Multimodal characterization and modulation of large-scale memory networks: Implications for cognitive aging

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declare and confirm that he has guided and supervised the doctoral thesis entitled: "**Multimodal characterization and modulation of large-scale memory networks: Implications for cognitive aging**", presented by **Dídac Vidal Piñeiro**. He thereby asserts that this thesis fulfils the requirements to present her defense to be defended for the Internacional Degree of Doctor in Biomedicine.

Signature,

Dr. David Bartrés Faz

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This thesis has been undertaken in the Neuropsychology Group of the Department of Psychiatry and Clinical Psychobiology, Faculty of Medicine, University of Barcelona. The group forms part of the Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS).

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Forework

This dissertation presented for the degree of Doctor by the University of Barcelona, is the result of three studies performed at the Department of Psychiatry and Clinical Psychobiology, Faculty of Medicine, University of Barcelona. The following articles have been published in international journals as a result of the work performed. The global impact of factor of the two published papers is 8,07 (ISI of Knowledge; Journal Citation Reports inferred from 2013):

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Glossary of abbreviations

AD Alzheimer's disease

(f)ALFF (fractional) Amplitude of Low Frequency Fluctuation

AMT Active Motor Threshold

ANOVA Analysis of Variance

ASL Arterial Spin Labeling

BOLD Blood Oxygen Level Dependent

βA Beta-Amyloid

CBF Cerebral Blood Flow

CR Cognitive Reserve

CRUNCH Compensation-Related Utilization of Neural Circuits

CTh Cortical Thickness

DAN Dorsal Attentional Network

DLPFC Dorsolateral Prefrontal Cortex

DMN Default-Mode Network

DTI Diffusion Tensor Imaging

EEG Electroencephalography

FA Fractional Anisotropy

FDI First Dorsal Interosseous

fMRI Functional Magnetic Resonance Imaging

FWE Family-Wise Error

GABA Gamma-Amminobutyric Acid

GLM General Linear Model

GWC Grey-White Matter Contrast

HAROLD Hemispheric Asymmetry Reduction in Older Adults

HERA Hemispheric Encoding-Retrieval Asymmetry

ICA Independent Component Analysis

IPL Inferior Parietal Lobe

LoP Level of Processing

LTD Long-Term Depression

LTP Long-Term Potentiation

MEP Motor-evoked Potential

MD Mean Diffusivity

MMSE MiniMental State Examination

mPFC Medial Prefrontal Cortex

MPRAGE Magnetization Prepared Rapid Gradient Echo

MRS Magnetic Resonance Spectroscopy

MTL Medial Temporal Lobe

(t)NAA (Total) N-Acetylaspartate

NIBS Non-Invasive Brain Stimulation

PAS Pair-Associative Stimulation

PCC Posterior Cingulate Cortex

PCU Precuneus

PET Positron Emission Tomography

PFC Prefrontal Cortex

ReHo Regional Homogeneity

RMT Resting Motor Threshold

ROI Regions of Interest

Rs-fMRI Resting State fMRI

RSN Resting- State Networks

rTMS Repetitive Transcranial Magnetic Stimulation

PASA Posterior-to-Anterior Shift in Aging

PPI Psychophysiological Interaction

STAC Scaffolding Theory of Aging and Cognition

(i/c)TBS (Intermittent/Continuous) Theta-Burst Stimulation

tCR Total Creatine

TDCS Transcranial Direct Current Stimulation

TFCE Threshold-Free Cluster Enhancement

TMS Transcranial Magnetic Stimulation

TTBS Tract-Based Spatial Statistics

VBM Voxel-Based Morphometry

VOI Voxel-of-Interest

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Chapter 1: Introduction

1. Cognitive Aging

a. General overview

Developed countries in the world are experiencing substantial increases in the proportion of elderly adults in the population (Cohen, 2003). By 2049, 30% of the population in Spain may be older than 64 years of age doubling the proportion of elders than in 2009 (INE, 2010). While maturity represents an enormous capital of experience and knowledge, ageing entails cognitive and motor decline and, most importantly, represents the greatest risk factor for several neurodegenerative disorders, especially Alzheimer's Disesase (AD; i.e. Hebert et al., 2003). It is of utmost socioeconomic importance to promote functional independence and quality of life in this group while it is fair to declare that, at present, neurocognitive frailty represents the biggest threat to successful aging in our society as well as it demands a high economic effort to affected individuals, families and, most generally, society.

In the first section of the introduction, cognition decline in elders will be reviewed focusing on episodic memory function. Next, a brief overview of main neuroimaging contributions towards the understanding of the ageing brain will be introduced with special focus to those related to memory performance.

b. Cognitive function in aging

There is extensive amount of knowledge about how cognition changes with age (Glisky, 2007; Park and Schwarz, 1999; Reuter-Lorenz and Park, 2014) mostly englobed in the cognitive psychology field. As individuals age many aspects of information processing become less efficient. Many cognitive domains are affected by age including speed of processing (Salthouse, 1996), working memory capacity (Rajah and D'Esposito, 2005; Reuter-Lorenz and Sylvester, 2005), inhibitory function (Hasher and Zacks, 1979; Hasher et al., 2007) and long-term memory encoding and

retrieval (Craik et al., 1987; Zacks et al., 2000). Instead, other cognitive functions, such as implicit memory and world knowledge remain preserved (Park et al., 2002) in cognitive aging. **Figure 1**: 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 (from Park and Reuter-Lorenz, 2009). depicts behavioral performance in different cognitive functions across lifespan being evident the decline in aging. Cognitive health has consistently been cited as an important factor for quality of life (CDC, 2010) and is widely recognized as an important contributor to late life functioning (Depp and Jeste, 2006; Rowe and Kahn, 1987), also predicting everyday functioning of elderly population (Allaire and Marsiske, 1999; Weatherbee and Allaire, 2008).

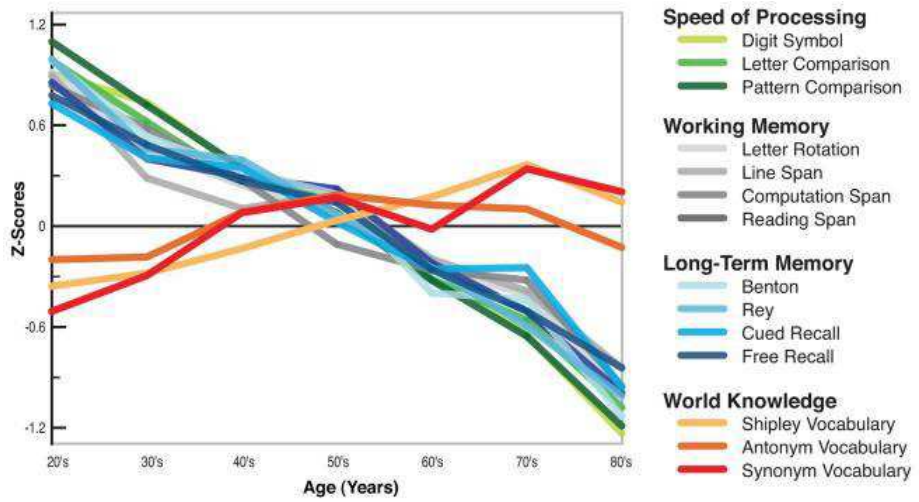

Figure 1: Cross-sectional aging data showing behavioral performance on measures of speed of processing, working memory, long-term memory, and world knowledge. Almost all measures of cognitive function show decline with age, except world knowledge (from Park and Reuter-Lorenz, 2009).

i. Neuropsychology of aging episodic memory

Episodic memory refers to memory for personally experienced events that occurred in a particular place and at a particular time and requires retrieval of information that is no longer present or being maintained in an active state. Episodic memory is one of the most affected cognitive functions in aging (Park and Gutchess, 2000; Zacks et al., 2000), and prominent scientific effort in this field successfully characterized memory changes during lifespan. It has been reported that memory functions decreases linearly since early adulthood (Park et al., 2002; Rönnlund et al., 2005) though in a population basis the changes might become evident in the sixth decade of life (Nyberg et al., 2012).

Memory decline in aging, though, might be affected at different stages such as encoding, storage or retrieval processes. At the input stage, older adults may encode less meaningful or less elaborated information (Craik et al., 1983). Also aged people might have difficulties to spontaneously recruit effortful encoding strategies (Logan et al., 2002), while when provided, their performance might remain relatively preserved compared to young adults. Alternatively, older people may attend to salient information while failing to integrate peripheral or contextual aspects, sometimes referred as source memory (Glisky et al., 2001). Older adults may also experience problems at the level of storage or consolidation, which critically depends on the medial temporal lobe, and involve bindings of various aspects of experience into durable memory trace. Memory decline in memory might be reflected by associative memory deficits. Considerable evidence also points to retrieval as a source of episodic memory problems in aging as effortful processes seems to be specially impaired in aging. Older adults tend to show deficits on tests of free recall, to a lesser degree in cued recall and even less in recognition memory. Also compared to recollection processes automatic judgments or familiarity remains almost intact (Jennings and Jacoby, 1997).

As suggested, the magnitude of age-related memory decline is highly dependent of the specific subprocesses engaged which in the neuroimaging field have usually been underrated. As paradigmatical examples it is the increased impairment for context, or source memory, when compared to content or item memory (Spencer and Raz,

1995). Another clear example is the abovementioned differential memory decline in aging as function of the specific recall process (Craik and McDowd, 1987). Such differential declines are probably mediated by diferent areas and neural systems supporting the cognitive demands. A last clear example is the observation that encoding decline might be differential according the Level of Processsing (LoP) information is encoded. That is, different LoP at encoding, such as semantic or perceptual analysis of the incoming information, result in more durable traces, therefore affecting the probability of a successful retrieval (Craik and Lockhart, 1972; Craik and Tulving, 1975). Similarly association memory is also specially affected by aging decline as well as recollective memory. Other types of memory that partially involve episodic memory such as autobiographical memory, some types of emotional memory or prospective memory are also affected by aging but to a lesser extent (see Park and Gutchess, 2000 for a review).

c. Basic neuroanatomical correlates of episodic memory

Based on a wealth of human and animal literature, evidence strongly supports the hypothesis that memory function relies on anatomically distributed networks of brain regions. Specifically interactions between cortical regions and medial temporal lobe structures (MTL) such as Hippocampus, Parahippocampal cortex and Entorhinal cortex are crucial to generate new episodic memory traces and new associations (Cohen and Eichenbaum, 1995; Squire and Zola-Morgan, 1991). A seminal report regarding the importance of MTL structures is the HM case, where medial temporal lobectomy led the patient with a severe anterograde amnesia (as well as retrograde amnesia) being unable to form new long-term memories of new event or semantic knowledge (Beecher Scoville and Milner, 1957). Initial evidence of implication of this area in episodic memory has been repeatedly confirmed by lesional, neuroimaging and animal literature.

