a higher WM performance would be associated with higher WM load-dependent adaptability of neural activity (Nagel et al. 2008, 2009; Nyberg et al. 2009; Steffener and Stern 2012). While caution is needed, the low activity identified for the SLP at both time points, even compared to decliners at follow-up, supported the hypothesis that a highly demanding task could give rise a shift to the 'under-activation', suggesting a neural resource limitation, or reduced neural capacity (Stern 2009), amongst low-performing elders. In this line, the age-related low activity seems to be partly explained by a low educational level (Archer et al. 2018), which is in accordance with the fact that in our sample the SLP was the less educated group. Another noteworthy finding was that neural activity was also higher for the decliners compared to the SHP at follow-up. However, the neuroanatomical location of these results indicated that the increased activation occurred partially over the left temporo-parietal cortex and posterior cingulate and lingual gyrus, outside the typical WM pattern areas of brain activity but instead overlapping with core default mode network (DMN) regions. Such observations may indicate that a reduced activation balance between the default mode and the task-related pattern activation may be characteristic of a WM decline (Grady et al. 2006; Miller et al. 2008; Sala-Llonch et al. 2012; Spreng et al. 2016).

Decliners showed higher rates of cortical atrophy

We identified a pattern of CTh atrophy after 2 years for all the participants, in accordance with the described widespread age-related grey matter reductions with aging (Fjell et al. 2014a, b; Storsve et al. 2014). There was a significant difference between groups as regards atrophy rate. This finding is in line with former reports describing slower rates of cortical atrophy sustaining cognition in successful aging subjects compared to average-agers (Harrison et al. 2012; Cook et al. 2017). Concretely, the interaction cluster comprised the inferior temporal gyrus and the lateral occipital cortex, areas relatively preserved against the typical age-related structural impairment (Lee et al. 2018). When considering each group separately, although atrophy clusters are scattered in different regions of the brain, we observed that the decliner group displayed a more generalized pattern of atrophy comprising extended temporal and lateral occipital areas in both hemispheres. Interestingly, this group also exhibited a significant cortical reduction in the left entorhinal cortex, a feature that has been suggested as a marker of incipient Alzheimer's disease pathology (Dickerson et al. 2001). Albeit this, our study does not permit to associate the

observed longitudinal findings with absence or evidence of impending brain pathology.

Limitations

A first limitation of the present study was the sample size, especially when the stable group was stratified. For this reason, the cluster-wise threshold set at $z > 2.3$ for the fMRI analyses should be considered lenient (see Eklund et al. 2016). Hence, and albeit present results are novel in that they offer first evidence of the functional and structural brain correlates associated with WM stability and decline in aging, it is important to highlight that they should be interpreted with caution, and further investigations with larger samples are needed. In this regard, a larger group could have also allowed a better understanding of the role of CR and its putative influence of being stable or decliner, as well as stratification of the decliner group into high- and low-performer subgroups. Further, additional time point measurements may be important to better identify subjects with stable trajectories and to discern whether the WM age-related changes follow a linear function. Moreover, the inclusion of a young reference group could have provided a powerful approach to detect successful cognitive aging. Also, the stratification criteria could be considered a limitation, but previous studies do not propose a unified method to identify 'brain stability' using two time point measures. In addition, the cross-sectional findings suggested that the low level of activation for the low-performing group is associated with the fact that this group may no longer be following a liner response by load as the cognitive demand increase. In this sense, the use of a linear task contrast supposes a limitation for the analysis of this concrete subgroup and further studies should overcome this constraint using a non-linear approach. Finally, the lack of significant associations between the functional and structural brain measures supposed a limitation to deeply discuss our results.

Conclusions

Our results provide new evidence on the underlying functional mechanisms and structural characteristics of WM cognitive stability in older age. First, we observed increased activity at follow-up compared to baseline for the whole sample. Nevertheless, this over-activation was only detected for the decliner group, highlighting the importance of conducting longitudinal studies and stratifying the elders according to their trajectories, as an approach to obtain fMRI-based data reflecting distinct cognitive profiles in aging. A novel finding of our study was that within the cognitively stable individuals, baseline WM performance suggested different age-related trajectories at a functional level. The high-performing and stable older adults showed a reduced brain activity across time, while the decliners expressed a longitudinal activity increase, evidencing neural efficiency and attempted compensation mechanisms, respectively. On the other hand, the cross-sectional approach highlighted an 'under-activation' for the SLP, suggesting a disrupted neural load-dependent adaptability. Furthermore, although structural decline occurred across time in the whole sample, the rate of atrophy was higher for the decliner group compared to stables. Finally, it should be noted that these results were found in the context of lower years of attained education in SLP, probably uncovering that these subjects are not able to engage plastic neural responses related to cognitive and/or brain reserve mechanisms, but can maintain the performance (even though at a low level) because they experiment low rates of structural atrophy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

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Study 4

Vaqué-Alcázar, L., Abellaneda-Pérez, K., Solé-Padullés, C., Ruffini, G., Bargalló, N., Ros, E., Sala-Llonch, R. & Bartrés-Faz, D. Effects of multifocal transcranial direct current stimulation on working memory functional patterns in healthy older adults. *In preparation.*

Effects of multifocal transcranial direct current stimulation on working memory functional patterns in healthy older adults

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Background: The combination of transcranial direct current stimulation (tDCS) with functional magnetic resonance imaging (fMRI) can provide original data about brain changes in aging. Furthermore, tDCS procedures might moderate age-related brain functional changes associated to cognitive decline. In the present study, we aimed to investigate how two multifocal tDCS procedures based on distinct working memory (WM) patterns can modulate neural activity in older adults either showing cognitive stability or decline in this cognitive domain. **Method:** We studied 4-year longitudinal changes in fMRI, as well as differences across the tDCS montages in a sample of 24 healthy elders (mean age: 71.73 ± 2.65 years, 14 females), that were divided into cognitively stables (N=12) or decliners (N=12) according to the scores of a verbal N-back task performed while fMRI data was acquired. Two multifocal tDCS montages were designed to generate two distinct electric field distributions fitting either compensatory or maintenance fMRI patterns underlying WM performance, described in a previous publication from our group (Fernández-Cabello et al., 2016). We designed a sham-controlled crossover study on three separate experimental sessions. During the fMRI acquisitions, tDCS was applied using compensatory, maintenance or sham stimulation in a randomized counter-balanced order. **Results:** There was a significant time*group interaction after 4 years, where the decliners evidenced over-activation of non-related WM areas. As regards the effects of the simulation, we identified significant interactions between condition (sham- vs. *compensatory*-tDCS and sham- vs. *maintenance*-tDCS) and group (stables vs. decliners). Regarding the specific effects for each group and montage, only in the decliner group the *compensatory*-tDCS reduced the activity over the posterior regions where these subjects showed longitudinal hyperactivation. **Conclusion:** The present results reinforce the notion that tDCS effects depend on the underlying age-related functional organization and that these effects might be larger in more compromised systems. Moreover, our findings are also in accordance with the hypothesis that tDCS is able to 'normalize' functional patterns sustaining cognitive decline in aging.

Keywords: aging; functional magnetic resonance imaging (fMRI); transcranial direct current stimulation (tDCS); working memory (WM); compensation; maintenance.

Highlights

- Combining tDCS and fMRI in aging is a powerful approach in order to understand the tDCS-induced effects and to better characterize age-related functional changes.
- tDCS modulations depend on the underlying age-related functional organization and are of greater magnitude in more compromised systems.
- The application of tDCS may normalize functional WM patterns sustaining cognitive decline in aging.

1. INTRODUCTION

Non-invasive brain stimulation (NIBS) techniques such as transcranial direct current stimulation (tDCS) allow modifying brain functioning and cognition in the elderly (Holland et al., 2011; Meinzer et al., 2013; for a review see Abellaneda-Pérez et al., 2019a). More precisely, these techniques permit to interrogate and characterize in a controlled manner the underlying brain functional patterns sustaining cognitive functions in humans (Abellaneda-Pérez et al., 2019b; Solé-Padullés et al., 2006; Vidal-Piñeiro et al., 2014). Noteworthy, inter-individual variability in response to distinct NIBS procedures have been described (i.e., Hamada et al., 2013; López-Alonso et al., 2014). Potential variables contributing to the mentioned variability include differences in the cognitive baseline performance (Krause & Kadosh, 2014; Lukasik et al., 2018); anatomical dissimilarities between subjects (Kim et al., 2014), distinct functional connectivity profiles (Nettekoven et al., 2015), differences in cortical excitability (Jannati et al., 2017), and even genetic background (i.e., Di Lazzaro et al., 2015; Stephens et al., 2017). As great efforts are being carried out to improve brain function and cognition in aging (i.e., Antonenko et al., 2018; Sandrini et al., 2014, 2016), it becomes necessary to identify the individual predictors of responsiveness at the large-scale brain level to improve the potential of NIBS procedures (de Lara et al., 2017; Thair et al., 2017).

The high variability reported among NIBS effects might allow characterizing individual factors at the neural level that might not be detectable with descriptive neuroimaging techniques (Abellaneda-Pérez et al., 2019b). In this sense, investigating how dissimilar brain features are related to differential brain stimulation effects appear to be of particular relevance in the aging process since increasing inter-individual differences in brain and cognition is the most noticeable defining characteristic of the aged brain (Lindenberger, 2014; Nyberg et al., 2012). Here, and albeit at a population level, cognitive abilities such as episodic memory, speed of processing or working memory (WM) are generally negatively impacted by advancing age (Park & Reuter-Lorenz, 2009), cognitive dysfunction is not a universal phenomenon in aging (Nyberg & Pudas, 2019) and some elders are able to achieve stable performance (Gorbach et al., 2017; Lin et al., 2017; Nyberg et al., 2012). The available data suggest that successful cognitive aging may be possible through the emergence of functional reorganizations that face age-related brain changes (i.e. by *compensation*, Cabeza et al., 2018; Stern et al., 2018) or, alternatively because there are minimal detectable brain changes with age, including little brain pathology, in some individuals (i.e. *maintenance*, Nyberg et al., 2012). In this line, functional magnetic resonance imaging (fMRI) studies hold up that some elders are able to compensate by engaging additional brain resources in order to counteract the age-related damaged functioning (Cabeza, 2002; Cabeza et al., 2004, 2018; Cabeza & Dennis, 2013; Grady et al., 2006; Mattay et al., 2006; Reuter-Lorenz & Park, 2014). However, in many cases, the interpretation of this over-activation is not straightforward and some investigations have suggested that these additional recruitments could be reflecting a less efficient use of typical neural resources (Morcom et al., 2007; Rypma et al., 2007; Zarahn et al., 2007), not necessarily leading to better performance. Further, longitudinal approaches revealed that under the brain maintenance hypothesis (Nyberg et al., 2012), cognitive stability is associated with a lack of longitudinal changes, or even activity reductions for those subjects with high performance (Nyberg et al., 2010; Vaqué-Alcázar et al., 2020).

Within the cognitive aging field, it has been demonstrated that tDCS may be useful to modulate age-related functional reorganizations (Meinzer et al., 2013, 2014; Perceval et al., 2016) and hence holds potential to be considered a powerful intervention in this population (i.e., Tatti et al., 2016). WM, a cognitive domain typically affected with advancing age, has been frequently targeted with tDCS in aging although its effects are not clear yet (Jones et al., 2015; Nilsson et al., 2015, 2017; Park et al., 2014; Stephens & Berryhill, 2016). It has been suggested that tDCS might modulate WM differentially in subjects with distinct profiles (Berryhill & Jones, 2012). However, the neural substrates of tDCS over WM-related neural patterns are greatly unknown (i.e., Abellaneda-Pérez et al., 2020; Orlov et al., 2017), particularly in aged subjects, were no previous studies have been conducted evaluating this question. In this light, in the present study we conducted a cross-over sham-controlled design applying two distinct multifocal tDCS montages based on a previous study from our group (Fernández-Cabello et al., 2016), one emulating *compensation* and the other one fitting a *maintenance* WM pattern, during the performance of an N-back task inside the MRI scan. Our aims were (1) to explore if these two modelings impact differentially on the two distinct aging population groups (stables vs. decliners) and (2) to elucidate if these tDCS-induced effects are related to the 4-year follow-up fMRI trajectories underlying stability or decline in WM performance. Thus, we hypothesized that subjects who decline will be more clearly modulated than the stable group (Abellaneda-Pérez et al., 2019b; Nilakantan et al., 2019; Peña-Gómez et al., 2012), and that tDCS-induced effects will be primarily driven via a normalization process of functional patterns, which could be related to a performance improvement (i.e., Meinzer et al., 2013, 2015).

2. METHODS

2.1. Participants and group classification

Twenty-four subjects aged (mean: 71.73 ± standard deviation, SD: 2.65) with normal cognitive functioning were recruited by a phone call from a larger cohort (Rajaram et al., 2017; Sala-Vila et al., 2020) in order to be enrolled in this study. Exclusion criteria were illiteracy or inability to understand the protocol or to undergo neuropsychological tests, any relevant psychiatric illness, dementia or other neurodegenerative diseases (i.e., Parkinson's disease), and any NIBS contraindication (Antal et al., 2017; Rossi et al., 2009). Before the stimulation sessions (detailed in the next section) a set of neuropsychological tests was administered in order to ensure that all participants had a normal cognitive profile with mini-mental state examination (MMSE) scores > 25 (Mitchell, 2009) and performances not more than 1.5 SD below normative scores adjusted for age, gender and years of education on any of the neuropsychological tests administered (i.e., they did not fulfill cognitive criteria for mild cognitive impairment, Petersen & Morris, 2005). The study was approved by the University of Barcelona's Bioethical Committee and has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from each participant prior to enrollment.

For the classification criteria (i.e., stables vs. decliners) we applied the same approach used in our previous publication (Vaqué-Alcázar et al., 2020), which is based on the performance of the N-back fMRI task. We undertook a principal component analysis (PCA) to create a composite scale which represented the WM factor (WMf), calculated using the *d prime* (d') scores from the 0-back, 1-back, 2-back and 3-back conditions during the fMRI task (for further details of the task see section 2.3.3

below). We calculated the change in WMf, using data acquired at the time of the present study and 4 years before. We classified subjects as stables (N = 12) or decliners (N = 12), according to the change in WMf (above/below zero, respectively).