Key cortical regions for subserving memory processes are mainly located in the prefrontal cortex (PFC; i.e. Fletcher et al., 1998a, 1998b; Wheeler et al., 1995); a

finding demonstrated ini multiple lines of evidence including animals, neuropsychological, non-invasive brain stimulation (NIBS) and neuroimaging studies. More specifically the interaction between PFC and MTL has been long hypothesized as a crucial system for episodic memory encoding suggested by structural connectivity (Braak et al., 1996), functional connectivity (Grady et al., 2003) or theta-rythm couplings (Anderson et al., 2010).

Further neuroimaging evidences revealed the existence of a set of functionally connected brain areas, so-called Default-Mode-Network (DMN, Raichle et al., 2001), comprising posterior midline structures (Precuneus/Posterior Cingulate Cortex (PCU/PCC), medial prefrontal cortex (mPFC), bilateral Inferior Parietal Lobes (IPL) and Middle Temporal lobe, which are also implicated in episodic memory (Andrews-Hanna et al., 2007; Greicius et al., 2004; Sestieri et al., 2011). A subsystem of this network also entails MTL regions such as hippocampus and parahippocampal cortex, which, at rest, are linked with posterior nodes of the DMN (Buckner et al., 2008; Campbell et al., 2013; Eldaief et al., 2011). Relationship between memory and DMN nodes has been most evidenced by neuroimaging literature. Early DMN research proposed that the network was involved in internal processing including memory processes (Fransson, 2005; Greicius et al., 2003; Vincent et al., 2007). Also, posteriormedial nodes of the DMN have consistenly related to episodic memory processes (Miller et al., 2008).

d. Main anatomofunctional correlates of aging

The human brain is continuously changing during lifespan from mollecullar to macronetwork levels (Pascual-Leone et al., 2005). From a general perspective, aging brain is characterized by grey mater cortical thinning (Fjell et al., 2009b, 2009c; Salat et al., 2004) and volumetric shrinkage (DeCarli et al., 2005; Good et al., 2001; Zimmerman et al., 2006) together with ventricular expansion (Earnest et al., 1979; see Fjell and Walhovd, 2010 for a review). Also, decreased density of white matter fibers, reflected by measures such as white matter hyperintensities (Wen and

Sachdev, 2004) or diffusion imaging techniques (Bennett et al., 2010; Davis et al., 2009; Sala et al., 2012), neurotransmitters depletion, especially loss of dopaminergic receptors (Reeves et al., 2002; Rinne et al., 1990), and alteration of functional brain networks (Ferreira and Busatto, 2013; Spreng et al., 2010) are also main features of the aging brain which have been extensively revealed by neuroimaging techniques.

Beyond neuroimaging techniques and *in vivo* observation findings, basic neuroscience studies have also characterized the aging human brain (see Burke and Barnes, 2010; Morrison and Baxter, 2012; Rosenzweig and Barnes, 2003; Samson and Barnes, 2013; Uylings and de Brabander, 2002 for reviews in the topic). This literature mostly contributed to increase our understanding of the chemical, cellular and molecular changes occurring during the ageing process, as these alterations cannot be specified at the resolution of neuroimaging techniques. A body of literature in this regard, has mostly focused on Dorsolateral Prefrontal Cortex (DLPFC) and medial temporal as areas as these are both regions of high age-related susceptibility and critical implication in learning and memory processes. Surprisingly, physiological aging is not characterized by dell death. Instead, it seems that cell number is relatively well preserved in healthy aging in most brain structures (Morrison and Baxter, 2012; Morrison and Hof, 1997). Instead, morphological changes have been detected such as dendritic arborization regression especially in pyramidal neortical neurons (Dickstein et al., 2013; Samson and Barnes, 2013). Aging is also characterized by changes in spine density and morphology, which is more marked in thin spines (Kabaso et al., 2009; Samson and Barnes, 2013).

The investigation of the underlying neural underpinnings that subtend learning and memory as key cognitive processes afected by aging, has payed great attention to the study of brain plasticity mechanisms. Indeed, one of the most significant changes in ageing is found at synaptic level, where altered mechanisms of plasticity in older subjects have been repeatedly documented (Burke and Barnes, 2006; Rosenzweig and Barnes, 2003), where altered plasticity in medial temporal regions is evident. Long Term Potentiation (LTP) induction and maintenance is significantly reduced in aging (Barnes, 1979) while alterations in Long-Term Depression (LTD) mechanisms are also documented (Burke and Barnes, 2010). Synaptic changes might be related to

another well-established finding in aging such as modifications in Ca^{2+} homeostasis processes (Burke and Barnes, 2010, 2006). Canonical microcircuits are also affected, as interactions between glutmatergic and gamma-amminobutyric acid (GABA)ergic cells is abnormal in healthy aging (Insel et al., 2012; Wang et al., 2010a). At a molecular level, the role of kinases or cyclic-adenosine monoposphate, Insulin signaling proteins and mitochondrial dysfunctions in aging are well documented (Burke and Barnes, 2010; Dickstein et al., 2013; Gkikas et al., 2014) while age-related genetic expression changes also significantly contribute to the brain aging process (Sibille, 2013). Finnally, neurochemical changes have also been widely studied. Both dopaminergic system and glutamatergic systems are greatly affected in aging while minor changes are also evident in serotonergic and cholinergic systems.

Detailed revision of such brain changes across lifespan, such as its modulators and its spatial patterns is beyond the scope of the thesis and have been previously addressed in several neuroimage-based reviews in the literature (Bennett and Rypma, 2013; Fjell and Walhovd, 2010; Govoni et al., 1988; Grady, 2012a; Salthouse, 2011). However it is important to consider such changes, as well as molecular and neuronal alterations, when studying functional networks dysfunctions in aging. Understanding these changes might help to explain how functional network alterations emerge and the specific impact of its dysfunction during lifespan. Anatomical changes during senescence have often been related to cognitive performance as well as to activity markers. Grey matter shrinkage, white matter hyperintensities and dopamine, as well as vascular changes are known to have a partial impact over functional conectome, as assessed by functional Magnetic Ressonance Imaging (fMRI), that often it is underestimated. However rhe exact relationship between aging, those physiological changes, resting-state networks and age-related cognitive decline remains to be fully elucidated and it is probable that may be moderated by several other variables. Mediation analyses (Salthouse, 2011) might which are increasingly been used (i.e. Sala-Llonch et al., 2014; Steffener et al., 2014) might help to clarify such relations.

Most of the abovementioned brain changes in aging where linked to “biological” deterioration. However, a consistent finding in cognitive aging studies is that brain

also subserves modifications not related to decline *per se*, but as a response to it. Hence several neural changes observed during lifespan are reflecting a *homeostatic* response, by engagement of mechanisms of plasticity. Such compensatory changes are particularly relevant, though not exclusive, at a functional network level. It is widely accepted that brain is a dynamic and adaptive organ that attempts to maintain "homeostatic" cognitive control and that is dynamically changing by plasticity mechanisms engageent during all the lifespan (Oberman and Pascual-Leone, 2013; Pascual-Leone et al., 2005). The consequence of such changes may be especially visible when brain is responding to neural insults such as those biological mechanisms related to age decline (see **Plasticity section**).

A last introductory note must be highlighted in any study focused on healthy aging and related to the possible contamination of samples by undetected pathology. That is, the study of brain and cognition in aging can be hampered by undetected neuropathological changes to dementing illnesses, though biomarkers tests can highly limit such limitation, and one has to take special caution when extracting conclusions if normal and pathological changes have similar biological fingerprints.

i. Main structural neuroimaging findings to memory in aging

Cognitive neuroscience in aging have tried to unify findings from both neuroscience and cognitive aging by studying the brain mechanisms associated cognitive changes in aging; that is to understand the brain mechanisms that might underlie better or worse performance in old adults. In this regard neuroimaging techniques qlso represents an outstanding method to investigate this correlates in elders given the possibility to study the human brain *in vivo*. Given the relevance of memory decline in aging highlighted during decades by cognitive aging scientists this field have attracted a great interest. The main findings from in this area are briefly overviewed.

Brain imaging supports a number of hypothesized mechanisms for memory decline in older age. Before the advent of functional neuroimaging, the predominant perspective assumed that episodic memory declines in aging was mostly due to loss of function in critical regions and networks, particularly in PFC and MTL. Indeed, in

aging, reduced grey matter integrity in PFC (Nyberg et al., 2010; Rajah et al., 2011) has been related to episodic memory performance though contradictory evidence exist (Maillet and Rajah, 2013; Salat et al., 2002). PFC is the brain region that shows the steepest rate of age-related decline (Fjell et al., 2009c; Fjell and Walhovd, 2010). More consistently, reduced MTL volumes in aging has been related to worse memory performance, both in the entorhinal cortex (Raz et al., 2004; Yonelinas et al., 2007), the hippocampus (Lye et al., 2006; Tisserand et al., 2000; Yonelinas et al., 2007) and the parahippocampal cortex (Tisserand et al., 2000). In this sense hippocampus shows considerable volume decline (Fjell et al., 2009b; Fjell and Walhovd, 2010) which might be steeper at older ages (Raz et al., 2005) in contrast with other subcortical structures and cortical areas.

Also, white matter integrity in prefrontal and temporal areas as well as tracts connecting frontotemporal areas (Charlton et al., 2010; Davis et al., 2009; Kantarci et al., 2011; Madden et al., 2004; Persson et al., 2006) have been related to memory performance. Healthy aging, is also associated with pronounced white matter atrophy, showing an anterior-to-posterior gradient of susceptibility (Bennett et al., 2010; Davis et al., 2009; Sala et al., 2012). However loss of white matter integrity in aging has mostly been associated with executive functions and speed processing decline more often than with memory.

Functionally, abnormal patterns of activation in aging, during episodic memory encoding and retrieval tasks have been observed both by Positron Emision Tomography (PET) and fMRI neuroimaging techniques (i.e. Spreng et al., 2010). Those changes in neural networks, involving increases and decreases of activation, over-recruitment of additional areas, and connectivity changes have been related to memory performance and will be discussed later on detail (see **Brain networks section**). fMRI due to its characteristics has become one of the most popular tools to explore memory function in aging leading to a number of existing theories and helping to understand the underlying mechanisms of declined cognition in aging. Hence, findings reviewed are mostly based in fMRI technique though it is fair to underscore the relevance of other methods to study age-related brain changes in resting-state networks such as electroencephalography (EEG), PET or functional near

infrared spectroscopy have made important contribution to this field, introducing influent brain aging models, which, unfortunately are out of the scope of this document. In the last few years, though, multimodal studies taking into account both structural and functional information have appeared representing an exciting attempt to better understand brain changes in aging. As example Daselaar and colleagues (Daselaar et al., 2013) found that greater PFC activity in older adults compensated for impaired white matter connectivitybetween temporal and anterior regions.

2. Brain networks

a. Functional Magnetic Resonance Imaging

Since the introduction in the early 90s (Ogawa et al., 1992, 1990), fMRI has become a pillar of neuroimaging in cognitive neuroscience. The most common use of fMRI consists in the measurement of the Blood Oxygen Level Dependent (BOLD) signal, a technique which was developed in the 1990s after the BOLD effect was first described (Ogawa et al., 1990). This effect reflects the dependence of T2*-weighted contrasts on the amount of blood deoxygenation which is a consequence of different magnetic properties of oxygenated and deoxygenated hemoglobin (in the hemodynamic response oxygen is released to the blood to a greater degree than needed). Thus, changes in the ratio of oxygenated to deoxygenated blood reflects metabolic activity which is dependent on the level of activation of the surrounding tissue; then BOLD signal can be used as an indirect indicator of neural activity. Neurophysiologically, fMRI BOLD signal is best correlated with local field potentials, that is, the complex signals arising from the integrated electrical activity in pre- and postsynaptic terminals of the brain (Logothetis, 2008). Main advantages come from its noninvasive nature and its good spatial resolution while main disadvantage is

that, like all hemodynamic-based modalities, it measures a surrogate signal whose spatial specificity and temporal response are subject to both physical and biological constraints. For more information about basis, pitfalls and research history see Logothetis (2012, 2008).

Initially, fMRI in cognitive neuroscience used model-based, task-related fMRI designs to assess brain activity. Block design, based on the subtraction paradigm, and later, event-related and mixed designs dominated fMRI studies. Advances in fMRI analysis led to the arising of new methods, which are being increasingly used, such as multivariate pattern analyses, psychophysiological interaction, causal analyses, or data-driven analyses. However, in the last years designs without experimental paradigms are being increasingly used (Snyder and Raichle, 2012), also known as resting-state fMRI (rs-fMRI) protocol. Task-free functional connectivity MRI detects interregional correlations in spontaneous BOLD fluctuations (Biswal et al., 1995) with neurophysiological significance. One of the main findings were that at rest, brain is organized in several Resting-State Networks (RSN), such as the DMN, relatively stable across time and subjects (Damoiseaux et al., 2006; Guo et al., 2012; Zuo et al., 2010). This RSNs are partially reflecting the underlying structural arquitecture (Honey et al., 2009; Horn et al., 2013), and also have a high degree of similitude to those neural networks found when subjects are performing tasks (Smith et al., 2009). Methodologically, several methods have been used to investigate rs-fMRI, of which seed-based correlation, independent component analysis (ICA) and graph-based methods are probably the most widely used (see Cole et al., 2010; Margulies et al., 2010 for detailed information of main analytical tools).

b. Neural networks and aging

In this section, we will review main studies of theories and models that have attempted to explain age-related cognitive changes focusing on a network perspective level by employing fMRI. These age-related changes might be grouped into activation and deactivation alteratios and connectivity abnormalities from task-

related studies as well as a body of knowledge originated from resting-state studies. The section will conclude with a review of neural network correlates of memory function in aging.

i. Recruitment patterns

In the past two decades fMRI studies have provided vast evidence of age differences in task-related activity (Eyler et al., 2011; Grady, 2012 for reviews; Spreng et al., 2010 for meta-analysis). While these studies provided interesting knowledge to understand neuropsychological aging, heterogeneous findings, such as, both, increases and decreases in activity in the PFC under several patterns of cognitive decline, revealed the necessity of interpretations and theories capable to take into account disparate findings.

As expected, some of the earlier studies found decreased brain activity in elders during task, accompanied by poorer performance (i.e. Grady et al., 1995), which has typically been interpreted as a reflection of cognitive deficits in older adults and a lack of efficiency in the use of neural resources (Grady et al., 2006; Rypma and D'Esposito, 2000). A typical pattern displayed by aged people, consist in reduced activity in posterior areas across several cognitive tasks (Ansado et al., 2012; Grady et al., 1994; Kehoe et al., 2013), as observed by Davis and colleagues (Davis et al., 2008) whom evidenced decreased activation in posterior areas both during episodic retrieval and visual perceptual tasks in elders compared to young participants. Decreased response in the hippocampus was also shown in earlier block-design studies of episodic memory (Daselaar et al., 2003; Grady et al., 1995; Gutchess et al., 2005) in elder subjects. Similarly, equivalent activity, but lower performance in the old group may also be interpreted as a less effective use of neural resources (Zarahn et al., 2007).

Increased activity, a recurrent finding in cognitive neuroimaging of aging (Grady, 2012a; Spreng et al., 2010), poses a major challenge of interpretation. Such increased activity has both been observed in areas typically engaged by young subjects, but also, these increases involve "over-recruitment" of additional areas

which are not usually engaged in young subjects (see reviews and metaanalysis Eyler et al., 2011; Grady, 2012; Park and Reuter-Lorenz, 2009; Spreng et al., 2010).

Increased activity in the areas primarily engaged by young subjects has frequently been interpreted as increased demands for performing the same cognitive task, suggesting lack of efficiency when recruiting neural responses (Cabeza et al., 2008; Stevens et al., 2008). However, as early as in the 90s, increased activity, especially "over-recruitment" of additional areas, generally in the PFC, was thought to be compensatory recruitment for reduced or inefficient activity elsewhere (Cabeza et al., 1997; Grady et al., 1994). Much of the subsequent work has continued to explore this idea as these findings illustrates the capacity of the brain to plastically adapt in order to maintain its function. Such interpretation is usually assumed when elders "over-recruits" areas tha are not engaged in young adults (Cabeza et al., 1997; Grady, 2012a; Grady et al., 1994; Reuter-Lorenz et al., 2000). However, such interpretations necessarily need behavioral support. Lack of cognitive correlates implies that other interpretations cannot be ruled-out such as the results are reflecting inefficient use of brain activity. Hence, interpretation of compensatory, PFC, activity has been proposed when high-performing elders shows "over-recruitment" compared to young and low-performers (Cabeza et al., 2002) or when activity in this additional areas is positively related to cognitive function (Greenwood, 2007). Hemispheric Asymmetry Reduction in Older Adults (HAROLD, Cabeza, 2002, **Figure 2.** A) HAROLD MODEL. PFC activity during source memory was right lateralized in Young and Old-Low participants but bilateral in Old-High participants (from Cabeza et al., 2002)) refers to those patterns of "compensatory" activity consisting in inverrecruitment of the homologous region in the opposite hemisphere from the region typically responsive in the young group; consequently presenting a less lateralized pattern of activation. However when an "over-recruitment" in elders is negatively associated or unrelated to performance, it has often been interpreted as increased cognitive effort, a need to allocate greater neural resources in general or as a non-selective engagement or brain areas (Logan et al., 2002). The so-called dedifferentiation pattern presents a more diffusive pattern of activation in older adults and less selective activity in task-relevant regions

across a variety of tasks (Dennis and Cabeza, 2011; Grady, 2002; Townsend et al., 2006).

Another model that accounts for age-related changes in brain activity is the to Posterior-to-Anterior shift in aging (PASA, **Figure 2.** A) HAROLD MODEL. PFC activity during source memory was right lateralized in Young and Old-Low participants but bilateral in Old-High participants (from Cabeza et al., 2002)) model (Davis et al., 2008). This model integrates two frequent findings in neuroimaging literature of aging; that is increased activity in PFC (i.e. Cabeza, 2002; Morcom et al., 2003) and reduced activity in posterior visual areas (i.e. Davis et al., 2008; Madden et al., 2007). PASA is a compensatory model that sustains that older people compensate with more activity in the PFC for reduced activity in visual processing regions. In this regard, in an early study Cabeza and colleagues (1997) observed increased engagement in PFC and decreased engagement in posterior areas during memory encoding and retrieval tasks assessed with PET. Such age-related changes are though to be produced by increased top-down processing compensating for decreased bottom-up processing. To note, most of the models described here are not incompatible rather patterns of task-related activity in elders can fit with different models at the same time such as HAROLD and PASA. A review by Eyler and colleagues (Eyler et al., 2011) found that almost 50% of studies examining neural correlates of cognitive performance in aging found results that are either consistent with PASA and/or HAROLD model. Such results are present in a variety of cognitive task, mostly working (Cabeza et al., 2002) and episodic memory tasks (Daselaar et al., 2003). More evidence supporting the PFC compensatory models has come from other techniques, such as NIBS (i.e. Manenti et al., 2011; Rossi et al., 2004).

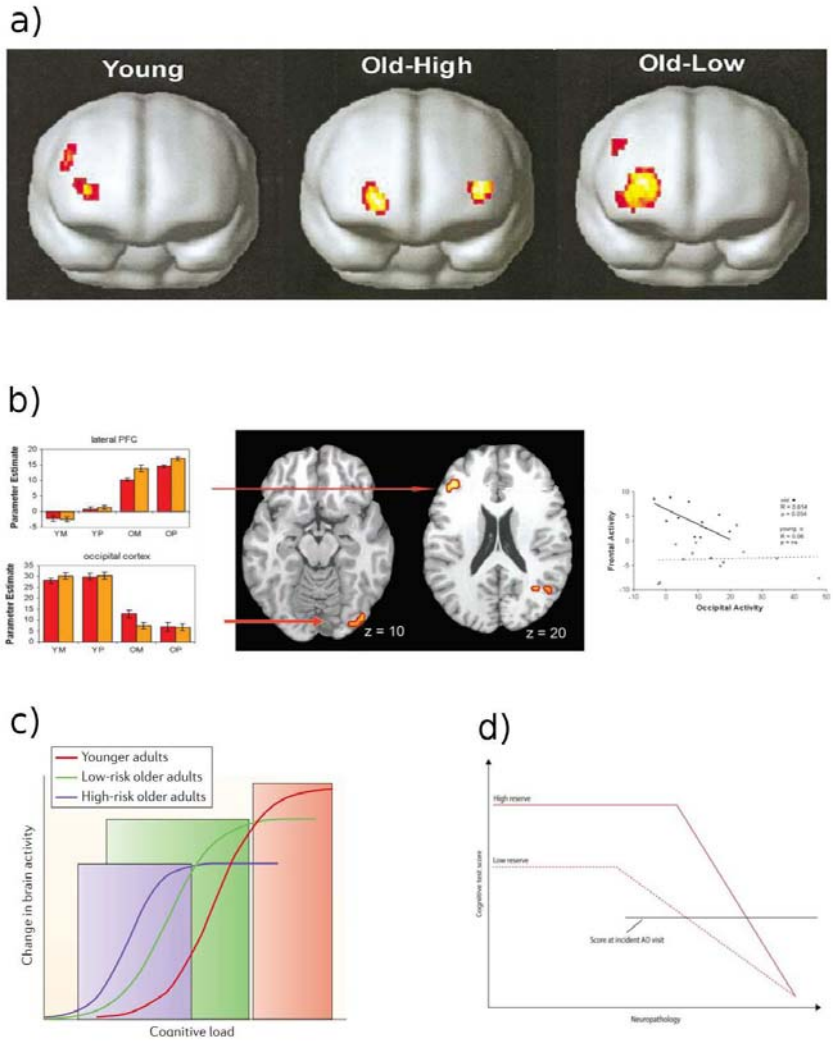

Figure 2. A) HAROLD MODEL. PFC activity during source memory was right lateralized in Young and Old-Low participants but bilateral in Old-High participants (from Cabeza et al., 2002). B) PASA model. Across 2 different tasks and 2 conditions, the occipital cortex showed greater activity in younger than in older adults whereas PFC showed the opposite pattern. In elders occipital and frontal activations were correlated suggesting compensatory mechanisms (from Davis et al., 2008). C) CRUNCH model. The function relating the change in brain activity to levels of cognitive load is shown for young adults, old adults with a low risk of developing Alzheimer's disease and old adults with a high risk of developing dementia. The function in low-risk older adults would be shifted to the left relative to that seen in younger adults. At relatively low levels of cognitive load this shift would result in higher activity in older relative to younger adults. However, activity in older adults would reach its peak and level off while younger adults' activity is still increasing, so that at higher load levels there would be no age difference in activity or younger adults would have higher activity. A similar effect would be seen when high-risk older adults are compared to low-risk older adults, with the reverse seen at higher levels of load (from Grady, 2012). D) CR

hypothesis. Hypothesised change in memory function over time in individuals with high and low cognitive reserve. AD pathology begins to advance before changes in memory performance are observed. Decline is seen later in individuals with high cognitive reserve because pathology is tolerated longer than by people with low cognitive reserve. The rate of decline, differs between groups, and is more rapid in individuals with high reserve than in those with low reserve (from Stern, 2012).