2.2. Multifocal tDCS parameters

We designed two multifocal tDCS montages in collaboration with Neuroelectrics® (Barcelona, Spain) planning system to obtain the electric field distribution in the brain cortical surface (Ruffini et al., 2014) fitting two fMRI patterns identified previously using the same N-back task (Fernández-Cabello et al., 2016). The first pattern describes age-related over-activation in fronto-parietal regions (i.e. suggesting a *compensatory* pattern, Fig. 1A), whereas the other showed no differences compared with young individuals (i.e. suggesting a possible *maintenance* mediation effect of preserved WM function, Fig. 1B). Based on these findings, we designed two multifocal tDCS montages to modulate accordingly the expression of brain regions observed in each of the fMRI patterns: *compensation* (see Fig. 1C) vs. *maintenance* (see Fig. 1D).

tDCS was delivered through a MRI-compatible StarStim Neuroelectrics® system using 8 cm^2 circular MRI Sponstim electrodes placed inside a sponge and into the holes of a neoprene cap corresponding to the 10/10 international system for electrode placement, with the central Cz position aligned to the vertex of the in every subject to ensure an accurate placement. The stimulator was situated outside the MRI room and the electrodes' sponges were soaked with saline solution and a thin layer of Ten20 conductive paste to ensure good conductivity and stability throughout the MRI acquisition. In the compensation-tDCS condition, which attempted to promote over-activation, the 8 electrodes were positioned over the scalp at AF7, F4, FC5, P3, P4, P7, P8 and Cz. On the other hand, in the *maintenance*-tDCS pattern, which aimed to exert both activation and deactivation, the electrodes were placed over the AF3, C3, C4, F4, FC6, FPZ, OZ and Cz (Fig. 1C & 1D). The current was delivered to each electrode with a wireless neurostimulator connected to a computer via Bluetooth. The maximum current delivered by any electrode was 2mA, while the maximum current injected through all the electrodes was 4 mA. In the sham condition, either the compensation or maintenance electrode distribution were randomly used. In all groups the current was delivered during 25min and it was initially increased and finally decreased in a ramp-up and ramp-down of 30s. For the sham condition, either compensation or maintenance electrode distributions was randomly used and the current dosage was composed of a ramp-up of 30s immediately followed by a 1min ramp-down at the beginning and at the end of the stimulations, in order to simulate the real stimulation fade-in/out.

2.3. Experimental setups

2.3.1. Retrospective longitudinal analysis

Longitudinal fMRI data was available for all subjects. First, we used the performance scores of this longitudinal data in order to establish the groups (stables vs. decliners). Then, we carried out a retrospective longitudinal fMRI analysis in order to elucidate the underlying task-related patterns of activation for each group, as showed in Fig. 1E. We compared the time point 1, as the baseline, with the sham-tDCS session in the tDCS study, as the follow-up measure. Both acquisitions were separated 4 years (mean: 3.88 years ± SD: 0.68).

2.3.2. Cross-over design

We designed a single-blind, sham-controlled, crossover study where the participants completed a verbal N-back task inside the

scan on three separate days. During the fMRI WM task, participants were stimulated in an online fashion with compensatory, maintenance and sham condition. The participants were randomly assigned to one of the three experimental conditions on each day and all of them underwent the three sessions at the end of the experiment with a wash-out period of ~1 month (mean: 32.5 days ± SD: 4.35). Each fMRI WM session was proceeded by a short N-back training outside the scan to ensure that all the participants were able to correctly answer equal or above the 50% in the most difficult load of the task (i.e., 3-Back task). The N-back task inside the scan was performed during the last 11 min of the stimulation period (14 minutes after the beginning of the electrical induction, Fig. 1E).

2.3.3. N-back task

Subjects performed a letter N-back task with different levels of memory load (from 1 to 3 letters to be retained) that were presented in a pseudo-random order during 11 min inside the MR scan. As described in Sala-Llonch, et al., we used a block-designed task where each N-back condition lasted 26s, followed by inter-block fixation periods of 13s. Before any N-back block, an instruction screen was shown to inform the subject about the upcoming block. Each stimulus (capital letter A-J) was presented in white in the center of a black screen during 500ms, with an inter-stimulus interval of 1500ms. Subjects were instructed to press a button when the letter shown matched the one seen one (1-back), two (2-back) or three (3-back) stimuli before or when the letter "X" appeared (0-back). The individual performance was recorded and scores were calculated using the d' measure (which accounts for correct responses and false alarms), computed: Z(hit rate) - Z(false alarm rate) where function Z(p), $p \in [0,1]$, as the inverse of the cumulative distribution function of the Gaussian distribution of the hits and false alarms rates. Mean response time (RT) in each condition were also recorded (Sala-Llonch, et al., 2012).

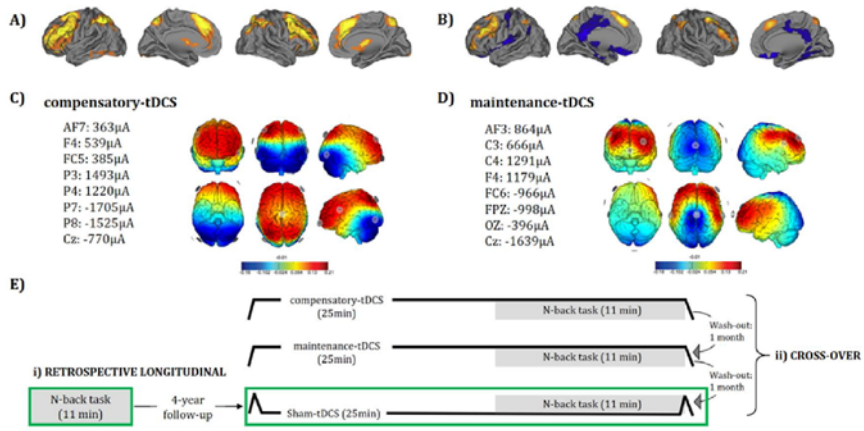

Figure 1. Target fMRI patterns in A) compensation and B) maintenance (adapted with permission from Fernández-Cabello et al., 2016). Current intensities, electrode positioning and electric field distribution in the brain cortical surface for both multichannel stimulation montages using the Neuroelectrics Instrument Controller (NIC) engine software in C) *compensatory*-tDCS and D) *maintenance*-tDCS montages. Positive intensity values are shown in red-yellow and negative in blue-light-blue. E) Experimental setups combining (i) retrospective longitudinal analysis (measures in green) and the (ii) cross-over approach to elucidate the tDCS-induced effects.

2.4. MRI acquisition

MRI was acquired in a 3T Siemens scanner (Magnetom Trio Tim syngo) at the *Unitat d'Imatge per Ressonància Magnètica IDIBAPS (Hospital Clínic)*, Barcelona, at all sessions. The participants underwent an fMRI acquisition (T2*weighted EPI scans, repetition time [TR] = 2000 ms, echo time [TE] = 16 ms, 336 volumes, 40 slices, slice thickness = 3 mm, interslice gap = 25%, field of view [FOV] = 220 mm, matrix size = 128 × 128) during the performance of the N-back task. For all participants, MRI images were examined by a senior neuroradiologist (N.B.) for any clinically significant pathology (none found). Then, all the acquisitions were visually inspected before analysis by the first author (L.V.-A.) to ensure that they did not contain significant MRI artifacts or excessive motion. At this level, no images were excluded.

2.5. Neuroimaging analyses

2.5.1. Functional magnetic resonance imaging (fMRI)

For the image analysis we used the non-parametrical tool FEAT-FSL (FMRIB's Software Library version 5.0.6; http://fsl.fmrib.ox.ac.uk/fsl/; Jenkinson et al., 2012). Individual fMRI scans were pre-processed, including non-brain tissue removal, motion correction with MCFLIRT (Jenkinson et al., 2002), spatial smoothing with a Gaussian kernel of 5 mm of FWHM, temporal filtering with a high pass filter of 160 s, and a registration to a 2mm standard template. Then, at the first level analysis (Woolrich et al., 2001), data were fit to a general lineal model (GLM) containing the task time-series with a gamma convolution of the hemodynamic response function as well as the motion parameters estimated previously, as confounding variables. In this GLM, four regressors and their first temporal derivatives were modeled: 0-back, 1-back, 2-back and 3-back. By including the derivatives, we aimed to correct for shifting in the time series as well as for slice timing effects. We defined a single contrast of interest combining the previous four regressors as the difference of brain activity between the highest and lowest loads (3-back > 2-back > 1-back > 0-back), by using weights of 0.375, 0.125, -0.125 and -0.375.

The results of the first level analyses were further fit into a higher-level analysis, performed using the FMRIB's Local Analysis of Mixed Effects (FLAME; Woolrich et al., 2004). The following variables were evaluated in the group-level statistics: group (stables, decliners), time (for the longitudinal evaluation: baseline, sham-tDCS), and tDCS session (sham-tDCS, *compensatory*-tDCS, *maintenance*-tDCS). Summarizing, the fMRI analyses could be grouped in two substudies:

Retrospective longitudinal study

We first calculated the 4-year follow-up trajectory for the whole sample (i.e., longitudinal differences between baseline and sham-tDCS fMRI sessions). Then, we created a group GLM designs to evaluate: (1) time (baseline vs. sham-tDCS) * group (stables vs. decliners) interaction; (2) longitudinal trajectories for each group; and (3) differences between groups at each time point.

Study of tDCS effects in a cross-over design

Following a similar procedure, we compared each active tDCS montage (compensatory and maintenance) vs. sham for the whole sample. Subsequently, we tested: (1) condition (sham- vs. *compensatory*-tDCS and sham- vs. *maintenance*-tDCS) * group (stables vs. decliners) interactions; (2) changes induced by each tDCS montage at each group; and (3) differences between groups at each tDCS session.

All the previously described analyses were performed in the whole brain at a voxel-wise

level, and a $z > 2.3$ was used to define contiguous clusters of activity. Then, cluster significance levels were estimated and corrected using Family-Wise Error (FWE) correction. The significance threshold was set at a corrected level of $p < 0.05$. Finally, we computed individual mean BOLD signal values within the significant region of interest (ROI) derived from the functional analyses in order to plot the fMRI data and to evaluate correlations with performance (see the following section).

2.6. Statistical analyses

Statistical analyses for non-imaging data were performed using IBM SPSS Statistics (Statistical Package for Social Sciences. Version 24.0. Armonk, NY: IBM Corporation). Demographic data was described as mean ± SD (Table 1). For categorical data, differences between groups were evaluated using the Fisher's exact test. For quantitative data, we evaluated differences between groups (stables vs. decliners) at each fMRI session (baseline, sham-tDCS, *compensatory*-tDCS and *maintenance*-tDCS) using independent-samples *t*-tests. In addition, when the normal distribution assumptions were not met as indicated by the Shapiro-Wilk test, was used Mann-Whitney's U-test pairwise comparisons. Similar to neuroimaging approach, the differences among group trajectories (differences between scan sessions) were investigated using repeated measures ANOVAs. As post-hoc pairwise analyses, differences between conditions (baseline vs. sham-tDCS, sham-tDCS vs. *compensatory*-tDCS, and sham-tDCS vs. *maintenance*-tDCS) for each group and across the whole sample were calculated using paired-samples *t*-tests. Finally, in order to identify possible associations between the changes at fMRI and at cognitive level, we performed correlations between the mean BOLD signal values within the significant ROIs derived from the imaging analyses and the WM scores (d' and RT at 3-back). The statistically significant difference for all the analyses was considered at $p < 0.05$. The graphical representations were performed using GraphPad Prism (version 6.00, GraphPad Software, La Jolla, CA, USA).

3. RESULTS

3.1. Demographics and N-back performance

First of all, we split the sample into stables, those showing WM scores stability in the 4-year follow-up, and decliners, whose performance declined after this period. The main demographic characteristics for the whole sample and for each group are shown in Table 1. In summary, there were no group differences regarding age, gender or years of education. Nevertheless, as expected due to the stratification criteria, there were differences between groups showing that WMf scores were higher for the stables than for decliners participants at the sham-tDCS session (t=2.276, p=0.038). For further details regarding the cognitive group trajectories evaluated within the retrospective longitudinal approach see Supplementary Fig. 1.

3.2. Differential fMRI trajectories underlying WM stability and decline

We evaluated BOLD activity associated with the 'cognitive load' contrast in the WM task (3-back > 2-back > 1-back > 0-back). As compared to baseline, during sham-tDCS condition (4-year follow-up) there was an increased task-related activity on left areas comprising the frontal-orbital, insular and temporal cortex for the whole sample (Supplementary Fig. 2A). Then, regarding the comparison of fMRI trajectories between stables and decliners, we detected a significant time*group interaction (Fig. 2A).

The stable group showed a reduction of activity at 4-year follow-up, while the decliners exhibited increased activation in a cluster encompassing the left occipital and lateral-occipital cortex. Furthermore, the longitudinal progressions for each group were studied independently and the decliners showed increased activation within the same are identified in the time*group interaction (Fig. 2B, in red) and a significant reduction over the superior frontal gyrus after the 4-year follow-up (Fig. 2B, in blue).

No significant longitudinal differences were found considering only the stables participants. Finally, we detected a significant correlation between the occipital over-activation identified for the decliner group as post-hoc analysis (Fig. 2B, in red) and the decline of d' scores during the 3-back load of the task (r=0.621, p=0.031).

	TOTAL (N=24)	Stables (N=12)	Decliners (N=12)	Difference *stables vs. decliners*
Age	71.62 ± 2.65	72.25 ± 3.33	71.00 ± 1.65	p=0.551[a]
Gender (F/M)	14/10	8/4	6/6	p=0.680[b]
Years of education	13.38 ± 3.29	13.08 ± 4.23	13.67 ± 2.14	p>0.999[a]
MMSE	28.75 ± 0.94	29.00 ± 0.95	28.50 ± 0.90	p=0.219[a]

Table 1.
Demographic characterization. Data are presented as mean ± standard deviation for the whole sample and considering the two groups (stables and decliners).
Abbreviations: M/F, male/female.
[a] Mann-Whitney U test.
[b] Fisher's exact test.

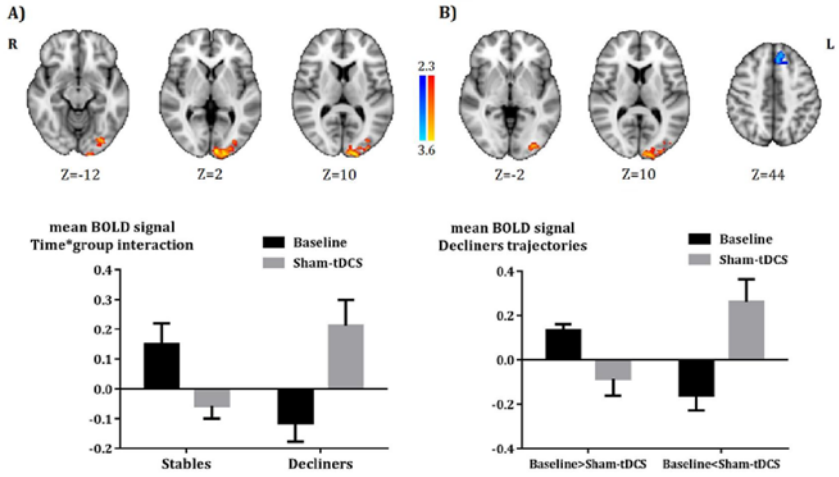

Figure 2. Comparisons in fMRI between stables and decliners. **A)** Time*group interaction, showing increases in decliners compared with stables after 4-year follow-up and plot of mean BOLD signal values at the ROIs in the two groups separated by baseline and sham-tDCS measures. **B)** Significant activity increases in the decliner group between baseline and sham-tDCS (in red), activity reductions (in blue), and the respective plot of mean BOLD signal values for the decliner group at the ROIs, separated by baseline and sham-tDCS measures. Error bars: ± 1 standard error of the mean (SEM).

3.3. Differential tDCS-induced changes in fMRI in stables and decliners

Considering stables and decliners, a significant condition*group interaction emerged for the *compensatory*-tDCS montage compared to sham-tDCS (Fig. 3A), showing differences in activity over the right parietal operculum cortex and Heschl's gyrus. In addition, when we investigated the *compensatory*-tDCS effect compared to sham-tDCS for each group separately, the decliners showed a significant activity reduction over posterior areas comprising the right lingual gyrus and bilateral occipital and lateral-occipital cortex, while no differences were detected for the stable participants (Fig. 3B).

Regarding the *maintenance*-tDCS montage, we also identified a significant condition*group interaction. However, in this case, the stable group showed an activity reduction, while the decliners' activity increased in a non-related WM area comprising the left central opercular cortex, caudate and placed partially over the white matter tissue (Fig. 4). The post-hoc analyses, revealed no significant differences during the *maintenance*-tDCS montage as compared to sham-tDCS when considering each group (stables and decliners) separately.

There were no group differences between stables and decliners measured in direct group comparison at any stimulation condition (sham-tDCS, *compensatory*-tDCS and *maintenance*-tDCS). In addition, we did not identify associations between the changes induced by tDCS measured with fMRI and the N-back task scores.

4. DISCUSSION

In the present study, our goals were to elucidate if two tDCS configurations (compensatory and maintenance) impact differentially on the two distinct aging population groups (stables and decliners) and to elucidate whether if tDCS-induced effects in older adults are related to the fMRI patterns sustaining stable or decline trajectories in the context of WM. For these proposes, we separated the sample into stables and decliners according to their 4-year follow-up performance trajectory. The retrospective 4-year longitudinal analysis revealed a significant time*group interaction, with the stables showing an activity reduction, while the decliners significantly increased their activation.

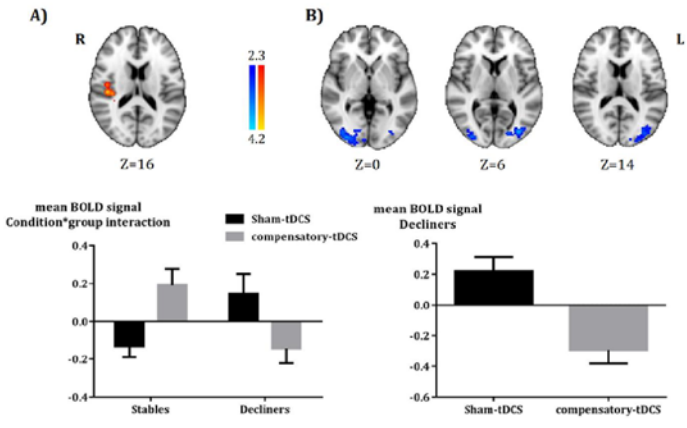

Figure 3. *Compensatory*-tDCS-induced effects in fMRI. **A)** Condition*group interaction, showing increases in stables and reductions in the decliner group induced by the *compensatory*-tDCS compared to sham-tDCS condition. Plot of mean BOLD signal values at the ROIs in the two groups separated by each stimulation session. **B)** Significant activity reductions in the decliner group in *compensatory*-tDCS condition as compared to sham-tDCS, and the respective plot of mean BOLD signal values of the decliner group at the ROI separated by each stimulation condition. Error bars: ± 1 SEM.

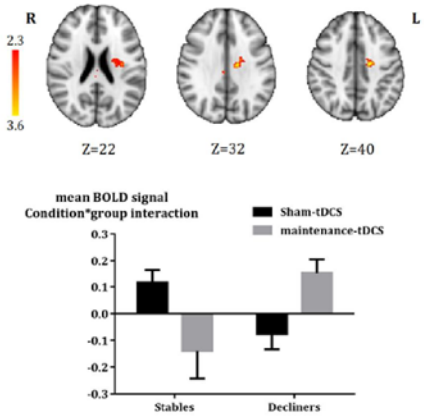

Figure 4. *Maintenance*-tDCS-induced effects in fMRI. Condition*group interaction, showing decreases in stables and increases for decliners after the *maintenance*-tDCS compared to sham-tDCS condition. Plot of mean BOLD signal values at the ROIs in the two groups separated by each stimulation condition. Error bars: ± 1 SEM.

At the cross-over approach to evaluate tDCS effects, there were two significant condition*group interactions under each active tDCS session (compensatory and maintenance) vs. sham. Post-hoc analyses revealed that under the *compensatory*-tDCS configuration, the decliners exhibited brain activity reductions in posterior cortical regions, overlapping the areas over-activated longitudinally for this group and thus, suggesting a tDCS-induced 'normalization' of brain activity WM-related pattern in this group.

Longitudinal fMRI changes sustaining stability and decline in WM

The retrospective longitudinal findings results evidenced differential progressions along time for each group supporting the 'brain maintenance' hypothesis (Nyberg et al., 2012), which should be cautiously interpreted given the small sample size. It should be noted, that we identify similar results in a former publication with a larger sample partially overlapping with the sample used in the present study but assessing differences in 2-year follow-up (Vaqué-Alcázar et al., 2020). The stable participants did not show significant longitudinal changes at fMRI level (Pudas et al., 2017; Rieckmann et al., 2017; Vaqué-Alcázar et al., 2020) stressing a preserved neural integrity (Nyberg et al., 2010), while the decliners reduce their activity in a left frontal region (i.e., WM-related area) and exhibited additional occipital over-recruitments (i.e., non-related WM area; Nyberg et al., 2012; Pudas et al., 2017; Rieckmann et al., 2017; Vaqué-Alcázar et al., 2020). The activity reduction could be interpreted as a resource limitation and as a compromised capacity for the decliners participants, whose are not able to engage the typically task-related areas (Stern, 2009), suggesting a loss of load-dependent adaptability of neural activity (Nagel et al., 2009, 2011; Nyberg et al., 2009; Steffener & Stern, 2012). The present results suppose new longitudinal evidence in the field supporting that over-activation patterns may be reproducing unsuccessful compensation (Cabeza & Dennis, 2013) or dedifferentiation mechanisms (Carp et al., 2011; Park et al., 2004; Rajah & D'Esposito, 2005), rather than successful compensation (Cabeza, 2002; Cabeza et al., 2004, 2018; Grady et al., 2006; Reuter-Lorenz & Cappell, 2008; Reuter-Lorenz & Park, 2014). Moreover, this idea was reinforced by the correlation identified between the greater activation of posterior regions and the WM decline (Eyler et al., 2011).

Differential tDCS-related fMRI effects between stables and decliners

We did not identify changes induced by tDCS for the whole sample, for any of the two configurations. We hypothesized that this lack of results could be due to heterogeneity in the individual response to tDCS (Dayan et al., 2013; Horvath et al., 2015), which, as described before, could be even larger among

elderly people due to the differential age-related impact (Tatti et al., 2016). However, when the sample was split into two separate more homogeneous groups according, to the 4-year follow-up WM stability or decline, we identified significant condition*group interactions for both the *compensatory*-tDCS and the *maintenance*-tDCS compared with sham-tDCS. The post-hoc analyses revealed that only decliners reduced brain activity when the *compensatory*-tDCS was applied. For that reason, the classification according to the cognitive trajectories (based on the N-back scores) supposed an interesting approach in order to uncover tDCS effects during this task, supporting the notion that the trajectory of cognitive changes is one of the individual characteristics influencing neural responses to tDCS interventions. Also, the present results reinforced the idea that the effectiveness of stimulation depends on the brain state (Krause & Kadosh, 2014). Similarly, previous studies have already found that baseline performance levels may predict stimulation response to tDCS (Berryhill & Jones, 2012; Martin et al., 2017; Meinzer et al., 2013; Peña-Gómez et al., 2012), but from our knowledge, this is the first approach focusing on the cognitive trajectories. Most notably, for the decliner group, the activity reduction after the *compensatory*-tDCS occurred in those brain areas where there was a longitudinal change. This is a relevant result because emphasizes that tDCS has the capacity of acting in those systems with larger age-related changes underlying suboptimal performance (Meinzer 2013, 2015; Nilakantan 2019), highlighting the importance of taking into account the specific individual age-related functional reorganization strategy in future tDCS interventions (reviewed in Abellaneda-Pérez et al., 2019a; see also Martin et al., 2017; Perceval et al., 2016). In addition, this tDCS-induced effect described for the decliner group, suggests that the combination of tDCS and fMRI may provide more information about brain functional patterns of suboptimal reorganization in aging compared with cross-sectional fMRI data alone. During the last years, the number of studies assessing the impact of tDCS on neural functioning during task-based fMRI has increased and in this light, it seems that age-related changes not only affect the magnitude of tDCS-induced modulation but also the pattern of underlying functional reorganization (Antonenko et al., 2018; Fiori et al., 2017; Martin et al., 2017). Thus, it seems that different electrode montages affecting specific brain areas interact with ongoing neural processes, accentuating the importance of considering the brain state underlying the specific task that we want to improve. Despite little is known about the explicit properties of tDCS on brain function in aging, our findings are in accordance with previous reports where anodal tDCS may boost neural efficiency by inducing a 'normalization' of task-related patterns through activity reductions (Holland et al., 2011; Martin et al., 2017; Meinzer et al., 2013, 2014). In addition, this finding for the decliner group was in accordance with the previously suggested knowledge that NIBS could exert a greater impact on those subjects that need it the most (i.e. Nilakantan et al., 2019), which is in line with previous hypothesis supporting that 'the more affected is a system, the larger are the NIBS effect' (Hsu et al., 2015). On the other hand, despite the condition*group interaction detected for the *maintenance*-tDCS session, the cluster result was small and comprised white matter areas, suggesting an unspecific effect of this tDCS configuration.

No significant WM performance changes induced by tDCS

There were no tDCS-induced effects on participants' N-back accuracy neither for the RT scores, which seems to be more easily modified by tDCS (for a meta-analysis see

Brunoni & Vanderhasselt, 2014). In the light of previous studies, the lack of significant changes in performance could partially be explained by the existence of larger delayed effects of tDCS in aging (e.g., Flöel et al., 2008; Jones et al., 2015; Park et al., 2014; Sandrini et al., 2014, 2016). In addition, mixed cognitive effects induced by tDCS coexist in the literature. Some studies reported improvements only for those subjects with high education (Berryhill & Jones, 2012) or high performance (Learmonth et al., 2015), others showed that high performance is related to a slight deterioration of accuracy induced by tDCS (Lukasik et al., 2018), while other studies have reported that low-performing participants would benefit more from tDCS while high-performers would not (Hsu et al., 2014, 2016; Tseng et al., 2012; Wu et al., 2016). It is also worth noting that we evaluated only the most difficult load of the N-back task and previous tDCS studies focusing on visuospatial WM have shown that response to stimulation may vary depending on the cognitive load (Wu et al., 2014). Our decision of studying the 3-back level in order to evaluate the cognitive effects of the tDCS was motivated by the fact that both tDCS active configurations were based on the previous findings identified considering the cognitive load (Fernández-Cabello et al., 2016). In turn, other authors also reported limited evidence of the WM gains induced by tDCS used alone (Nilsson et al., 2015), and also in combination with specific cognitive training programs (Jones et al., 2015; Nilsson et al., 2017; Park et al., 2014). Overall, this difficult to identify clear gains in WM performance could be explained by the complexity of this cognitive domain and the cognitive sub-processes that interact (Kessler & Meiran, 2008), which could be differentially sensitive to the potential modulation effects of tDCS.