The above reported "over-recruitments" observed during cognitive demands in several studies of cognitive ageing may be interpreted as evidences of functional brain resources expressing adaptive plastic mechanisms, in the line with brain plasticity models proposed by Pascual-Leone and collaborators (Oberman and Pascual-Leone, 2013; Pascual-Leone et al., 2005). That is, age-related increases in activity observed by neuroimaging methods are a consequence of plasticity mechanisms engagement in an effort to compensate for brain decline, but activity is not necessarily compensatory *per se,* in the sense that such activity not necessarily relates to improved performance), either because brain engages unsuccessful compensatory mechanisms or either because compensatory mechanisms can only partially take into account brain deterioration (Grady, 2012a). According to this latter author, successful, unsuccessful or attempted compensation are the terms referred to the increased age-related activity depending on the subsequent memory performance. Such changes are mostly observed in PFC over-recruitments though other areas in task-positive networks have also been reported (Frings et al., 2010; Jenkins and Ranganath, 2010; Staresina and Davachi, 2010) such as superior parietal areas. Finally, age-related changes in activity can be caused by engagement of different strategies to perform the task. As specific cognition processes declines with age, elders seems to rely more in alternative strategies to maintain task performance (Daselaar et al., 2006; Kirchhoff et al., 2012; Logan et al., 2002). A paradigmatic example, especially relevant during memory encoding strategies, relates to level of processing and effortful encoding and retrieval strategies of recollection compared to familiarity.

Another compensation-based model which tries to account for increased age-related activity is the 'compensation-related utilization of neural circuits hypothesis' (**Figure 2.** A) HAROLD MODEL. PFC activity during source memory was right lateralized in

Young and Old-Low participants but bilateral in Old-High participants (from Cabeza et al., 2002), CRUNCH; Reuter-Lorenz and Cappell, 2008). The core proposition of CRUNCH is that more neural resources are recruited by older adults at low levels of cognitive load — that is, when tasks are easier — than by younger adults, who do not need them (Rypma et al., 2007). At higher levels of load, this compensatory mechanism is no longer effective, leading to equivalent or less activation in older adults relative to young adults. Data consistent with this idea have been reported mostly in the PFC (Cappell et al., 2010; Schneider-Garces et al., 2010). From another theoretical perspective Scaffolding theory of aging and cognition (STAC, Park and Reuter-Lorenz, 2009) and Cognitive Reserve hypothesis (**Figure 2.** A) HAROLD MODEL. PFC activity during source memory was right lateralized in Young and Old-Low participants but bilateral in Old-High participants (from Cabeza et al., 2002), CR, Stern, 2009) also takes into account such age-related compensatory changes, by explaining disfunction between degree of brain insult and cognitive (and clinical) outocome. A torough descprition of models that take into account the mismatch between biological pathology and clinical/behavioral outcome are described in several papers of our group (Arenaza-Urquijo et al., 2013; Bartrés-Faz and Arenaza-Urquijo, 2011; Bosch et al., 2012).

ii. Deactivation patterns

Age-related changes in activity during cognitive tasks are not only observed in task-positive networks, such as PFC, but also in task-negative networks, which in most-cognitive external-oriented tasks resembles the topology of DMN. When subjects perform a cognitive task, these areas deactivate, a reduction of activity is observed compared to baseline/rest levels (Anticevic et al., 2012a; Raichle et al., 2001). Greater deactivation in those areas has been repeatedly related to cognitive performance (Anticevic et al., 2010; Daselaar et al., 2004; Shulman et al., 2007; White et al., 2013) which has frequently been interpreted as an effective reallocation of neural resources essential for efficient cognitive processing.

Several studies have found that old adults show less reduction of default mode activity during cognitive task (de Chastelaine et al., 2011; Duverne et al., 2009; Grady et al., 2006; Lustig et al., 2003; Miller et al., 2008; Prakash et al., 2012; Sambataro et al., 2012, 2010; Spreng and Schacter, 2012), especially during working memory and episodic memory encoding tasks which is often accompanied by alterations in connectivity in this network. Such failure to suppress activation in regions of the DMN, particularly the posteromedial cortices, has been associated with poorer performance (Duverne et al., 2009; Miller et al., 2008). Lack of suppression of task-negative networks, being posterior DMN midline structures the most affected by age, have been usually interpreted as deficient reallocation of neural resources. However other complementary explanations exists such as elders showing lower degree of network flexibility (Spreng and Schacter, 2012), reduced shifting capacity (Gould et al., 2006) or increased sensorial noise (Stevens et al., 2008). Similar to CRUNCH model, such age-deactivation might be moderated by cognitive load (Prakash et al., 2012). Aging also produces topological changes in task-negative networks beyond changes in magnitude and connectivity. Some areas that deactivate in young adults are recruited as task-positive networks in aging, which have been hypothesized to be part of a compensatory mechanisms (Sala-Llonch et al., 2012a). Indeed, negative relationship between performance and activity in such areas is reversed or disappears in the aging group (de Chastelaine et al., 2011; Duverne et al., 2009).

iii. Task-related connectivity patterns

Cognitive neuroscientists are becoming increasingly interested in assessing the integrated activity among groups of brain regions. Such view implies that cognitive processes are not localized to brain regions functioning in isolation, but rather are emergent properties of neural networks interactions. The capacity of engage and disengage different cortical networks and the precise moment to correctly perform is essential for optimal functioning. Task-related studies have shown age-related differences in brain connectivity during cognitive processing which has been further related to cognitive performance. Task-related connectivity patterns are highly

depending on the specific psychological state of the subjects which implies an enormous difficulty to overview these changes; still main common characteristics of age-related changes in connectivity will be delineated.

The most prominent age-related change in connectivity is dysfunctional DMN synchrony during tasks. Less connectivity between DMN nodes has been repeatedly observed in elders performing cognitive tasks (Grady et al., 2010; Lustig et al., 2003; Miller et al., 2008; Persson et al., 2006). Connectivity between posterior and anterior DMN subsystems as well as connectivity between posterior and MTL subsystems are most disrupted in aging (Grady, 2012a). Relationship between task positive and task negative (DMN) networks may also be altered which lead to the suggestion that the balance between the default and task positive networks is critical for effective cognitive processing (Grady et al., 2010). Aging brain is characterized to have less plasticity and flexibility to adapt for external demands, and that becomes evident when a change of networks is required to cope the cognitive demands (Prakash et al., 2012). Temporal inability to synchronize areas has shown critical for cognitive performance (Düzel et al., 2011; Persson et al., 2007).

As changes in functional recruitment, age-related connectivity changes might also be compensatory in nature. Hence, to compensate for brain decline, additional networks might be recruited in order to maintain cognitive performance (for experimental designs see: Lee and D'Esposito, 2012; O'Shea et al., 2007). Couplings that were essential for cognitive function in young healthy subjecs, might not be critical in elders that may rely in other *new* compensatory couplings instead (Grady et al., 2010). Such findings are highly consistent with the observations of recruitment of additional areas in elders. Changes of connectivity between groups, may be interpreted in some cases as a reflection of a different cognitive strategies by the aging group such as in Daselaar et al., (2006) where old adults had reduced functional connectivity within a hippocampal–parietotemporal network relative to young adults, but increased connectivity within a parahippocampal–frontal network.

Such finding was interpreted as olders relied more in familiarity-based memory retrieval.

Another recurrent findings in cognitive aging, is impaired top-down inhibition (Gazzaley and D'Esposito, 2007), which its main evidences come not only from fMRI but also from Transcranial Magnetic Stimulation (TMS) and EEG evidences. Top-down modulation is the mechanism by which people enhance neural activity associated with relevant information and suppress activity for irrelevant information, thus establishing a foundation for both attention and memory processes. Healthy older adults exhibit a selective inability to effectively suppress neural activity associated with distracting information which is correlated with memory impairment as well as has been related to inhibition and speed processing deficits (Gazzaley et al., 2008; Gazzaley and D'Esposito, 2007; Kalkstein et al., 2011; Zanto et al., 2010). Such top-down deficits are known to depend of neural connections subserving dynamic interactions between widely distributed brain regions at the "top" (i.e. PFC) and the "bottom" (i.e. Visual Cortex) and fits well with several neuroimaging models of aging, especially with dedifferentiation in *lower cognitive* areas. PFC-MTL alterations in aging, known to be essential in aging can be considered as top-down modulations most of the times.

Brain flexibility has pinpointed as an important indicator of cognitive variability (Arieli et al., 1996; Fjell et al., 2009a) which in turn is a good predictor of cognitive performance in aging (MacDonald et al., 2003). In the last years, ageing has been found to be related with a loss of variability of brain signals (Fjell et al., 2009a; Garrett et al., 2013, 2012, 2011; Mella et al., 2013) showing an inverted U-shaped across lifespan and that is related with a U-shape curve of variability in behavioral responses during lifespan. Networks that are more variable may be more robust to disruption and may explore more neural states, thus enhancing learning and promoting optimal performance (Deco et al., 2011; Stein et al., 2005). Such field remains mostly unexplored but may be related to several models explained above helping to understand difficulties in engaging and disengaging networks during task performance.

iv. Resting-state findings

The emergence of resting-state fMRI and its physiological relevance opened a new window to study the idiosyncratic characteristics of the aging brain. Such studies have been appearing in the last few years (Ferreira and Busatto, 2013) but several commonalities might be outlined mostly referred to rs-fMRI connectivity changes. Age has been found to be a significant determinant of inter-individual variability in rs-fMRI connectivity, hence attracting considerable attention (see Ferreira and Busatto, 2013; Hafkemeijer et al., 2012; Nakagawa et al., 2013 for recent reviews).