Limitations

A first limitation of the present study was the sample size. Especially for this reason, the cluster-wise threshold set at $z = 2.3$ for the fMRI analyses should be considered lenient (see Eklund et al., 2016). Hence, and albeit present results are novel in that they offer first evidence of online functional brain correlates underlying multifocal tDCS effects in aging, it is important to consider that they should be interpreted with caution, and further investigations with larger samples are needed. According to the fact that WM is built as the combination of different cognitive processes linked to most areas of the brain (see Eriksson et al., 2015 for a review) we predicted that multifocal tDCS could modulate the WM-related systems in a more precise way than the conventional bifocal stimulation systems, which result in relatively non-focal stimulation and may affect the brain areas in between the two electrodes (Bortoletto et al., 2016; Kuo et al., 2013). Unfortunately, because we did not perform a direct comparation between methodologies we could not evidence whether multifocal tDCS supposes an advantage in front of the conventional bifocal tDCS setup.

Conclusions

In conclusion, research into the effects of tDCS in aging seems to be highly relevant to reach a better understanding of the neural mechanisms underlying cognitive aging. Thus, future studies combining NIBS and functional imaging techniques are needed to scrutinize the tDCS effects (or the lack of thereof) and the cognitive relevance of these changes. At the same time, the combination of both methodologies is leading to a more detailed understanding of brain-behavior association in aging, along with the inherent processes triggered to counteract age-related neural adjustments (Abellaneda-Pérez et al., 2019b; Bergmann et al., 2016; Habib et al.,

2007). Moreover, the present study suggested that stimulation technique' effects depend on the underlying age-related organization of functional brain patterns, stressing that among the large variability in advanced age mediating tDCS effects, the 'brain state' is a crucial factor to consider in order to predict the tDCS action (Krause & Kadosh, 2014; Polanía et al., 2018). Finally, the larger tDCS induced changes for the decliner group reinforce the idea that NIBS-related effects are greater in the more compromised system and are linked with activity ameliorations that allow returning the brain dynamics to a more youthful profile of activity. In this light, it remains to be elucidated if the capacity of tDCS protocols to restore functional patterns would be an effective intervention to improve performance in those subjects who suffer the greatest age-related cognitive decline or on the contrary, modifying the functional reorganization adopted could be related to worse cognitive outcomes.

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CHAPTER 5

General Discussion

In the present Doctoral Thesis, which includes 4 studies, we have used advanced neuroimaging approaches and other novel techniques such as tDCS and brain-microarray measurements of regional gene expression, to investigate the brain structural, functional and molecular characteristics sustaining cognition in healthy aging. First, at the structural level, from different MRI modalities (i.e., CTh estimated from T1w-structural MRI and DTI from dMRI), we have described distinct effects of the level of education (as a proxy of CR) underlying preserved cognition in a healthy elder's population. These effects seemed to be related to the differential age-linked impacts on GM and WM. Higher rates of CR were associated with higher capacity to tolerate generalized WM disruption (i.e., which may suggest a compensatory mechanism in the context of high cognitive performance) and with higher CTh in the medial PFC (i.e., which may suggest BR and/or neuroprotection). In addition, in the second study, we found that such structural advantage was associated with a gene expression profile characterized by relative upregulation of gene sets implicated in neurotransmission and immune responses, proposing some of the biological mechanisms through which CR may operate. As regards cognition, we identified positive correlations between CR and the cognitive domains studied. Nevertheless, our findings corroborate that cognitive stability along time was not explained by different levels of CR. Then, using longitudinal functional analyses, we describe the distinct fMRI WMem-related patterns sustaining stability and decline in aging. The follow-up trajectories evidenced that the recruitment of non WMem-related regions and greater cortical atrophy seem to be underlying WMem decline in healthy aging. Moreover, preservation of a high cognitive level was related to no-changes in fMRI and CTh measures, in accordance with the BM hypothesis. Finally, combining fMRI and tDCS we found that the stimulation technique' effects depend on the underlying age-related organization of functional brain patterns, being greater in the more compromised systems and producing activity ameliorations that allow returning the brain dynamics to a more youthful-like activity pattern.

Aging is characterized by a wide heterogeneity among neuropsychological profiles, emphasizing the capacity of some elders to maintain a high level of performance. Our results revealed that elders with higher years of education (i.e., those with high CR) performed better on speed of processing, declarative memory, 'frontal lobe function' and WMem domains, in accordance with the classic postulates of CR (Stern, 2002, 2009). However, the CR effects seem to be related to distinct mechanisms in view of different

associations with MRI correlates and cognition. As regards the memory domain, we did not find any direct correlation between performance and CTh, maybe due to the relatively narrow age-range of our sample and/or the low variability in memory performance scores due to the fact that this cognitive domain starts to decline around 65s, while others, such as the speed of processing show early disruptions. In contrast to memory, the cognitive advantage of high education in speed of processing was directly related to greater CTh in PFC areas and higher generalized FA values (Kerchner et al., 2012; Madden et al., 2004; Salthouse, 2000; Sasson et al., 2013). Furthermore, through a mediation analyses, we confirmed that FA acts as a mediator of the association between education and speed of processing, indicating the impotence of the WM microstructure's role in explaining the high performance among highly educated elders. Notwithstanding the clear positive association between CR and cognition reported in our results, the longitudinal trajectories were not explained by different levels of CR. Concretely, in study 3, according to the classification into stables with high and low performance and decliners, a higher educational level was associated with higher initial WMem scores, whereas cognitive stability equally occurred amongst both high- and low-educated elders. These results concur with those of larger longitudinal studies suggesting that education mainly contributes positively to higher cognitive level in the intercept, rather than modulate the slope (i.e., being associated with a slower rate of decline; Seblova et al., 2019; Vemuri et al., 2014; Wilson et al., 2019).

Despite the cross-sectional nature of study 1, our results indicate that the slopes between chronological age and indicators of brain integrity were modulated by CR estimates, suggesting the existence of a putative neuroprotective effect of education on GM of the medial PFC including anterior cingulate areas (Arenaza-Urquijo et al., 2013; Boller et al., 2017; Kim et al., 2015; Lee et al., 2016), in parallel to a compensatory role in the maintenance of memory performance in the context of a greater age impact on WM integrity (Arenaza-Urquijo et al., 2011; Dufouil et al., 2003; Nebes et al., 2006). WM alterations in aging have been extensively reported (Bennett et al., 2010; Laukka et al., 2013; Salat, 2011) and the fast FA decline in this age range studied is in accordance with prior findings (Sala et al., 2012; Sexton et al., 2014b; Westlye et al., 2010). On the other hand, the lack of negative correlations between CTh and age is supported by other authors (Hogstrom et al., 2013; Storsve et al., 2014), who propose that the CTh lifespan progression shows a dynamic relationship with surface area changes, as well as the existence of both age-related linear

and non-linear CTh changes described (Fjell et al., 2014b). However, study 3, revealed clear patterns of cortical atrophy among the whole sample, suggesting a more sensitivity of longitudinal approaches. Indeed, the differences among rates of CTh decline between stables and decliners could partially explain our failure in order to obtain direct associations between age and CTh.

Besides the results observed using structural MRI approximations over the PFC area, study 2 revealed that these regions associated with higher CR estimates were related to the upregulation of genes involved in cell communication and neurotransmission processes. The majority of the upregulated leading-edge genes encode for key ionotropic glutamate receptors, nicotinic acetylcholine receptors, as well as GABAA receptor subunits, suggesting an important role for these brain areas in fast excitatory and inhibitory signaling. This molecular characteristic corresponds to the well-described role of the PFC areas as highly active, functionally connected network hubs subserving transmodal information integration (Braga et al., 2013; Buckner et al., 2009; Sepulcre et al., 2012). Moreover, neurotransmitter signaling through calcium-permeable ion channels were identified, particularly glutamatergic AMPA and NMDA receptors as well as nicotinic acetylcholine receptors, which is considered to be one of the most important molecular features of postsynaptic plasticity (Anggono & Huganir, 2012; Pankratov & Lalo, 2014). In addition, we also identified several other upregulated gene set families involved in G-protein-coupled transmembrane signaling through neuropeptide and purinergic receptor binding. Beyond their role in homeostatic and neuroendocrine processes, neuropeptide/G-coupled receptor signaling is increasingly recognized as a mediator of activity-dependent refinement of local brain circuits and plasticity (McClard & Arenkiel, 2018), including important roles for learning and memory (Gøtzsche & Woldbye, 2016). A second category of genes set that were found related to immune processes, suggesting a sensitivity of the identified cortical regions for microglial activation (Hopperton et al., 2018), that transiently seems to play an important role in the clearance and degradation of misfolded protein aggregates associated with neurodegenerative diseases, such as the extracellular Aβ fibrils (Cai et al., 2014; Cho et al., 2014). In summary, our results suggest that the gene expression profile and enriched biological pathways of the PFC CR-related regions may entail fundamental mechanisms to account for why these brain areas might develop a central role mediating the positive physiological and cognitive effects of high educational attainment.

Regarding functional findings, we identified that the subjects who significantly loss performance showed increased task-related brain activity at follow-up compared to baseline, while those stables with high performance showed reduced spatial extend of activity. The WMem-related fMRI trajectories from study 3 and study 4 should be cautiously interpreted given the small sample sizes, but they support the BM hypothesis (Nyberg et al., 2012). That is, the stable participants did not show significant longitudinal changes at fMRI level (Pudas et al., 2017; Rieckmann et al., 2017) stressing a preserved neural integrity (Nyberg et al., 2010). Even those showing higher level of performance were able to reduce the activation suggesting a more efficient use of their neural resources (Nyberg et al., 2010). On the contrary, the decliners tend to reduce their activity in WMem-related areas and exhibited additional over-recruitments in non-related WMem regions (Archer et al., 2018; Nyberg et al., 2012; Pudas et al., 2017; Rieckmann et al., 2017). The activity reduction could be interpreted as a resource limitation and as a compromised capacity for the decliner's participants, who are not able to engage typically task-related areas (Stern, 2009). The present results suppose new longitudinal evidence in the field supporting that over-activation patterns far to reflect a successful compensatory mechanism (Cabeza 2002; Cabeza et al., 2004, 2019; Grady et al., 2006; Reuter-Lorenz & Cappell, 2008; Reuter-Lorenz & Park, 2014), may be reproducing unsuccessful compensation (Cabeza & Dennis, 2013) or dedifferentiation (Carp et al., 2011; Park et al., 2004; Rajah & D'Esposito, 2005). Most notably, the cross-sectional group comparisons evidenced reduced activation in task-related areas for the group of stables with low performance, supporting the hypothesis that a highly demanding task could give rise a shift to the 'under-activation' (i.e., CRUNCH; Reuter-Lorenz & Cappell, 2008). The latter finding could be partly explained by the low educational level (Archer et al., 2018) of this group, which may be related to a reduced neural capacity (Stern, 2009). Altogether, as pointed out by Nyberg et al., (2010), the combination of cross-section and longitudinal approaches supposed a powerful advantage in order to better characterize the fMRI mechanisms sustaining cognitive aging. In our case, from a cross-sectional prospective, results suggested that a higher WMem performance would be associated with higher WMem load-dependent adaptability of neural activity (Nagel et al., 2009, 2011; Nyberg et al., 2009; Steffener & Stern, 2012). Furthermore, using a longitudinal approach, our results contributed to the idea that preserving a youthful functional signature is a crucial aspect for a well-maintained cognition (Johansson et al., 2020; Nyberg et al., 2012; Pudas et al., 2013; Vidal-Piñeiro et al., 2019), rather than

activating compensatory mechanisms to counteract the damaged normal functioning (Morcom & Henson, 2018). In addition, we reported differences across time as regards atrophy rates over a left temporo-occipital area, where the decliners showed a more noticeable and generalized pattern of change than subjects exhibiting WMem performance stability (Cook et al., 2017; Harrison et al., 2012), indicating differences in the temporal evolution of brain structure between these groups.

Finally, combining fMRI and NIBS, we only detected significant tDCS-induced changes for those subjects who showed cognitive decline. In concrete, through the application of multifocal tDCS using the compensatory configuration, we described an activity reduction over occipital areas, where there were identified 4-year longitudinal over-activations for this group. This result was in accordance with previous reports where anodal tDCS seems to boost neural efficiency by inducing a 'normalization' of task-related patterns through activity reductions (Holland et al., 2011; Martin et al., 2017; Meinzer et al., 2013, 2014). This is a relevant result because emphasizes that tDCS has the capacity to modifying those systems with larger age-related changes underlying suboptimal performance (Meinzer et al., 2013, 2015; Nilakantan, 2019), which is in line with the previous hypothesis supporting that 'the more affected is a system, the larger are the NIBS effect' (Hsu et al., 2015). Thus, it seems that at least partially the large variability in advancing age mediating tDCS properties was explained by the specific individual age-related functional reorganization strategies (reviewed in Abellaneda-Pérez et al., 2019a; Perceval et al., 2016). In this vein, the new knowledge in the field should be useful to facilitate the development of more personalized interventions aiming to promote successful cognition in aged populations (Cattaneo et al., 2018; Sheng et al., 2018). Remarkably, this tDCS-induced effect described for the decliner group, suggests that the combination of tDCS and fMRI may provide more information about brain functional patterns of suboptimal reorganization in aging than cross-sectional fMRI data alone, suggesting the potential of combining these techniques as a possible prognostic marker of cognitive trajectories (as proposed with TMS by Abellaneda-Peréz et al., 2019b).

Overall, we put forward an integrative model with different paths, including genetic contributions and lifestyle factors as modifiers of the relationship between age-related changes at brain structural and functional level and cognitive stability or decline (Figure 21). Since it is well-established that compensation processes are related to successful

cognition, this association is not as clear for longitudinal cognitive trajectories. In this line, our investigations emphasized that cognitive stability is supported by preserved brain structural and functional characteristics rather than by trigger compensatory mechanisms. As regards the modifiers of these relationships, there is compelling evidence suggesting that genetic factors can affect brain features or cognition in a direct sense and/or indirectly via epigenetic mechanisms through which environmental and lifestyle factors can modify gene expression (Nyberg & Pudas, 2019). It is also important to acknowledge that, lifestyle factors such as education are able to modulate all the elements illustrated in Figure 21 and their relationships. Notwithstanding years of education is a variable most often considered as a lifestyle factor, it is also partially genetically determined (Branigan et al., 2013; Okbay et al., 2016) because of gene-environment correlations imply that individuals with higher innate ability tend to acquire more years of education. Moreover, these genetic determinants likely overlap with those for general cognitive function (Trampush et al., 2017). In addition, it is relevant to consider that some of the same life exposures associated with different CR levels have also been found to be active in BM (Stern, 2017) and it has been predicted that older individuals with exceptionally good cognition have both low genetic susceptibility to biological aging and effective compensatory mechanisms to counteract the age-related impairments (Park & Reuter-Lorenz, 2009).