Connectivity changes in aging have been found in terms of changes in global brain connectivity patterns such as path length or clustering (Meunier et al., 2009; Tomasi and Volkow, 2012). However Dorsal Attentional Network (DAN; similar to Task-Positive Network as defined by Fox and colleagues [2005]) and more especially the DMN consistenly shown affectation in aging. These networks are related to different cognitive domains such as attention, memory and executive processes and have high susceptibility to brain insults. Decreased DMN synchrony is almost unequivocally observed in aging people (**Figure 3**; Achard and Bullmore, 2007; Andrews-Hanna et al., 2007; Biswal et al., 2010; Campbell et al., 2013; Damoiseaux et al., 2008; Esposito et al., 2008; Koch et al., 2010; Tomasi and Volkow, 2012; Wang et al., 2010a) which has been assessed by different methodologies such as seed-based, independent component or graph approximations. Only a handful of studies showed no evidence of decreased connectivity (Beason-Held et al., 2009; Westlye et al., 2011) or positive association (Jones et al., 2011) of DMN rs-fMRI connectivity with age. Such divergent results, though, might be explained by specific design and samples. Additionally some studies (Bluhm et al., 2008; Koch et al., 2010; Razlighi et al., 2014) suggested that the methodologies used to evaluate connectivity have a great impact to results. One example of altered DMN synchrony in aging was revealed by Damoiseaux and colleagues (2008) that identified age differences specifically in DMN network while no significant age-differences were found in other investigated RSN suggesting a preferential susceptibility of the DMN rs-fMRI in elderly. DMN vulnerability might be exacerbated between posterior structures (posterior midline structures and inferior

parietal lobes) with temporal structures (middle and medial temporal lobes; Wang et al., 2010a) and connectivity between posterior structures to medial prefrontal cortex (Andrews-Hanna et al., 2007; Campbell et al., 2013; Meunier et al., 2009; Wu et al., 2011). Such finding is paralleled for the abovementioned anterio-posterior disconnection in task-related contexts (Grady et al., 2010; Kalkstein et al., 2011; Sambataro et al., 2010). The specific cause explaining the increased rs-fMRI vulnerability to ageing of this network remains to be fully elucidated but it might be related to neuroplasticity as DMN nodes are amongst those with more plastic capacities. A recent provocative review (Fjell et al., 2014) suggests that regions characterized by a high degree of life-long plasticity are vulnerable to detrimental effects of normal aging (and that this age-vulnerability renders them more susceptible to additional AD-related changes). DMN nodes are highly affected by both age-related and AD-related cortical atrophy (i.e. Fjell et al., 2014, 2009b) and Beta-Amyloid (βA)-deposition (i.e. Buckner et al., 2008, 2005). However, alteration in DMN connectivity seems to be partially independent from both cortical atrophy (Damoiseaux et al., 2008) and βA load (Andrews-Hanna et al., 2007).

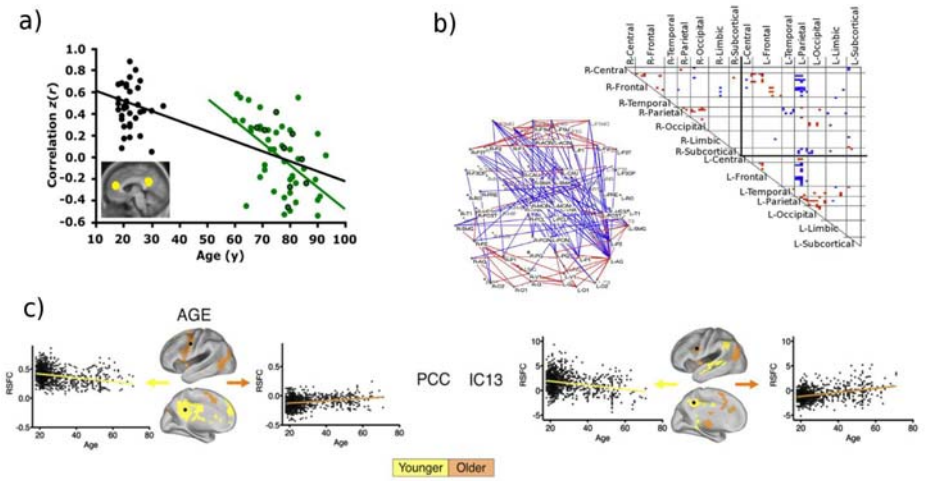

Figure 3. Anteroposterior DMN connectivity is highly reduced in aging. A) mPFC-PCC cingulate correlations were computed for each participats. Seed-to-Seed connectivity is decreased in elders (from Andrews-Hanna et al., 2007). B) Graph-theory based analysis. Node pairs showing positive (red) and negative (blue) correlations with age with matrix

representation of edges (Sala-Llonch et al., 2014a) C) Areas exhibiting age-related variation in rs-fMRI. In yellow decreased connectivity in elders; in orange increased connectivity in elders. Left side shows PCC-brain connectivity. Right side shows DMN network connectivity (from Biswal et al., 2010).

Rs-fMRI connectivity alterations have been described in other networks beyond DMN. DAN's functional connectivity has also been found to decline with age (Andrews-Hanna et al., 2007; Tomasi and Volkow, 2012). In this later study the authors demonstrated age-related functional connectivity alterations in a sample of 913 healthy subjects using functional connectivity density mapping analysis. Functional connectivity decreases in DMN and DAN networks were found while increases where observed in somatosensory and subcortical networks. Moreover, this study indicated that long-range connections are more prone to age effects than short-range connections, conclusion derived also from other studies (Meier et al., 2012; Sala-Llonch et al., 2014a).

Age-related deficits have also been reported in salience and motor networks (Allen et al., 2011; Onoda et al., 2012) but this findings contrast with increases in rs-fMRI connectivity in the same systems (Biswal et al., 2010; Meier et al., 2012; Tomasi and Volkow, 2012). Connectivity increases in aging are often explained as reduced global integrity, which causes isolation of hubs (Meunier et al., 2009) and increased regional connectivity (Sala-Llonch et al., 2014a). Such differences in rs-fMRI measures, and other graph-derived measures such as cost-efficieny coefficient (Achard and Bullmore, 2007) have been consistently related to differences in general cognitive performance and in specific cognitive domains such as memory, speed processing or executive functions (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; He et al., 2012; Wang et al., 2010a). Such differences are not only observed in healthy elder population but also in healthy young subjects and clinical populations. However, beyond the DMN, greater connectivity does not necessarily reflect greater network integrity nor imply better task performance (Antonenko et al., 2012; Ystad et al., 2010), as not only synchrony, but also segregation between different nodes plays a relevant role fostering effective behavior.

A number of hypotheses have been formulated to explain age-related functional connectivity deficits associated with cognitive decline in healthy elders. After several studies found that reduced DMN connectivity was not fully explained by grey matter integrity (Damoiseaux et al., 2008; Onoda et al., 2012), three potential causal factors have been highlighted: loss of white matter integrity, dopaminergic deficits, and amyloid deposition. Convergent evidence supports the notion that rs-fMRI connectivity reflects the underlying architecture of anatomical connectivity (Park and Friston, 2013; van den Heuvel et al., 2009; van den Heuvel and Sporns, 2013), and that it is positively correlated with indices of structural connectivity (Damoiseaux and Greicius, 2009), especially in the DMN (Andrews-Hanna et al., 2007; Khalsa et al., 2013; Teipel et al., 2010) which is the network with highest correlation between structural and functional connectivity (Horn et al., 2013; Skudlarski et al., 2008). However negative reports linking strength of functional and structural connectivity are also frequent in the literature which might partially be explained by known difficulties and limitations to assess structural connectivity especially in crossing fiber regions (see Jbabdi and Johansen-Berg, 2011 for a review). On the other side, the relationship between patterns of functional connectivity at rest and indices of dopaminergic transmission has also been shown by a number of independent fMRI studies (Diaconescu et al., 2010; Honey et al., 2009; Krajcovicova et al., 2012), which suggest an important contribution to age-related alterations of rs-fMRI connectivity, particularly within frontostriatal circuits. Further, Aβ deposition has also been linked to rs-fMRI connectivity especially with coupling reductions within DMN nodes (Drzezga et al., 2011; Hedden et al., 2009; Mormino et al., 2011; Sheline et al., 2010). Finally, rs-fMRI connectivity modifications in aging have been found after controlled interventions such as exercise (Voss et al., 2010), comprehensive cognitive/behavioral training (Pieramico et al., 2012) non-invasive stimulation (Lindenberg et al., 2013; Meinzer et al., 2013). It is thought that such network modulations might somehow mediate the relationship between intervention and cognitive benefits.

Other studies made use of local methods to assess age-related brain differences during rest, such amplitude of low frequency fluctuation (ALFF, i.e. Biswal et al.,

2010; Yan et al., 2011) or Regional Homogeneity (ReHo, i.e. Wu et al., 2007; Yan et al., 2011) techniques. Such measures are usually considered complementary to rs-fMRI connectivity changes and most frequent findings are reduced fluctuation and homogeneity in aging in several highlighted systems such as DMN and DAN networks as well as subcortical structures.

c. Memory networks in aging.

This section consists on a summarized revision of the scientific literature that studied the relationship between network dynamics and episodic memory function in aging will be described. The section comprises MTL, PFC and DMN activity-related findings. The last section will examine the relevance of network connectivity in episodic memory in aging. It is fair to announce that such divisions are arbitrarily and exist on a pragmatic basis as it is known that findings in distal structures, as well as connectivity changes are, at least, partially dependent one from the other.

i. Medial Temporal Lobe findings

As medial temporal structures have a critical role for memory formation and retrieval (see **Basic neuroanatomical correlates of episodic memory section**), neuroimaging researchers initially focused the study of age-related declines in memory towards MTL structures such as hippocampus, entorhinal and parahipocampal cortex.

Age-related under recruitment of the MTL has often been observed in a variety of episodic encoding memory tasks (i.e. Daselaar et al., 2003; Dennis and Cabeza, 2011; Dennis et al., 2007; Grady et al., 2002, 1995; Gutchess et al., 2005), especially associative memory paradigms which have shown to be particularly useful in detecting memory impairments. Such observation has often been viewed as a contributing factor to age-related deficits in episodic performance. Indeed, decreased activation during MTL engagement, specifically, reduced hippocampal

activity during memory encoding, has been related to impaired memory performance (Daselaar et al., 2003; Weis et al., 2011; Zamboni et al., 2013). Interestingly, such relationship is often seen in aged people but it is more uncommon in younger populations suggesting that age might also exert a moderator effect in such relationship (Salthouse, 2011). As an example, in a level of processing paradigm, Daselaar (2003) found that older adults exhibited reduced hippocampal activation during deep memory encoding, which was characteristic of low-performing elders. Such changes were often related to increased PFC activity (Dennis et al., 2007; Gutchess et al., 2005) which it is generally interpreted as engagement of PFC compensatory mechanisms.

However, when subsequent memory/forgetting measures are selected as indices of interest, results regarding decreased activity in MTL are more conflictive. Subsequent memory/forgetting only concern the neural activity predicting events that will be remembered or forgotten by use of event-related encoding paradigms. Different groups have repeatedly shown that elders hippocampus is normally engaged during associative memory encoding, which have been further replicated by independent groups (Miller et al., 2008; Persson et al., 2011; Rand-Giovannetti et al., 2006). Taken together, it is suggested that age-related decrements in memory performance are not specially influenced by MTL activity abnormalities, as this areas are capable of activations of the same magnitude as healthy young subjects. Instead, this changes may be more related to alterations in other activity patterns in cortical regions or due to deficient synchrony between MTL and other several brain regions (Chhatwal and Sperling, 2012; Hedden and Gabrieli, 2004) that might cause dysfunctional hippocampal activations during episodic memory encoding.

Local changes in MTL in aging have also been related to dedifferentiation processes (Dennis and Cabeza, 2011; Rieckmann et al., 2010). In the former study, they contrast implicit and explicit memory. While young adults showed more activity in the hippocampus for explicit memory and in the striatum for implicit learning, elders showed equivalent activity in both areas.

Probably the most salient neural differences associated with episodic retrieval in aging are found within MTL (together with PASA phenomenon). Still though, it is unclear whether hippocampal activation during retrieval is associated with decreased hippocampal activation (Cabeza et al., 2004; Daselaar et al., 2006; Giovanello et al., 2010) or not (Miller et al., 2008; Persson et al., 2011; Ramsøy et al., 2012) which is probably dependent on the exact measure used and the specific demands of the retrieving task (Dennis and Peterson, 2012). A recurrent finding though, is a shift from hippocampal recruitment to other MTL areas, especially the parahippocampal cortex (Cabeza et al., 2004; Daselaar et al., 2006). This neural shift suggests that older adults may be relying on greater familiarity in order to compensate for deficits associated with recollection. However, it might be difficult to disentangle whether such changes represent a compensatory mechanism (Daselaar et al., 2006) or a dedifferentiation phenomenon (St-Laurent et al., 2011). Relationship of this MTL age-related changes with PFC suggest that top-down mechanisms might explain in a great degree MTL dysfunction in aging. Differences in the temporal lag of MTL recruitment have also been observed in aging adding additional support to the hypothesis that elders rely in different retrieval process than aging (Dew et al., 2012).

ii. Prefrontal Cortex Findings

Increased activity during episodic memory encoding task by elders soon attracted great attention from researchers in this field. Indeed, one of the most consistent findings in task-related memory tasks in aging is the observation of increased PFC recruitment in elders compared to a young group (Cabeza et al., 1997; Daselaar et al., 2003; Grady, 2002; Logan et al., 2002; Rosen et al., 2002; Stebbins et al., 2002) both in areas engaged by young adults and also additional “over-recruited” areas.

Generally overrecruitement of contralateral cortex (HAROLD model) has been considered to be compensatory as several studies found correlations of such patterns with posterior memory recognition (i.e. Cabeza et al., 1997; Logan et al., 2002). This pattern has also shown to be related with greater hippocampal atrophy

and greater white matter decline in a memory task (Persson et al., 2006). However, not all studies attribute bilateral over-recruitment in memory task to compensation as over-recruitment have also found to be negative correlated with memory performance (de Chastelaine et al., 2011; Duverne et al., 2009). At this moment, the most accepted explanations is that HAROLD-like observations are indicative of an attempted compensation, which tries to take into account both PFC and MTL age-related declines, though dedifferentiation phenomenon might complementary be observed (Logan et al., 2002). As de Chastelaine and Grady (2011; 2012) suggest, aging pattern of PFC overrecruitment during memory tasks is indicative of a compensation attempt which can be a successful, a partially successful or an unsuccessful engagement of plasticity mechanism, similarly as proposed elsewhere (Oberman and Pascual-Leone, 2013). An effort should be done to know how and when these plasticity mechanisms become compensatory and, alternatively, when does little to enhance memory performance.

Memory encoding changes in aging, though, may also be explained as a consequence of different strategies of encoding as shown by Logan and Kirchoff (2002; 2012). The first study found PFC under-recruitments in older adults during a self-initiated (intentional) memory encoding task. Elders showed less activity in anterior-ventral regions associated with controlled use of semantic information. Under-recruitment was reversed when elders performed a task that required semantic elaboration suggesting that BOLD differences between youngsters and elders stemmed from difficulty in spontaneous recruitment of available frontal resources. Kirchoff (2012) found comparable results after providing semantic training to elders. Age differences in recognition memory and in brain activity were mitigated after training in semantic encoding strategies. Both results showed that neural differences between aging groups could be caused by engagement of different cognitive strategies and that at least some some encoding-related networks, hence memory performance, might be relatively preserved in aging if proper strategies are provided.

Retrieval in aging is also associated with increases in frontal recruitment, which are usually accompanied with decreases of activation in posterior areas, according the

PASA model (Anderson et al., 2000; Cabeza et al., 2004, 2000; Davis et al., 2008). Similarly to memory encoding, such increases are often regarded as compensatory and positive correlations of memory with such patterns of activation have been found. As example, Davis (2008) found that the strength of the observed age-related increases correlated both with reduced activity in posterior areas and with memory performance. However, increases in the PFC during retrieval have also been associated to poorer performance (Grady et al., 2010). To sum up, it seems, similarly than during encoding, that PFC over-recruitments is reflecting an attempted compensation of posterior areas and MTL that may or may not be successful. The ability to compensate for such deficits, putatively by top-down regulation mechanisms will have a great impact on episodic retrieval processes.

iii. Default Mode Network findings

Decreased ability to deactivate DMN in aging during episodic encoding has been repeatedly revealed in the last few years (i.e. Duverne et al., 2009; Grady et al., 2006; Miller et al., 2008). Inability to deactivate DMN areas during memory encoding in aging, as in young healthy populations (Daselaar et al., 2004; White et al., 2013), has been related to worse memory performance. Such observations are coherent with the notion that DMN, or at least its cores, is a key element in memory encoding. In an elegant study, Miller (Miller et al., 2008) found that old adults did not show a greater deactivation pattern in midline posterior regions when successful encoding was compared to failed encoding which was evident in young subjects. This pattern of reduced deactivation was even more evident in low-performing elders that additionally demonstrated greater hippocampal and prefrontal activation further ineterpreted as compensatory. Another study (de Chastelaine et al., 2011) found that several areas of the DMN that showed a negative memory effect (greater deactivation for remembered compared to forgotten items) in in young subjects in elders displayed an inversed pattern, thus greater activity during remembered items. Additionally, in the old group, the reversed pattern negatively correlated with memory performance. This group of researchers, hence, found that some regions that usually deactivate in young adults when encoding demands are present, were

recruited by the old subjects (de Chastelaine et al., 2011; Duverne et al., 2009). Authors deduced that aging brain recruits task-negative areas in order to compensate for loss of function (Mattson et al., 2013), similarly to HAROLD-like processes described elsewhere. As an example, Duverne (2009), identified posterior midline areas with subsequent negative memory effects in young adults. Instead, this effect was reversed in the old group. In fact, bad performers showed a significant correlation between greater activity in such areas (deactivated in young) and memory. Such results are partially in agreement with other evidence that also show moderator effects of age in negative subsequent effect in DMN areas (Gutchess et al., 2005; Kukolja et al., 2009; Miller et al., 2008; Morcom et al., 2003).

Similarly to other cognitive domains, it is though that decreased deactivation during memory encoding performance is due difficulty to reallocate neural resources by old adults (Miller et al., 2008). Complementarily, it has been suggested that worse memory performance in aging could be provoked by a failure of disengagement between task-irrelevant (i.e. auditory cortex) regions and DMN, mediated by the last circuit (Stevens et al., 2008). Similarly it is proposed that a choreographed interplay between task-positive and task-negative networks is essential to engage neural substrates of episodic memory.

In conclusion, these results suggest that failures of DMN deactivation in older subjects is a central feature for decreased memory performance and that the interplay of this network with other task-positive networks, such as consequent disruption of reciprocal activity between MTL and parietal memory systems, may underlie (part of the) age-associated memory impairment.

To a lesser extend DMN has shown to be affected during retrieval phases in aging (Braskie et al., 2009; Oedekoven et al., 2013; Wang et al., 2009). DMN avtivation, especially posterior midline areas are shown to be necessary for retrieval success opposed to deactivation of this areas during encoding (Buckner and Carroll, 2007; Daselaar et al., 2004; Kim et al., 2010), a phenomenon also known as encoding/retrieval flip (Huijbers et al., 2013). Some authors proposed that DMN

might engage compensatory mechanisms to maintain function during retrieval (Martinelli et al., 2013; Oedekoven et al., 2013) but more research is needed.

iv. Connectivity findings

It is increasingly been accepted that cognitive processes, and consequently age-related changes in episodic memory function, are mediated by a complex interplay between neural systems. Meanwhile fMRI has been revealed as a good measure to rflect such network interactivity, thus helping to understand the underlying organization of memory networks.

Functional connectivity:

Much of the examination of age-related functional connectivity during memory tasks has focused, unsurprisingly, on connectivity between MTL, essentially the hippocampus, and PFC regions. A typical aging pattern found both in encoding and retrieval tasks relates to PASA-like connectivity shift (Addis et al., 2010; Murty et al., 2009; St Jacques et al., 2009). That is, a change in couplings towards a more anterior connectivity pattern is observed in aging during memory tasks; that is exemplified by a loss of connectivity between posterior areas and increased connectivity of MTL with PFC (Dennis et al., 2008). Such changes in activity are thought to reflect compensatory mechanisms (Grady et al., 2003) or at least an attempted compensation (Dennis et al., 2008) according to the relationship of PFC activity with subsequent memory performance. An interesting study (Gao et al., 2013) recently showed that during a visual memory encoding task, elders had increased frontotemporal network connectivity further related to compensatory function. Some authors sustain that these changes in couplings are indicating a shift between a perceptual-based processing in youngers and a higher-order, top-down, processing in advanced aging. Daselaar (2006) showed that age-related differences in recollection-related MTL activation (age shifting between recollection and familiarity) were also accompanied by age-related differences in MTL connectivity. This result was interpreted as evidence that older adults compensate for hippocampal deficits by relying more on the parahippocampal cortex. Oh and Jagust

(2013) found increased parahippocampal connectivity with PFC in elders during a visual memory encoding task. Coupling strength was related to memory performance however this compensatory coupling was no longer observed in subjects with positive βA deposition.

Regarding the DMN, MTL has often been found to be related with this network at rest. However, successful performance of encoding memory tasks leads to uncoupled activity within MTL and other DMN nodes. Failure in uncoupling such structures during encoding task has been proposed to be disrupted with age (Chhatwal and Sperling, 2012; Miller et al., 2008; Vannini et al., 2011) though more work remains to fully elucidate this hypothesis. DMN-task positive anticorrelation has also been found altered in an implicit task in elders (Sambataro et al., 2012).