So far, the present findings put in relevance the importance of studying cerebral changes from a multimodal perspective, which helps us to understand the aging process from a multifactorial approach. Similarly, the designs combining both cross-sectional and longitudinal data suppose a methodological advantage when interpreting and discussing the findings. For instance, regarding our specific results, higher education was associated with better WMem performance at baseline but not with a stable cognitive trajectory (Nyberg et al., 2010). Moreover, also based in our data, cognitive stability was linked with greater efficiency of brain circuits over time, whereas over-activations observed in cross-sectional approaches may reflect stable performance or decline. From all this, the multimodal study of brain characteristics supporting CR has allowed identifying the medial PFC as a valuable structural feature among subjects with high CR. Notwithstanding CR seems to not exert a protective effect against the age damage, the associated upregulation of genes related to plasticity mechanisms in CR-related areas, could underlie the functional efficiency described longitudinally for the stable subjects with higher CR estimates. In

addition, the identification of such structural, functional and molecular characteristics associated with CR could afford new targets for boosting potential cognitive interventions with NIBS (Abellaneda-Pérez et al., 2019a).

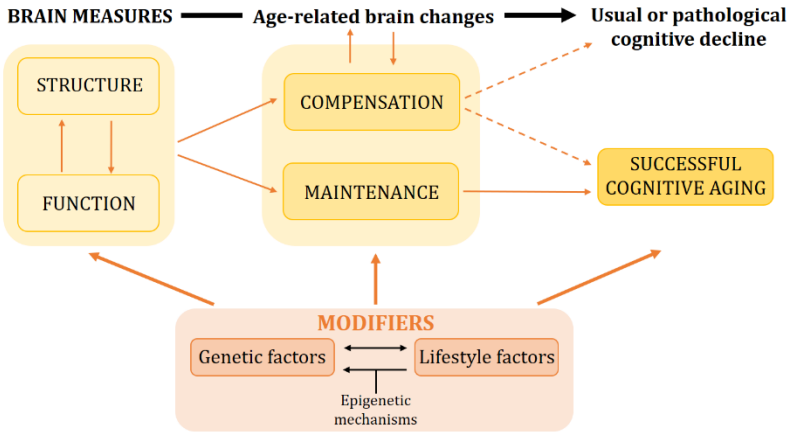

Figure 21. Model of life-course paths to different levels of cognition in elderly. The brain structural and functional characteristics may influence cognition through different paths. The modifiers' box, where genetic factors, lifestyle exposure and their interaction were included, can potentially have a direct effect on any box, or a moderating effect on any path (indicated with orange narrows). The dashed lines between compensation and cognition represent the discrepancies in this field, where compensation mechanisms had been related to both decline and successful cognitive aging.

CHAPTER 6

Conclusions

Through the combined analysis of the studies included in this Doctoral Thesis, we can conclude that:

1. CR, measured as years of education, is strongly associated with higher cognitive performance in aging, whereas it does not differentially influence cognitive trajectories.

2. Both neuroprotective and compensatory mechanisms linked with CR coexist amongst healthy elders, indicating that greater capacity to tolerate brain changes may be revealed in response to the age-related effects.

3. CR-related frontal areas exhibit a distinct gene expression profile characterized by relative upregulation of gene sets implicated in biological mechanisms that may underlie the CR's capacity for plastic changes, such as cell communication, neurotransmission and immune response.

4. Among WMem stable elders, there are different age-related fMRI trajectories according to the performance level. High performers show high CR, neural efficiency and preserved structure, while low-performers have lower CR rates and their fMRI patterns suggest disruption of load-dependent adaptability.

5. WMem decliners exhibit longitudinal fMRI over-activations in non-related task areas, evidencing unsuccessful compensation mechanisms together with higher rates of cortical atrophy.

6. tDCS effects depend on the underlying age-related organization of functional brain patterns, revealing that modulations are greater in more compromised systems and are linked with restoring brain dynamics into a more youthful activity profile.

APPENDIX

Supplementary Material

Supplementary material for:

Functional and structural correlates of working memory performance and stability in healthy older adults

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1. Demographics and cognitive performance

The study of the demographical variables revealed no group differences regarding age and APOE status. Nevertheless, differences in years of education when the sample was divided into three groups were detected (F = 7.723, p < 0.001), where the SLP group disclosed a lower educational level than the SHP (t = 3.824, p = 0.001) and decliners (t = 2.876, p = 0.007). Moreover, the three groups also differed regarding gender ($\chi 2$ = 8.313, p = 0.016) due to the small representation of females in the SHP compared to SLP (p = 0.039) and decliners (p = 0.011). No more differences were detected at baseline. At the follow-up, although MMSE punctuations differed between the three groups (F = 3.564, p = 0.037), the post-hoc comparisons did not evidence significant pairwise differences.

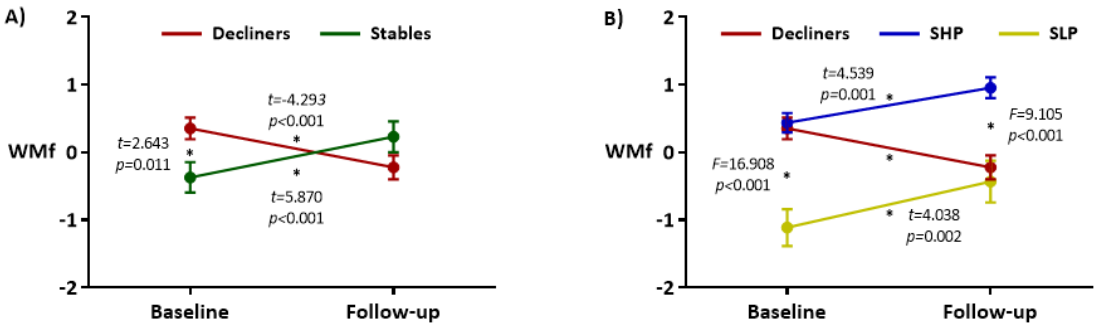
As expected, due to the stratification criteria, there were numerous differences in WMf scores between groups. The WMf did not decline significantly between baseline and 2-year follow-up assessment for the whole sample (p = 0.891). However, when considering stables and decliners, we identified a time-group interaction (F = 48.037, p < 0.001). Subsequent pairwise analyses revealed that, at baseline, decliners exhibited higher performance than stables (t = 2.643, p = 0.011). At follow-up, no significant differences were detected between these groups. When the sample was split into three groups, there was another time-group interaction (F = 23.942, p < 0.001), being the WMf scores for SLP lower than for SHP (t = -4.917, p < 0.001) and decliners (t = -4.964, p < 0.001) at baseline. At follow-up, the SHP group remained performing better than SLP (t = 3.907, p = 0.001) and a significant difference emerged between SHP and decliners (t = 4.164, p < 0.001). As regards longitudinal changes, there were significant differences at follow-up compared to baseline, evidenced by an increase of the WMf for stables (t = 5.870, p < 0.001) and their respective subgroups (SHP: t = 4.539, p = 0.001; SLP: t = 4.038, p=0.002), and a reduction for decliners (t = -4.293, p < 0.001), (Supplementary Table 1 and Supplementary Figure 1).

Finally, as a supplemental exploratory analysis, we investigated differences between group trajectories for each d' measure composing the WMf (0-back, 1-back, 2-back and 3-back). For this purpose, the same statistical analyses used for the WMf were carried out. Taking into account stables and decliners (Supplementary Figure 2), we identified time-group interactions for the d' 2-back (F = 15.048, p < 0.001) and d' 3-back (F = 20.206, p < 0.001). Subsequent pairwise analyses revealed that, regarding the d' 2-back scores at baseline,

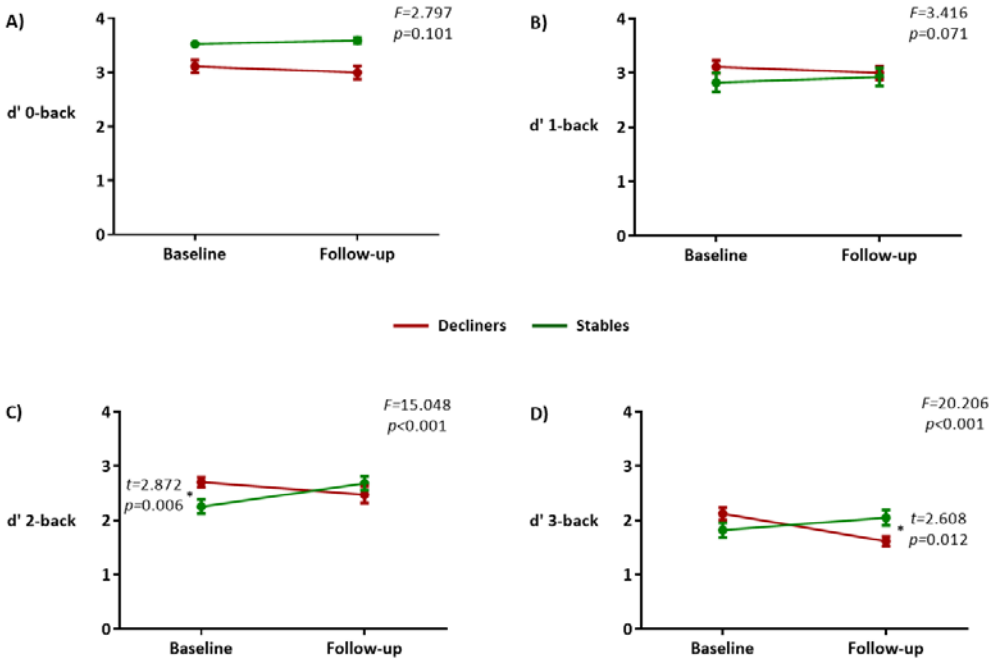
decliners exhibited higher performance than stables *(t* = 2.872, *p* = 0.006). For the d' 3-back measure the difference between groups emerged at follow-up, where the stables showed higher performance than decliners (*t* = 2.608, *p* = 0.012). Similar findings were detected considering the three groups (SHP, SLP and decliners): there were two time-group interactions (Supplementary Figure 3) as regards the d' 2-back (*F* = 7.357, *p* = 0.002) and d' 3-back (*F* = 9.964, *p* < 0.001). We identified differences between groups at baseline (d' 2-back: *F* = 9.484, *p* < 0.001; d' 3-back: *F* = 9.199, *p* = 0.006) and at follow-up (d' 3-back: *F* = 10.497, *p* < 0.001). The difference at baseline evidenced that SLP achieved lower scores than SHP (d' 2-back: *t* = -2.753, *p* = 0.012; d' 3-back: *t* = -4.057, *p* = 0.001) and decliners (d' 2-back: *t* = -4.310, *p* < 0.001; d' 3-back: *t* = -3.680, *p* = 0.001) at the highest task loads, while at follow-up the SHP group had better d' 3-back scores than SLP (*t* = 3.088, *p* = 0.006) and decliners (*t* = 5.018, *p* < 0.001).

	Baseline					Follow-up					Group differences	
	Total n=47	Decliners n=24	Stables n=23	SHP n=11	SLP n=12	Total n=47	Decliners n=24	Stables n=23	SHP n=11	SLP n=12	Stables, Decliners	SHP, SLP, Decliners
Age	68.40 ± 2.86	67.75 ± 2.66	69.09 ± 2.95	68.91 ± 3.30	69.25 ± 2.73	0.47 ± 2.97	69.88 ± 2.92	71.09 ± 2.95	70.91 ± 3.30	71.25 ± 2.73	*BL:* p=0.152 *FU:* p=0.175	*BL:* p=0.318 *FU:* p=0.354
Gender (F/M)	30/17	18/6	12/11	3/8	9/3	-	-	-	-	-	p=0.135	p=0.016*
APOE status (NC/C)	40/7	20/4	20/3	9/2	11/1	-	-	-	-	-	p=0.727	p=0.755
Educational level (years)	11.34 ± 3.97	11.63 ± 3.44	11.04 ± 4.51	14.00 ± 4.24	8.33 ± 2.77	-	-	-	-	-	p=0.696	p<0.001*
MMSE	29.06 ± 1.33	29.08 ± 1.28	29.04 ± 1.40	29.45 ± 0.93	28.67 ± 1.67	29.21 ± 1.00	29.42 ± 0.83	29.00 ± 1.13	29.45 ± 1.04	28.58 ± 1.08	*BL:* p=0.953 *FU:* p=0.230	*BL:* p=0.560 *FU:* p=0.037*
WMf	0 ± 1	0.36 ± 0.78	-0.37 ± 1.08	0.43 ± 0.47	-1.11 ± 0.94	0 ± 1	-0.22 ± 0.87	0.23 ± 1.09	0.96 ± 0.51	-0.43 ± 1.07	*BL:* p=0.011* *FU:* p=0.112	*BL:* p<0.001* *FU:* p<0.001*
d' 0-back	3.49 ± 0.16	3.46 ± 0.20	3.53 ± 0.92	3.52 ± 0.08	3.53 ± 0.11	3.47 ± 0.41	3.36 ± 0.49	3.59 ± 0.26	3.62 ± 0.35	3.56 ± 0.16	*BL:* p=0.330 *FU:* p=0.036*	*BL:* p=0.588 *FU:* p=0.105
d' 1-back	2.97 ± 0.72	3.11 ± 0.58	2.82 ± 0.84	3.39 ± 0.27	2.30 ± 0.85	2.96 ± 0.70	3.00 ± 0.60	2.93 ± 0.80	3.41 ± 0.33	2.48 ± 0.86	*BL:* p=0.310 *FU:* p=0.847	*BL:* p=0.002* *FU:* p=0.010*
d' 2-back	2.98 ± 0.58	2.70 ± 0.44	2.25 ± 0.62	2.58 ± 0.50	1.95 ± 0.58	2.57 ± 0.69	2.47 ± 0.75	2.68 ± 0.61	3.00 ± 0.46	2.38 ± 0.60	*BL:* p=0.036* *FU:* p=0.305	*BL:* p<0.001* *FU:* p=0.052
d' 3-back	1.97 ± 0.61	2.12 ± 0.55	1.81 ± 0.64	2.25 ± 0.47	1.42 ± 0.51	1.83 ± 0.61	1.61 ± 0.43	2.05 ± 0.69	2.44 ± 0.50	1.69 ± 0.65	*BL:* p=0.088 *FU:* p=0.014*	*BL:* p<0.001* *FU:* p<0.001*