Finally few studies investigated connectivity changes from a global approach by using graph theoretical approaches. One study (Wang et al., 2010b) analyzed steady-state data during an encoding task and found that aging was associated with loss of long-range connections, less centrality for prefrontal areas and increased centrality of several DMN areas. Such results correlated with memory performance. In a similar approach (Matthäus et al., 2012) found loss of small-worldness, with less specific, symmetric and localized activity in a group of elder participants. Increased parietal connectivity was further shown.

Resting-state fMRI connectivity:

Default mode networks have been intensively investigated in aging context both at rest and during tasks (as stated above). Integrity of DMN is an essential requirement for optimal functioning. Intrinsic connectivity alterations in several neuropsychiatric disorders (see Broyd et al., 2009 for a review) as well as relation of rs-fMRI with several cognitive functions such as general intelligence (Cole et al., 2012), speed processing (Damoiseaux et al., 2008), working memory (Sala-Llonch et al., 2012b) and memory function (Andrews-Hanna et al., 2007). In aging, decreased DMN synchrony has repeatedly linked to impoverished performance (Andrews-Hanna et al., 2010; He et al., 2012; Oh and Jagust, 2013; Wang et al., 2010a).

In an elegant study, Wang and colleagues (2010) found that stronger coupling between right hippocampus and bilateral posterior parietal cortex, both structure belong to DMN, predicted better memory performance across a number of memory tasks in a group of older adults. Another influential study (Andrews-Hanna et al., 2007) demonstrated, with a seed-based approach, that ageing was characterized by marked reductions in DMN systems (and also in DAN system), being antero-posterior connectivity most disrupted by age. Further DMN functional integrity, measured as the coupling between mPFC and posterior midline Regions-of-Interest (ROI)s, was related to cognition, especially with a memory factor. Moreover, neither connectivity reduction nor relation with cognition was influenced by βA load. Recently, Razlighi (2014) also found connectivity disruption between several DMN nodes in aging evaluated in native space. Moreover, antero-posterior couplings were found to be affected by aging, further related to memory scores and other cognitive functions (concretely connectivity between supramarginal gyrus and superior frontal cortices). Relationship in aging, between IPL-mPFC connectivity and episodic memory scores was also found by He and colleagues (2012). This relationship was independent from measures of structural integrity. These last studies are in agreement with Campbell (2013) that proposes that intrinsic connectivity between MTL and posterior midline structures are relatively preserved in aging while disruption is clearly evident in the coupling between mPFC and PCC/PCU nodes. The former DMN subsystem might result more affected in task-contexts (Chhatwal and Sperling, 2012; Stevens et al., 2008). Also, decreased connectivity at rest has been found in elders between MTL and PFC nodes which was further reversed during encoding tasks (Oh and Jagust, 2013). Finnally, a recent study by our group also found a relationship between functional DMN connectivity measures and memory using a sample of middle-aged and aged subjects and graph-based analysis (Sala-Llonch et al., 2014). Regional cluster coefficients were the main measure used in that study, a measure of segregation which reflects the prevalence of clustered connectivity around individual nodes. Regional cluster coefficients were significantly increased with age in numerous cortical regions, including main DMN nodes, which were negatively related to visual and verbal memory scores. This relationship persisted in posterior midline areas after regressing the effects of age, while, in

addition, greater age-related connectivity changes were found between parietal and frontal areas (which include DMN and dorsal attentional network intra-connections).

In aging, verbal memory encoding has also been reported to partially depend from subcortical structures connectivity at rest such as thalamus and putamen (Ystad et al., 2010). As negative correlations were found between these components and memory scores dedifferentiation processes were proposed; however the exact role of such areas in memory aging remains to be fully elucidated.

Overall, the findings reviewed in this section provide support for the, increasingly recognized hypothesis that episodic memory processes are subserved by large-scale brain networks.

3. Plasticity

a. General overview

Plasticity can be conceptualized as an intrinsic property of the human brain, retained throughout lifespan, representing "nature's" invention to enable nervous system to overcome limitations of the genome and adapt to environmental pressures, physiologic changes, and experiences (Oberman and Pascual-Leone, 2013; Pascual-Leone et al., 2005). Thus, it is not possible to understand any normal psychological function or, even, manifestations and consequences of disease without invoking the concept of brain plasticity. Hence, plasticity is the mechanism for learning and for development, that enables modification of function, structure and behavior through engagement of synaptic, cellular and molecular mechanisms (see Feldman, 2009 for a review). Such mechanisms include synaptic strengthening, weakening, pruning, synaptogenesis an also neurogenesis in concrete brain areas. As the brain is highly interconnected, plasticity plays out across multiple levels of nervous system

complexity, from cellular to large-scale networks. Activity in lower levels, then, may be reflected as changes in higher levels, and vice versa with the objective of keeping a fine tuned balance which optimizes functionality. In this sense several authors suggest that this interaction between plasticity mechanisms might be observed through lifespan (Freitas et al., 2011b; Oberman and Pascual-Leone, 2013; Pascual-Leone et al., 2011). Those authors suggest that decrements in local plasticity (Freitas et al., 2013) and neural flexibility (Garrett et al., 2011), due to several factor including age, could be compensated by engagement of higher network plasticity mechanisms (reviewed in **Brain networks section**).

As stated above, dynamic shifts in the strength of preexisting connections, changes in task-related network coherence and modifications of the mapping between behavior and neural activity take place in response to changes in input or demands. While such rapid ongoing changes may allow subjects adaptation to demands (see Pascual-Leone et al., 1995, 1994 for very elegant experiments using motor learning tasks) they can also evolve towards an unsuccessful pattern of activation leading to abnormal behavior. A system capable of such flexible reorganization harbors the risk of unwanted changes, which, in presence of certain predisposing factors may result in unwanted cortical rearrangement, leading to disease. Plasticity mechanisms, then, while being critical for development and learning, when aberrant, excessive, insufficient or mistimed, may represent a pathogenic cause of several brain disorders (Gogolla et al., 2009) such as Autism spectrum disorders, Alzheimer's disease or Schizophrenia (see Oberman and Pascual-Leone, 2013; Palop and Mucke, 2010; Voineskos et al., 2013 for reviews). Both deficits and excesses in plasticity mechanisms, which are known to be highly affected by inter-individual determinants (Ridding and Ziemann, 2010), may compromise cognition and behavior.

While plasticity is an inherent capacity of the nervous system (remains continuously active) it is in response to insults that its effect has been often observed and highlighted. Influence of plasticity mechanisms to clinical, cognitive and motor outcome have been highlighted after insults (Sharma et al., 2013). Ultimately, plasticity is probably the most efficient way to utilize brain's resources. In the neuroimaging field, multiple theories such as STAC, HAROLD, CRUNCH or CR, have

tried to shed light to those mechanisms, its predictors, and its outcomes mostly focused in healthy and pathological aging populations. However, these neuroimaging theories, discussed thoroughly elsewhere, are assessing the consequences of the engagement of plasticity mechanisms, more than the mechanisms itself. Such mechanisms might be measured by other techniques, being TMS a useful non-invasive technique that allows study of human function. Relationship between these observed consequences of plasticity and the plasticity mechanisms itself is still in its origins.

As changes in local and network plasticity may be a hallmark of early impairment in several disorders, measures of cortical plasticity may provide early biomarkers of neuropsychiatric diseases, being used as early predictors. Ultimately, modulating brain plasticity may minimize, delay or even prevent symptoms of brain disease. Such measures of cortical brain plasticity may thus serve to guide plasticity based therapeutic interventions. While animal and "in vitro" research shed light to the basic, cellular and molecular, mechanisms of plasticity (Bliss and Lomo, 1973), study of plasticity mechanisms in humans has clearly benefited from the introduction of non-invasive brain stimulation techniques (NIBS). NIBS techniques allows the experimentally-controlled characterization of local and network plasticity instead of studying its consequences (Oberman and Pascual-Leone, 2013).

b. Non-invasive brain stimulation

i. Introduction

In the last two decades, modern non-invasive brain stimulation techniques have made remarkable contributions to neuroscience, studying brain physiology (Di Lazzaro et al., 2008), brain-behavior relations (Sack et al., 2005) and treating a variety of neurologic and psychiatric disorders (i.e. depression; Pascual-Leone et al., 1996). TMS represents a useful physiological tool to evaluate central nervous system physiology representing a well-established clinical tool. Additionally, in contrast to

most imaging techniques NIBS are useful to identify causal links (Robertson et al., 2003) between specific brain structures supporting cognitive, affective, and behavioral functions, thus offering insight into local and global brain network organization, dynamics and experience-dependent plasticity (Dayan et al., 2013). NIBS techniques also represents a valuable tool for interventional neurophysiology applications as they are able to modulate brain activity, network functionality, neurochemistry or gene expression in a controlled fashion, which occasionally may yield a specific behavioral impact (Wagner et al., 2007). Abundant evidence supports the use of NIBS techniques as tools for enhancing/manipulating motor and cognitive functions in human subjects (Miniussi and Ruzzoli, 2013; Reis et al., 2008). Consequently NIBS have become a promising treatment tool for a variety of medical conditions and potential utility has been claimed for several disorders, both psychiatric (Aleman, 2013; such as depression, schizophrenia, posttraumatic stress disorder, drug craving) and neurologic disorders (Harris-Love and Cohen, 2006; such as Parkinson's disease, dystonia, tinnitus, stroke rehabilitation, migraine, Alzheimer's Disease or epilepsy among others).

Unfortunately, despite the rapid growth in interest and applications of these techniques, the specific underlying mechanisms of stimulation-induced behavioral and physiological effects remain largely unknown, especially those mediating excitability changes that outlast the stimulation duration. This problem, along with large inter-individual differences (López-Alonso et al., 2014; Maeda et al., 2000) has often limited the use and effectiveness of these tools. Biomedical engineering and computational approaches are appearing in the last few years (see Miranda, 2013 for a review) with the expectation to lead to more effective stimulation devices and protocols and to optimize the stimulation to handle different brain systems and states.

ii. Types:

NIBS can be divided according the manner to induce brain stimulation either by means of magnetic or electric induction. On the first side, TMS has to be highlighted

as the most important technique, and the one that has dominated human noninvasive brain stimulation field in the last two decades (Rossi et al., 2009). Techniques such as transcranial static magnetic field stimulation (Oliviero et al., 2011) or low field magnetic stimulation (Rohan et al., 2013) use electromagnetic induction principles in the respective tools. On the other side, we can gather those techniques eliciting electrical current. The main technique among those is transcranial direct current stimulation (tDCS), but other methods such as transcranial random noise stimulation and transcranial alternating current stimulation are emerging as useful techniques to study and intervene the human brain (Paulus, 2011). For more information about mechanism of actions and applications of the later techniques the reader is further addressed to the following reviews (Kuo and Nitsche, 2012; Nitsche and Paulus, 2011; Paulus, 2011; Rothwell, 2012; Stagg and Nitsche, 2011) as complete explanation of these techniques is beyond the scope of this document.

iii. Brief historical perspective

As early as in Julio-Claudian dynasty, in the early Roman Empire, use of electrical currents is recommended to threat headaches and by application an electric torpedo fish to the affected regions (Scribonius Largus, 1529). However, the use of electric stimulation did not emerge as a electricophysiologic tool until the late 18th and the 19th century with figures such as Luigi Galvani, Charles Le Roy, fathers of the electrophysiology, and Duchenne de Boulogne (1871) or Giovanni Aldini (1804) pioneers at using electric stimulation technique in clinical medicine. In the 1960's impact of weak direct currents to brain physiology was briefly recovered (Bindman et al., 1964, 1962; Purpura and McMurtry, 1965) but it was not until Priori (1998) and, essentially, with the seminal paper of Nitsche and Paulus (2000) in the Georg-August University (DE), where it was shown that tDCS induced cortex excitability changes in the human cortex outlasting the period of stimulation, that modern direct current stimulation began.