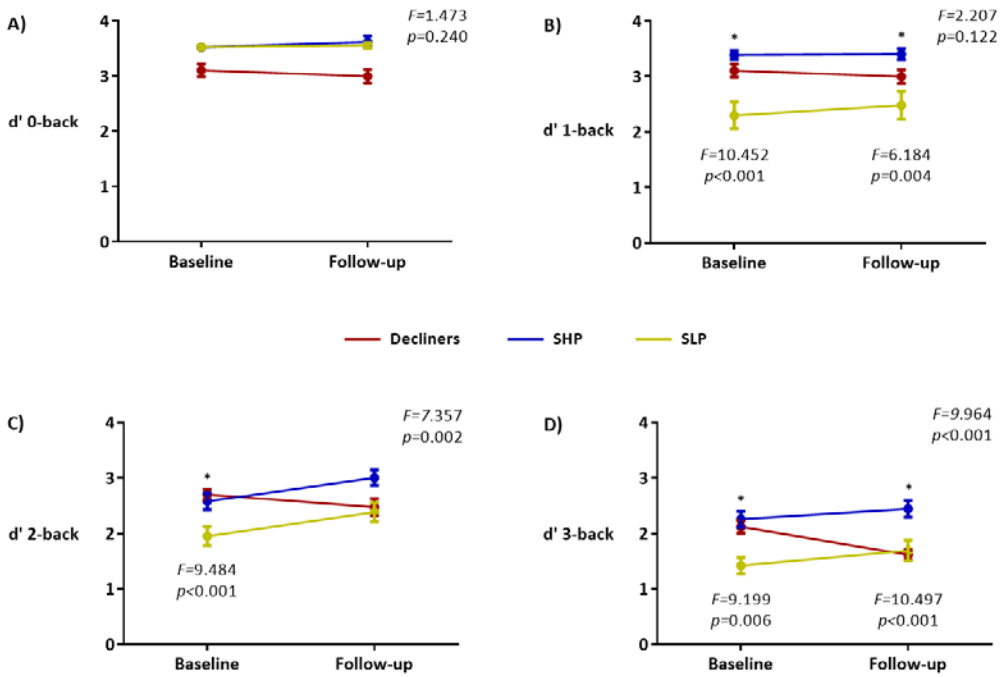
Supplementary Table 1. Demographic characterization and performance. Data are presented as mean ± standard deviation for the whole sample, considering the two groups (stables and decliners), and considering the subgroups (SHP and SLP). Abbreviations: M/F, male/female; NC/C, ε4 non-carriers/ε4 carriers; SHP, stables high performers; SLP, stables low performers; WMf, working memory factor; MMSE, mini-mental state examination; BL, baseline; FU, follow-up.
*Significant differences.



Supplementary Figure 1. WMf performance. Graphics showing the WMf scores in each group and time point. Error bars: ± standard error of the mean (SEM). A) Considering stables and decliners. B) Considering the three groups (SHP, SLP and decliners). Abbreviation: WMf, working memory factor; SHP, stables high performers; SLP, stables low performers.
*Significant differences.



Supplementary Figure 2. N-back task performance. Graphics showing the N-back scores in each group (stables and decliners) and time point. *F-values* correspond to the repeated measures ANOVA testing the time-group interaction. A) d' 0-back, B) d' 1-back, C) d' 2-back, D) d' 3-back. Error bars: ± 1 SEM.
*Significant differences.



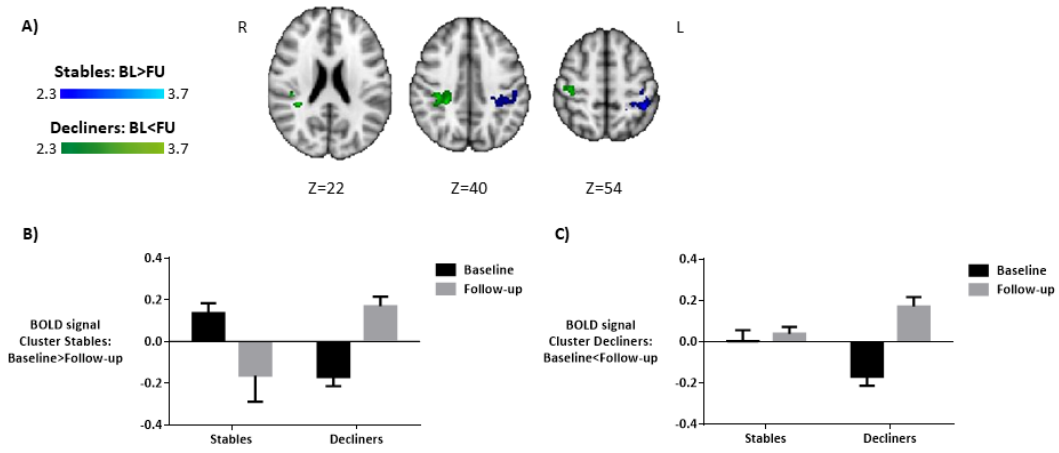
Supplementary Figure 3. N-back task performance. Graphics showing the N-back scores for each group (SHP, SLP and decliners) and time point. *F-values* correspond to the repeated measures ANOVA testing the time-group interaction. A) d' 0-back, B) d' 1-back, C) d' 2-back, D) d' 3-back. Error bars: ± 1 SEM. Abbreviations: SHP, stables high performers; SLP, stables low performers.
*Significant differences.

	d' 0-back	**d' 1-back**	**d' 2-back**	**d' 3-back**
Total (N=47)	*t*=-0.371 *p*=0.713	*t*=-0.098 *p*=0.924	*t*=0.943 *p*=0.350	*t*=-1.494 *p*=0.142
Decliners (N=24)	*t*=-1.247 *p*=0.225	*t*=-1.404 *p*=0.174	*t*=-1.852 *p*=0.077	*t*=-4.072 *p*<0.001*
Stables (N=23)	*t*=1.153 *p*=0.261	*t*=1.216 *p*=0.237	*t*=3.728 *p*=0.001*	*t*=2.178 *p*=0.040*
SHP (N=11)	*t*=0.955 *p*=0.362	*t*=0.137 *p*=0.894	*t*=3.163 *p*=0.010*	*t*=1.192 *p*=0.261
SLP (N=12)	*t*=0.610 *p*=0.554	*t*=1.580 *p*=0.142	*t*=2.281 *p*=0.043*	*t*=1.820 *p*=0.096

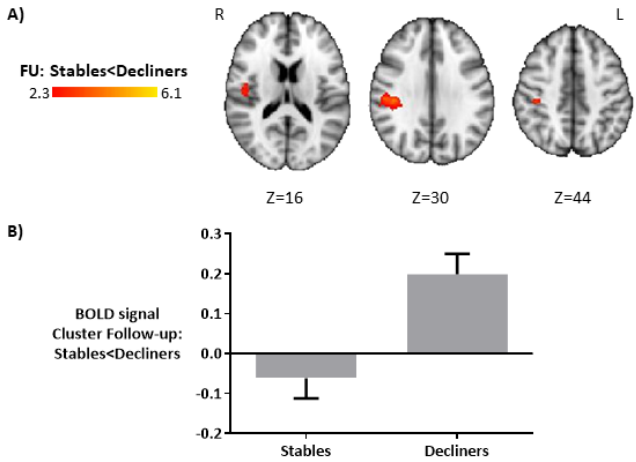
Supplementary Table 2. N-back task loads trajectories. Longitudinal differences for each group regarding the four levels of the N-back task (0-back, 1-back, 2-back, 3-back). Abbreviations: SHP, stables high performers; SLP, stables low performers.
*Significant differences

2. Stables vs. decliners

Regarding the fMRI data, despite there were no time-group interaction between stables and decliners, subsequent analysis in each group revealed different trajectories. The stables reduced activity across time in the left supramarginal gyrus (anterior and posterior division) and postcentral gyrus, while decliners increased activity at follow-up compared to baseline in the right parietal operculum cortex, the supramarginal gyrus (anterior and posterior division) and the superior parietal lobule (Supplementary Figure 4). As regards the group comparison at each time point, there were no differences in brain activity at baseline between stables and decliners. However, at follow-up, decliners exhibited higher brain activity than stables in an area comprising the central opercular cortex, parietal operculum cortex, supramarginal gyrus (anterior division) and postcentral gyrus (Supplementary Figure 5).



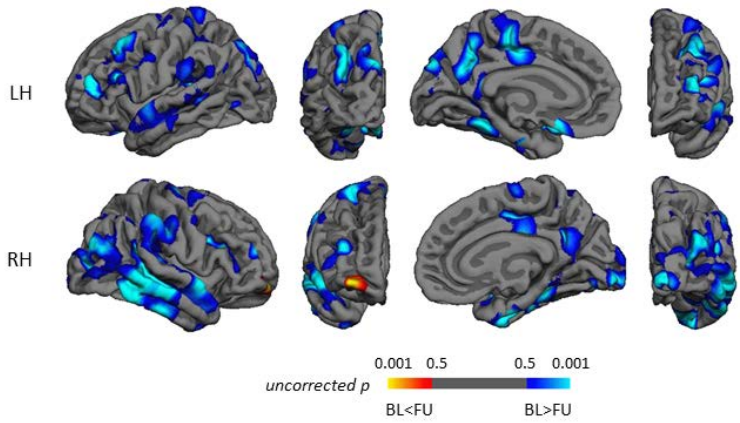
Supplementary Figure 4. Results from the fMRI activity change across time for each group. A) Significant activity reductions in the stables between baseline and follow-up (in blue) and activity increases in decliners (in green). B) Plot of mean BOLD signal values at the ROIs where stables reduced the activity at follow-up in stables and decliners separated by time point measures. C) Plot of mean BOLD signal values at the ROIs where decliners increased the activity at follow-up in stables and decliners separated by time point measures. Note that both groups were represented in each plot, although the correspondent voxel-wise statistical analyses were performed considering only one group. Error bars: ± 1 SEM. Abbreviations: BL, baseline; FU, follow-up.



Supplementary Figure 5. fMRI group differences between stables and decliners at follow-up. A) Significant activity map for the difference between groups is shown in red. B) Plot of baseline mean BOLD signal values at the ROI separated by stables and decliners. Error bars: ± 1 SEM. Abbreviations: FU, follow-up.

3. Cortical thickness atrophy at 2-year follow-up

The whole sample maps exhibited a generalized atrophy in both hemispheres comparing the baseline and follow-up measures. The clusters of atrophy comprised areas of the rostral middle frontal, posterior cingulate, temporal, precuneus, parietal and caudal middle frontal (Supplementary Figure 6).



Supplementary Figure 6. Vertex-wise symmetrized percent change (spc) uncorrected maps of atrophy at 2-year follow-up for the whole sample. The CTh increases in the 2-year follow-up are shown in red and CTh decreases are shown in blue. Abbreviations: LH, left hemisphere; RH, right hemisphere; BL, baseline; FU, follow-up.

Supplementary material for:

Effects of multifocal transcranial direct current stimulation on working memory functional patterns in healthy older adults

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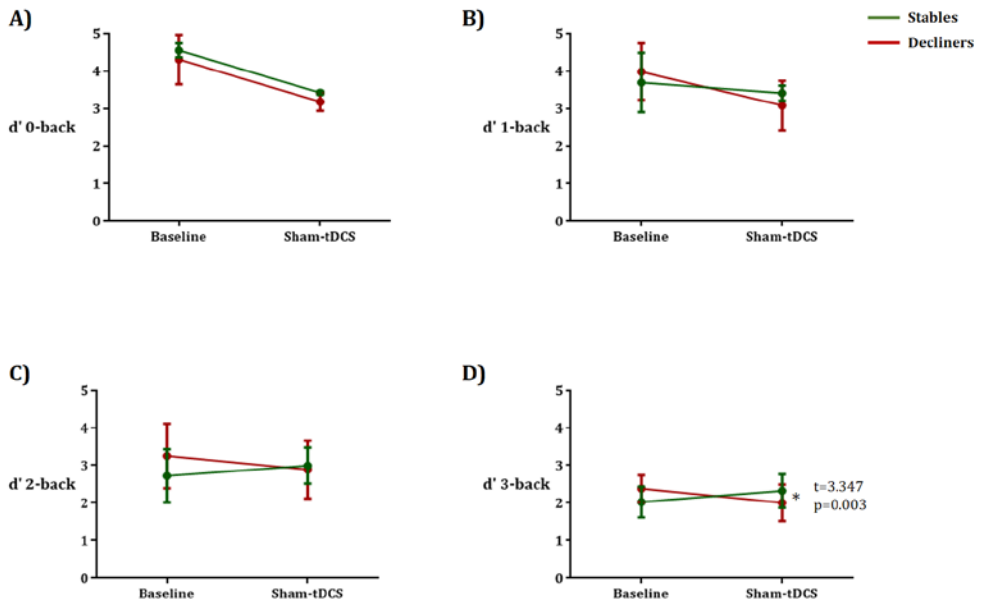
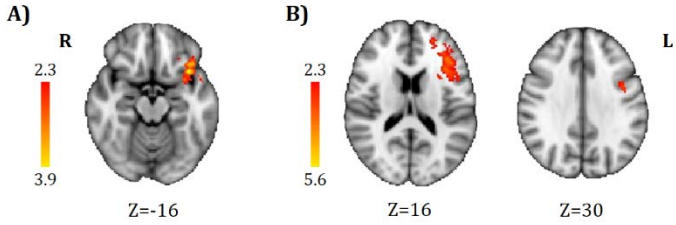


Supplementary Figure 1. Graphics showing the working memory performance measured as *d prime* (d') scores in each group (stables and decliners) and time point (baseline and sham-tDCS) at each load of the N-back task: **A)** 0-back, **B)** 1-back, **C)** 2-back, **D)** 3-back. Error bars: ± standard error of the mean (SEM). *Significant differences.



Supplementary Figure 2. Significant fMRI whole sample results (corrected $p < 0.05$ and $z > 2.3$), shown on the standard MNI map. **A)** Longitudinal differences for the whole sample between baseline and sham-tDCS acquisitions. **B)** Cross-sectional group differences at baseline, decliners showed greater activity than stables.

ABSTRACT

Background

As the aging population increases worldwide, the number of people with dementia and other conditions (i.e. cerebrovascular disease) leading to disability among elders is also growing (GBD Neurological Disorders Collaborative Group, 2017). However, according to the World Health Organization, there is strong evidence supporting that 'healthy aging' is possible, also from a cognitive point of view. For this reason, the challenge for the new decades of research is focused on developing new prevention strategies (Kivipelto et al., 2018; Livingston et al., 2017; Satizabal et al., 2016) and on characterizing this population of preserved elders.

The availability of diverse Magnetic Resonance Imaging (MRI) techniques have evidenced that among healthy people there is a generalized reduction of Gray Matter (GM; Fjell et al., 2014), and disruption of White Matter (WM) tracts (Salat, 2011). Nevertheless, it seems that there is an age-related selective vulnerability (i.e., some areas may be preserved against the aging effect, while others suffer severe atrophy; Lee et al., 2018). At least some portion of the age-related changes in cognitive function is associated with these structural changes. However, the relationship between cognitive function and brain anatomy is not straightforward in aging and measuring brain activity during cognitive demands, which can be undertaken through task-based functional MRI (fMRI) is critical to achieving a more comprehensive knowledge of brain-behavior associations. There are several theories that have been proposed in order to explain the variations observed during the aging process using fMRI. In general, decreased brain activity has typically been interpreted as a reflection of cognitive deficits in older adults (i.e. diminished neural capacity; Stern, 2009), while increased activity has usually been understood as a successful compensatory mechanism as response to aging-induced system failures (Cabeza, 2002; Grady et al., 2006; Mattay et al., 2006; Park & Reuter-Lorenz, 2009; Reuter-Lorenz & Park, 2014). However, these functional brain changes are not invariably linked to better cognitive performance (Rypma et al., 2007; Zarahn et al., 2007) and in these cases, increased activations have been conceived as attempted or unsuccessful compensatory mechanisms (Cabeza & Dennis, 2013) or dedifferentiation processes (Carp et al., 2011; Park et al., 2004), that are mainly characterized by more diffuse activation patterns and less engagement of

the typical task-related areas. In this framework, the specific use of Working Memory (WMem) tasks-based fMRI, have allowed revealing that those older adults with relatively high levels of performance increase brain activity as a function of load, whereas low-performing elders show flat or inverted-U shape activation profiles (according to the Compensation-Related Utilization of Neural Circuits Hypothesis - CRUNCH; Reuter-Lorenz & Cappell, 2008).

Despite the well-documented evidence that cognitive decline is a generalized process along the aging course, it should be noted that increasing inter-individual differences in cognition are the most noticeable defining characteristic of the aged brain (Lindenberger, 2014; Nyberg et al., 2012). The existence of performance heterogeneity among healthy elders (Habib et al., 2007; Lindenberger, 2014) and in the trajectories identified in longitudinal studies (Josefsson et al., 2012; Yaffe et al., 2009) have stressed that some elders are able to preserve optimal functioning in aging (Nyberg et al., 2012; Nyberg & Pudas, 2019). Thus, the previous research in the field has established that achieving a successful cognitive level in aging may be possible through two not-mutually exclusive general pathways: 1) by means of Cognitive Reserve (CR)-related mechanisms (Stern et al., 2018) or 2) associated with the Brain Maintenance (BM) hypothesis (Nyberg et al., 2012). CR is a theoretical construct proposed in order to account for the frequent discrepancy between a person's underlying level of age-related brain changes and the observed cognitive profile that is expected to result of that damage (Stern, 2009). Years of education are the most commonly used proxy variable of CR, which has been widely associated with higher cognition (Habib et al., 2007; Josefsson et al., 2012). Moreover, CR estimates have been related to neuroprotection and to greater capacity to tolerate brain damage in the face of aging effects by recruiting alternative neural mechanisms (Stern et al., 2017). On the other hand, BM refers to a relative lack of brain pathology measured longitudinally, which could be possible due to neural processes of reparation and plasticity (Nyberg et al., 2012). MRI studies evidence that adults who display stable cognitive performance have youthful brains showing relative flat trajectories at both functional and structural levels. Currently, these two concepts refer to rather macroscopic constructs that are not linked to identifiable biological pathways through which they suppose beneficial in terms of adaptability to cope with (i.e. CR), or not experience (i.e. BR) age-related brain changes.

In the light of all this, new methodologies to apprehend and enhance the mechanisms related to optimal brain function in advancing ages become vital. Hence, the combination of MRI and Non-Invasive Brain Stimulation (NIBS), such as transcranial Direct Current Stimulation (tDCS), might provide novel experimental data on the putative neurophysiological mechanisms underlying inter-individual differences in cognitive status among older adults, and also might help to reformulate the cognitive aging models proposed to date. Further, the NIBS procedures entail the capacity to modify the brain function in older adults, potentially leading to improvements in cognitive function which is relevant in order to design non-pharmacological interventions to boost cognition in aging (reviewed in Abellaneda-Pérez et al., 2019a).

Objectives and Hypotheses

Main objective:

The overarching objective was to characterize the structural and functional brain substrates underlying healthy cognitive aging and to reveal how CR estimates may modulate the relationship between the measures studied.

Specific objectives:

1. To explore how years of education, as the main proxy variable for CR, relates to the cognitive profiles amongst healthy elders.
2. To identify associations between CR estimations, GM and WM integrity MRI-based measures and to study if these are related to the impact of age on brain structure.
3. To describe the transcriptional genetic architecture of CR-related cortical regions using brain-wide gene expression data in combination with gene set enrichment analysis.
4. To investigate the brain properties underlying WMem stability and decline, using longitudinal functional and structural MRI.
5. To study whether combining NIBS and fMRI techniques could be a useful approach to better understand the functional mechanisms sustaining different cognitive trajectories in aging.

Hypotheses:

1. Higher educational attainment, as a main proxy of CR, will be related to better cognitive performance but will not have a clear modulatory effect on the longitudinal progression.
2. Amongst cognitively preserved elders, CR estimates will be positively associated with measures of brain integrity in those areas relatively preserved in aging and at the same time, CR will be related to greater rates of atrophy in brain measures suffering more abrupt age-related disruption.
3. We further expected that the cortical areas showing positive associations between CR and integrity will exhibit a distinct gene expression profile characterized by the upregulation of gene sets related to neuroprotective molecular pathways.
4. With age, distinct brain characteristics will underlie stability and decline in WMem performance, with those subjects showing decline being more likely to engage unsuccessfully compensatory fMRI patterns and greater age-related cortical atrophy.
5. Among elderly people showing age-related stability in cognition, only those high performers will exhibit longitudinal brain features fitting with the BM hypothesis.
6. During a WMem task, tDCS will result in dissimilar modulations of brain activity in those subjects showing cognitive stability vs. decline, providing insight into the fMRI correlates of these two groups with different trajectories.

Materials and Methods

The present Doctoral Thesis consists of four studies that were carried out in order to answer the above-mentioned objectives.

In study 1 we analyzed MRI structural data from 100 cognitively normal elders (aged: mean ± standard deviation, 68.35 ± 3.09 years), in order to investigate the associations and interactions between education (as a proxy of CR), speed of processing, memory and two MRI brain integrity measures: Cortical Thickness (CTh) of GM and Fractional Anisotropy (FA) of WM tracts.

In study 2 we first identified brain areas that showed an association between CTh and CR among 122 cognitively healthy older individuals (aged: 68.20 ± 3.51 years). We subsequently investigated the transcriptional architecture of these regions using brain-wide regional gene expression data from the Allen Institute Human Brain Atlas, in combination with Gene Set Enrichment Analysis (GSEA).

In study 3, a total of 47 subjects (aged: 68.40 ± 2.86 years) were categorized as WMem stables (N=23) or decliners (N=24) based on a 2-year longitudinal design. Stables were further subdivided into high and low performers, based on their N-back task scores. At both time points, fMRI during the performance of the N-back task and structural images were acquired.

In study 4, we retrospectively characterized 4-year longitudinal changes in fMRI, as well as differences across tDCS montages in a sample of 24 healthy elders (aged: 71.73 ± 2.65 years). The sample was divided into cognitive stables (N=12) or decliners (N=12) according to the scores of an N-back task performed while fMRI data were acquired (as described in study 3). Regarding the tDCS intervention, we designed a sham-controlled cross-over study on three separate experimental sessions. During the fMRI acquisitions, multifocal tDCS was concomitantly applied using sham and two distinct electric field distributions (fitting *compensatory* and *maintenance* fMRI patterns underlying successful WMem performance), in a randomized counter-balanced order.

Results

Our results revealed that elders with higher rates of years of education (i.e., those with high CR) performed better on speed of processing, declarative memory, 'frontal lobe function' and WMem domains. Notwithstanding, our findings reinforced the idea that longitudinal cognitive stable trajectories can occur equally amongst both high and low educated elders, suggesting that education mainly contributes positively to higher cognitive level in the intercept, rather than modulate the slope.

From different MRI modalities, we have described the neuroprotective and compensatory effects of CR underlying preserved cognition in a healthy elder's population,

which seem to be related to differential relationships between age and GM and WM measures. Higher rates of CR were associated with a higher capacity to maintain memory performance in the context of a greater age impact on WM. Furthermore, in parallel, CR was related to a putative neuroprotective effect on GM integrity of the prefrontal cortex area. In addition, such structural benefit was associated with a gene expression profile characterized by relative upregulation of gene sets implicated in cell communication, neurotransmission and immune responses, proposing some of the plasticity-related biological mechanisms through which CR may operate.

Focusing on the fMRI analyses, we identified that the subjects who significantly declined in WMem performance after 2-year follow-up, increased brain activity in non-related WMem areas in the presence of greater structural atrophy. On the other hand, those stable subjects with high performance reduced the activation. In addition, derived from the cross-sectional group comparisons, we observed a less activation in task-related areas for the group of stables with low performance, which also was the less educated group.

Finally, combining fMRI and tDCS we detected significant tDCS-induced changes for those subjects who showed WMem decline. Specifically, through the application of multifocal tDCS using the compensatory configuration, we described an activity reduction of occipital areas. The cluster identified was partially located over the same area characterized by over-activations detected through the retrospective 4-year longitudinal approach.

Conclusions

Through the combined analysis of the studies included in this Doctoral Thesis, we can conclude that:

1. CR, measured as years of education, is strongly associated with higher cognitive performance in aging, whereas it does not differentially influence cognitive trajectories.
2. Both neuroprotective and compensatory mechanisms linked with CR coexist amongst healthy elders, indicating that greater capacity to tolerate brain changes may be revealed in response to the age-related effects.

3. CR-related frontal areas exhibit a distinct gene expression profile characterized by relative upregulation of gene sets implicated in biological mechanisms that may underlie the CR's capacity for plastic changes, such as cell communication, neurotransmission and immune response.
4. Among WMem stable elders, there are different age-related fMRI trajectories according to the performance level. High performers show high CR, neural efficiency and preserved structure, while low-performers have lower CR rates and their fMRI patterns suggest disruption of load-dependent adaptability.
5. WMem decliners exhibit longitudinal fMRI over-activations in non-related task areas, evidencing unsuccessful compensation mechanisms together with higher rates of cortical atrophy.
6. tDCS effects depend on the underlying age-related organization of functional brain patterns, revealing that modulations are greater in more compromised systems and are linked with restoring brain dynamics into a more youthful activity profile.

RESUM EN CATALÀ

Marc Teòric

A mesura que la població envellida augmenta a tot el món, també creix el nombre de persones amb demència i altres patologies (com la malaltia cerebrovascular) que condueixen a discapacitat entre les persones d'edat avançada (GBD Neurological Disorders Collaborative Group, 2017). Tanmateix, segons l'Organització Mundial de la Salut, hi ha una forta evidència que demostra que un "envelliment saludable" és possible, també des del punt de vista cognitiu. Per aquest motiu, el repte de la recerca per les properes dècades està enfocat en desenvolupar noves estratègies de prevenció (Kivipelto et al., 2018; Livingston et al., 2017; Satizabal et al., 2016), així com caracteritzar aquesta població de gent gran preservada.

La disponibilitat de diverses tècniques de ressonància magnètica (RM) ha demostrat que entre les persones sanes d'edat avançada hi ha una reducció generalitzada de la Substància Grisa (SG; Fjell et al., 2014) i una alteració de la integritat de les fibres de Substància Blanca (SB; Salat, 2011) . Tot i així, sembla que hi ha una vulnerabilitat selectiva relacionada amb l'edat (és a dir, hi ha alguns regions cerebrals preservades de l'efecte de l'envelliment, mentre que d'altres pateixen una atròfia més severa; Lee et al., 2018). Com a mínim una part dels canvis relacionats amb l'edat en la funció cognitiva s'associen a aquests canvis estructurals. Tanmateix, la relació entre la cognició i l'anatomia del cervell no és senzilla en el marc de l'envelliment i la mesura d'activitat cerebral durant la realització de tasques cognitives demandants, que es pot dur a terme mitjançant RM funcional (RMf) amb tasca, és fonamental per aconseguir un coneixement més complet de les associacions comportament-cervell. Mitjançant RMf, hi ha diverses teories que s'han proposat per tal d'explicar les variacions observades durant el procés d'envelliment. En general, la disminució de l'activitat cerebral s'ha interpretat típicament com un reflex dels dèficits cognitius en adults d'edat avançada (és a dir, disminució de la capacitat neuronal; Stern, 2009), mentre que l'augment d'activitat s'acostuma a entendre com un mecanisme compensatori exitós com a resposta a disrupcions del sistema fruit de l'envelliment. (Cabeza, 2002; Grady et al., 2006; Mattay et al., 2006; Park & Reuter-Lorenz, 2009; Reuter-Lorenz & Park, 2014). Tanmateix, aquests canvis funcionals cerebrals no estan vinculats de

forma invariable a un millor rendiment cognitiu (Rypma et al., 2007; Zarahn et al., 2007) i, en aquests casos, els increments d'activitat s'han interpretat com a mecanismes d'intent de compensació o compensació infructuosa (Cabeza i Dennis, 2013), o processos de desdiferenciació (Carp et al., 2011; Park et al., 2004), que es caracteritzen principalment per patrons d'activació més difusos i menys implicació de les àrees relacionades amb la tasca. En aquest marc, l'ús específic de RMf durant la realització de tasques de Memòria de Treball (MT), ha permès revelar que les persones d'edat avançada amb nivells relativament alts de rendiment augmenten l'activitat cerebral com a funció de la càrrega cognitiva exigida, mentre que la gent gran amb baix rendiment mostra perfils d'activació plans o en forma d'U-invertida (segons la "Compensation-Related Utilization of Neural Circuits Hypothesis – CRUNCH"; Reuter-Lorenz & Cappell, 2008).

Malgrat l'evidència ben documentada que l'alteració de la cognició és un procés generalitzat al llarg del curs de l'envelliment, cal destacar que l'augment de diferències interindividuals és la característica definidora més notable del cervell envellit (Lindenberger, 2014; Nyberg et al., 2012). L'existència d'heterogeneïtat pel que fa al rendiment de la gent gran (Habib et al., 2007; Lindenberger, 2014), així com en les trajectòries identificades en estudis longitudinals (Josefsson et al., 2012; Yaffe et al., 2009), han posat de manifest que algunes persones tot i envellir són capaces de preservar un funcionament òptim (Nyberg et al., 2012; Nyberg i Pudas, 2019). Així, les investigacions prèvies en el camp han establert que l'assoliment d'un nivell cognitiu exitós en l'envelliment pot ser possible mitjançant dues vies generals no mútuament excloents: 1) a partir de mecanismes relacionats amb la Reserva Cognitiva (RC; Stern et al., 2018) o 2) associats a la hipòtesi del Manteniment Cerebral (MC; Nyberg et al., 2012). La RC és un constructe teòric proposat per tal de considerar les freqüentes discrepàncies entre el canvis cerebrals relacionats amb l'edat que experimenta una persona i el perfil cognitiu que s'espera que resulti d'aquest dany (Stern, 2009). Els anys d'educació són la variable *proxy* de RC més utilitzada, i han estat àmpliament associats a nivells elevats de cognició (Habib et al., 2007; Josefsson et al., 2012). A més, les estimacions de RC han estat relacionades amb la neuroprotecció i amb una capacitat major de tolerar el dany cerebral sofert davant l'envelliment, mitjançant la utilització de mecanismes neuronals alternatius (Stern et al., 2017). D'altra banda, el MC es refereix a una falta relativa de patologia cerebral mesurada longitudinalment, fet que podria ser possible a causa dels processos neurals de reparació i

plasticitat (Nyberg et al., 2012). Els estudis de RM han evidenciat que les persones d'edat avançada amb rendiment cognitiu estable tenen cervells més joves que mostren trajectòries planes relatives tant a la funció com a l'estructura. Actualment, aquests dos conceptes (RC i MC) es refereixen a constructes més aviat macroscòpics que no estan vinculats a mecanismes biològics identificables a través de les quals s'explicaria el seu efecte beneficiós en termes d'adaptabilitat per afrontar (RC), o no experimentar (MC) canvis cerebrals relacionats amb l'edat.

A la llum de tot això, es fa vital l'ús de noves tecnologies que ens permetin aprendre i potenciar els mecanismes relacionats amb la funció cerebral òptima en edats avançades. Per tant, la combinació RM i Estimulació Cerebral No Invasiva (ECNI), com l'Estimulació Transcranial per Corrent Directe (ETCD), podria proporcionar dades experimentals innovadores sobre els mecanismes neurofisiològics subjacents a diferències interindividuals en l'estat cognitiu entre la gent gran, i també podria ajudar a reformular els models d'envelliment cognitiu proposats fins ara. A més, els procediments ECNI tenen la capacitat de modificar la funció cerebral, provocant possibles millores en la funció cognitiva que són rellevants per tal de dissenyar intervencions no farmacològiques per impulsar la cognició en l'envelliment (Abellaneda-Pérez et al., 2019a).

Objectius i Hipòtesis

Objectiu principal:

L'objectiu general era caracteritzar els substrats cerebrals estructurals i funcionals subjacents a un envelliment cognitiu saludable i revelar com les estimacions de RC poden modular la relació entre les mesures estudiades.

Objectius específics:

1. Explorar com els anys d'educació, com a principal variable *proxy* de RC, es relacionen amb els perfils cognitius de persones sanes d'edat avançada.
2. Identificar associacions entre les estimacions de la RC, les mesures de RM d'integritat de SG i SB i estudiar si aquestes estan relacionades amb l'impacte de l'edat sobre l'estructura cerebral.

3. Descriure l'arquitectura transcripcional de les regions corticals relacionades amb la RC utilitzant dades d'expressió gènica de tot el cervell en combinació amb un anàlisi d'enriquiment del conjunt de gens.
4. Investigar les propietats cerebrals subjacents a l'estabilitat i la disminució de la MT, mitjançant mesures longitudinals de RM funcional i estructural.
5. Estudiar si combinar tècniques ECNI i RMf pot ser un enfocament útil per comprendre millor els mecanismes funcionals que sostenen diferents trajectòries cognitives en l'envelliment.

Hipòtesis:

1. Una major educació, com a principal representant de la RC, estarà relacionada amb un millor rendiment cognitiu, però no tindrà un efecte modulador clar en la progressió longitudinal.
2. Entre la gent gran preservada a nivell cognitiu, les estimacions de la RC s'associaran positivament a les mesures d'integritat cerebral en aquelles zones relativament conservades en l'envelliment i, alhora, la RC estarà relacionada amb majors taxes d'atròfia en les mesures cerebrals que pateixen una disrupció més brusca com a efecte de l'edat.
3. A més, preveiem que les zones corticals que mostren associacions positives entre la RC i la integritat presentaran un perfil d'expressió gènica diferent caracteritzat per la regulació de gens relacionats amb vies moleculars neuroprotectores.
4. Amb l'edat, l'estabilitat i la disminució del rendiment en MT estaran caracteritzades per mesures cerebrals diferents, sent aquells subjectes que declinen els que més probablement mostraran patrons de RM de tipus compensació infructuosa i una major atrofia cortical relacionada amb l'edat.
5. Entre les persones grans que mostren estabilitat cognitiva relacionada amb l'edat, només aquelles amb alt rendiment presentaran característiques cerebrals longitudinals que s'ajustin a la hipòtesi de MC.
6. Durant una tasca de MT, l'ETCD donarà lloc a modulacions diferents de l'activitat cerebral en aquells subjectes que mostren estabilitat cognitiva en front als que declinen, proporcionant informació dels correlats de RMf d'aquests dos grups amb trajectòries diferents.

Materials i Mètodes

La present Tesi Doctoral consta de quatre estudis realitzats per donar resposta als objectius esmentats anteriorment.

A l'estudi 1 es van analitzar les dades estructurals de RM de 100 ancians cognitivament sans (edat: mitjana ± desviació estàndard, 68.35 ± 3.09 anys), per tal d'investigar les associacions i interaccions entre l'educació (com a *proxy* de RC), velocitat de processament, memòria, i dues mesures d'integritat cerebral derivades de la RM: el gruix cortical de la SB i l'anisotropia fraccional dels tractes de SB.

A l'estudi 2, primer es van identificar àrees cerebrals que mostraven una associació entre els gruix cortical i la RC en 122 individus d'edat avançada cognitivament sans (edat: 68.20 ± 3.51 anys). Posteriorment, es va investigar l'arquitectura transcripcional d'aquestes regions mitjançant dades regionals d'expressió gènica a tot el cervell procedents de l'"Allen Brain Human Atlas Institute", en combinació amb "Gene Set Enrichment Analysis (GSEA)".

A l'estudi 3, un total de 47 subjectes (edat: 68.40 ± 2.86 anys) van ser classificats com a estables (N=23) o declinadors (N=24) en MT segons un disseny longitudinal de 2 anys de seguiment. El grup d'estables es va subdividir en subjectes amb alt i baix rendiment, en funció de les seves puntuacions en una tasca N-back. En ambdós punts de temps, es van adquirir RMf durant la realització d'una tasca N-back i imatges estructurals.

A l'estudi 4, es van caracteritzar retrospectivament canvis longitudinals de RMf durant 4 anys de seguiment, així com diferències entre dos muntatges d'ETCD en 24 persones d'edat avançada sanes (edat: 71.73 ± 2.65 anys). La mostra es va dividir en estables (N = 12) o declinadors (N = 12) segons les puntuacions de la tasca N-back realitzada mentre s'adquiriren dades de RMf (com es descriu a l'estudi 3). Pel que fa a la intervenció amb ETCD, es va dissenyar un estudi creuat controlat dividit en tres sessions experimentals separades. Durant les adquisicions de RMf, es va aplicar simultàniament ETCD multifocal mitjançant placebo i dues distribucions de corrent diferents (adequant patrons compensatoris i de manteniment de RMf subjacents a un bon rendiment en MT), en un ordre contra-balancejat aleatoritzat.

Resultats

Els nostres resultats van revelar que la gent gran amb més anys d'educació (és a dir, els que tenien més RC) mostraven un rendiment major en velocitat de processament, memòria declarativa, "funció del lòbul frontal" i MT. No obstant això, els nostres resultats van reforçar la idea que les trajectòries cognitives estables longitudinals es poden produir igualment entre persones d'edat avançada amb nivells alts i baixos d'educació, fet que suggereix que l'educació contribueix principalment a un nivell cognitiu més alt en el punt de partida, en lloc de modular el pendent.

A partir de diferents modalitats de RM, en una població de gent gran sana, vam descriure els efectes neuroprotectors i compensatoris de la RC que suporten una cognició preservada, i que semblen estar relacionats amb les associacions diferencials entre l'edat i les mesures de SG i SB. Els índexs més alts de RC es van associar amb una capacitat més elevada de mantenir el rendiment de la memòria en el context d'un major impacte de l'edat en la SB. En paral·lel, la RC va estar relacionada amb un efecte sobre la integritat de la SG de l'escorça prefrontal. A més, aquest benefici estructural es va associar a un perfil d'expressió gènica caracteritzat per la relativa regulació a l'alça d'un conjunt de gens implicats en la comunicació cel·lular, la neurotransmissió i les respostes immunes, proposant alguns dels mecanismes biològics relacionats amb la plasticitat mitjançant els qual la RC podria actuar.

Centrant-nos en les anàlisis de RMf, vam identificar que els subjectes que van disminuir significativament el rendiment de MT després de dos anys de seguiment, van augmentar l'activitat cerebral en àrees no relacionades amb MT en presència d'una atrofia estructural major. D'altra banda, aquells subjectes estables amb un alt rendiment van reduir l'activació. A més, derivat de les comparacions entre grups, es va observar una menor activació en àrees relacionades amb la tasca per part del grup d'estables amb baix rendiment, que també va resultar ser el grup menys educat.

Finalment, combinant RMf i ECNI, es van detectar canvis significatius induïts per l'ETCD en aquells subjectes que mostraven un declivi en MT. Concretament, a partir de l'aplicació d'ETCD multifocal mitjançant la configuració compensatòria, es va descriure una reducció d'activitat de les zones occipitals. La regió identificada se solapava parcialment amb l'àrea on es van detectar sobreactivacions mitjançant un enfocament longitudinal retrospectiu de 4 anys de seguiment.

Conclusions

Mitjançant l'anàlisi combinada dels estudis inclosos en aquesta Tesi Doctoral, podem concloure que:

1. La RC, mesurada com a anys d'educació, està fortament associada a un major rendiment cognitiu en l'envelliment, mentre que no té un impacte diferencial en les trajectòries cognitives.
2. Tant els mecanismes neuroprotectors com els compensatoris relacionats amb la RC conviuen en les persones sanes d'edat avançada, cosa que indica que es pot revelar una major capacitat de tolerar els canvis cerebrals en resposta als efectes relacionats amb l'edat.
3. Les àrees frontals relacionades amb la RC presenten un perfil d'expressió gènica diferencial caracteritzat per la relativa regulació a l'alça dels conjunts de gens implicats en mecanismes biològics que poden suportar la capacitat de RC per produir canvis plàstics, com la comunicació cel·lular, la neurotransmissió i la resposta immune.
4. Entre les persones d'edat avançada que mostren estabilitat en MT, hi ha diferents trajectòries de RMf relacionades amb l'edat, segons el nivell de rendiment. Els subjectes amb rendiment més alt mostren una elevada eficiència neuronal, major RC i una estructura preservada, mentre que aquells que rendeixen baix presenten taxes inferiors de RC i els seus patrons de RMf suggereixen una interrupció de l'adaptabilitat depenent de la càrrega cognitiva.
5. Els declinadors en MT presenten sobre-activacions longitudinals de RMf en àrees no relacionades amb la tasca, evidenciant mecanismes de compensació infructuosos juntament amb taxes més altes d'atròfia cortical.
6. Els efectes de l'ETCD depenen de l'organització subjacent relacionada amb l'edat dels patrons funcionals cerebrals, destacant que les modulacions són més grans en sistemes més compromesos i estan relacionades amb la restauració de la dinàmica cerebral cap a un perfil d'activació més jove.

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