Primitive transcranial magnetic stimulation studies can be placed in the late 19th century where figures such as Jaques-Arsene d'Arsonval (d'Arsonval, 1896) reported vertigo, and posphenes after placing subjects heads into a coil. Sporadic research took place during all the 20th century but, mostly due to technical limitations, results were quite discrete. Beginnings of modern era of magnetic stimulation can be set at 1985 (Barker et al., 1985) with the publication of a paper by Anthony Barker and his group in the University of Sheffield. After years of related research, they introduced Transcranial Magnetic Stimulation Technique, a non-invasive technique that uses the principles of electromagnetic induction to focus currents in the brain to subsequently modulate the function of the cortex. More information about history of NIBS can be found in the next references (Dayan et al., 2013; Priori, 2003; Vidal-Dourado et al., 2014; Wagner et al., 2007 SI).

c. Transcranial Magnetic Stimulation

i. Biophysical Principles

Currently, is considered that TMS is a standard stimulation technique for the non-invasive investigation of cognitive function, whereby neural tissue is stimulated by using the principles of electromagnetic induction to generate electrical currents in the brain (Wagner et al., 2007). Magnetic stimulators consist of two main components, a waveform generator and an electromagnet coil positioned on the head (**Figure 4**). The waveform generator consists on a capacitive high voltage, high-current charge-discharge system, composed of a charging unit, a bank of storage capacitors, switching circuitry, and control electronics. The electromagnet coil, made of wound copper wire, produces brief pulsed strong magnetic fields and it is the only component that comes in direct contact with the subject undergoing stimulation (Wagner et al., 2007). Currently, figure-eight shaped (butterfly coils) are the most common types of coils used as showed increased focality (Deng et al., 2013); however, classic circular loop coils or H-coils are still being used. Maximum TMS

currents are at cortical surface, and with the exception of H-coils, depth of stimulation is relatively small, up to 2cm (Rudiak and Marg, 1994). TMS has then, relatively good spatial resolution (**Figure 4**) that depends on the specific protocol used, and high temporal resolution as single TMS pulses effects can last roughly 40-60ms (Brasil-Neto et al., 1992).

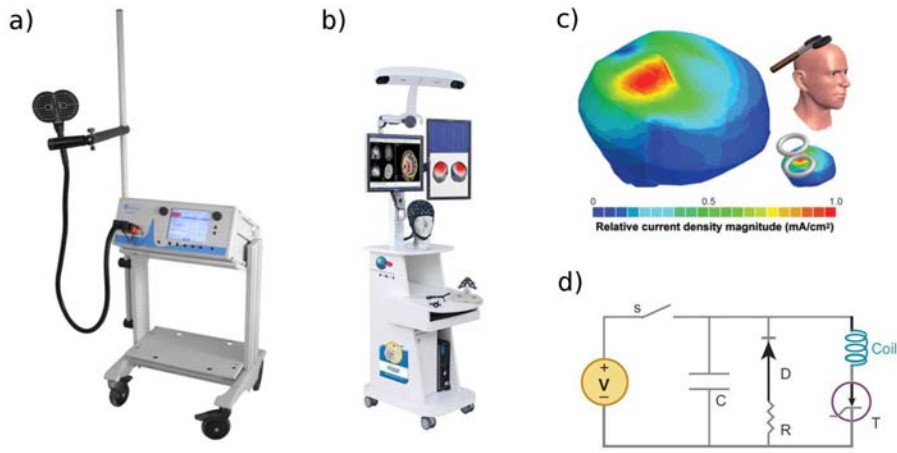

Figure 4. A) Transcranial Magnetic Stimulation device with a waveform generator and an electromagnet coil. B) Infrared-based Neuronaviagtion device. C) Plots of the current density magnitudes on the cortical surface for the TMS solutions (from Wagner et al., 2007). D) A simplified circuit diagram of a single-pulse magnetic stimulator (from Wagner et al., 2007).

Physiological response is highly affected by parameters linked with the spatial distribution of the electromagnetic field such as shape, size, position, and electrical properties of the coil, and parameters of the current waveform including pulse shape, amplitude, width, repetition frequency, duration, interstimulus interval, patterns of stimulation and number of pulses (Peterchev et al., 2012). Interaction of these variables with biological properties of the brain will determinate the effect of TMS in the brain. The importance of predicting brain impact of TMS is reflected by the increased number of models, such as electromagnetic modeling trying to predict

location and distribution of induced currents (i.e. Wagner et al., 2014; Wilson et al., 2013).

ii. TMS basic mechanisms of action

The investigations that have helped to illuminate our understanding as regards the mechanisms of action of TMS are mostly based onphysiological (Vincenzo Di Lazzaro et al., 2008) and pharmacological (Nitsche et al., 2012) studies (Di Lazzaro et al., 2008), but, also from the necessary contributions of animal research (i.e. Allen et al., 2007). TMS is both a neurostimulation and neuromodulation technique. TMS uses the principle of electromagnetic induction to focus induced currents in the brain. Rapidly changing magnetic fields easily penetrate scalp and skull and induce an electrical field of enough magnitude to stimulate neuronal activity and change the pre-stimulus dynamics of neuronal firing in the stimulated region. While mechanisms of action are still poorly understood, the prevailing hypothesis is that axonal boundaries (such as axonal-soma, axonal-bouton boundaries) or fiber bends of individual cells are preferentially affected by the TMS pulse, as are the areas at which stimulating currents have their maximal impact (**Figure 5** ; Huerta and Volpe, 2009; Ridding and Rothwell, 2007; Tranchina and Nicholson, 1986). TMS may suppress neural signal (Mantovani et al., 2006), generate random noise (Moliadze et al., 2003), or disrupt temporal relation between neurons (Pasley et al., 2009) as well as probably affects both inhibitory and excitatory neural populations. TMS, though, may also manifest its effects through the induced modification of membrane resting potentials and thresholds, channel properties with subsequent alterations in spontaneous activity, synaptic connectivity, timing dynamics of cellular gating components and/or other cellular and mollecular mechanisms.

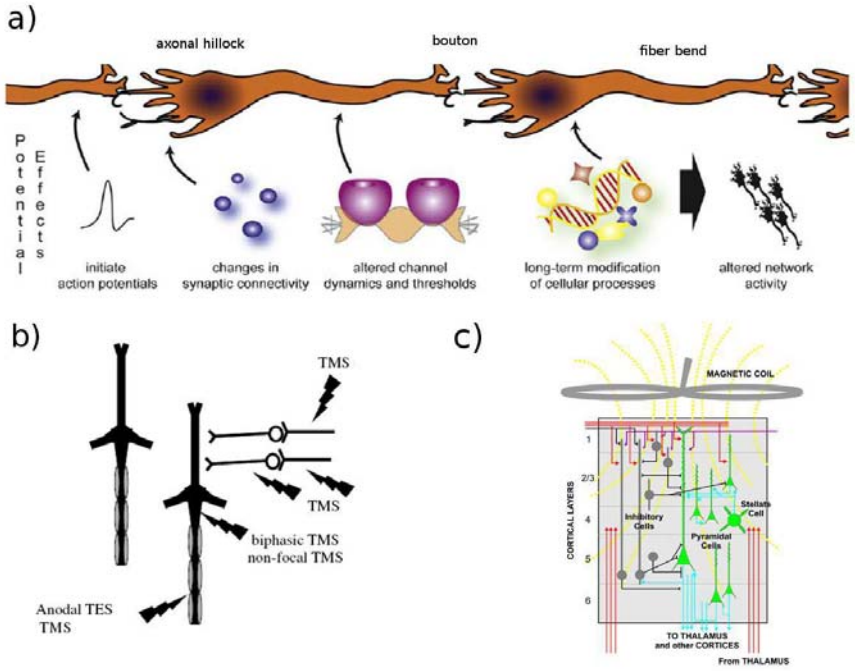

Figure 5. A) Locations of TMS induced current-cell interactions which can drive both immediate and long term effects in the cellular activity and integrated network behavior. Many of these concepts still need to be experimentally verified (from Wagner et al., 2007). B) Possible sites of direct and indirect activation of corticospinal cells using TMS. Two pyramidal tract neurons and first and second-order excitatory interneurons are represented (from Di Lazzaro et al., 2008). C) Schematic representation of the human cerebral cortex and magnetic pulses effects inducing electrical currents across the six layers of the cerebral cortex. TMS-related activations are potentially located in excitatory cells (green with blue axons) inhibitory cells (gray with black axons), which contain the highest density of ion channels, as well as axons from other cortical areas and the thalamus (indicated in red) are also activated. Pulse outcome is the synaptic activation of a chain of neurons, which generate feed-forward and feedback loops of excitation and inhibition (from Huerta and Volpe, 2009)

Although there is still debate and it is likely that TMS is impacting brain in a multifactorial way, it is known that online supra-threshold pulses actively initiate action potentials and that both online supra- and subthreshold TMS pulses are capable to alter integrated network activity. Offline TMS effects are thought to result from an alteration of the long-term excitability of neural cells and networks following stimulation (Thickbroom, 2007). For further information, the author addresses to several papers that discuss all this questions in detail (Di Lazzaro, 2013;

Miranda, 2013; Peterchev et al., 2012; Thickbroom, 2007; Wagner et al., 2009, 2007).

Two ideas have to be outlined in reference to the TMS physiological effect. One is the concept of TMS state-dependency (**Figure 7**); that is the neural impact of TMS external stimulus will highly depend of the ongoing brain activity at the time of the stimulation (i.e. Siebner et al., 2004; Silvanto et al., 2008). These different states, may be driven by cognitive task engagement (Bestmann et al., 2008; Feredoes et al., 2011; Sack et al., 2007), muscle contraction (Huang et al., 2008), history of previous plasticity (Fung and Robinson, 2014; Siebner et al., 2004), or adaptation (Silvanto et al., 2008) and will affect neural responses, as well as behavioral outcome. The second concept refers to the idea of transynaptical propagation. TMS pulses are only able to induce depolarization to the underlying neurons, but these ones, in turn, might activate other neurons via synaptic transmission both in the same underlying cortex (Di Lazzaro et al., 2003) or in distal areas of the brain (Bestmann et al., 2008; Paus et al., 1997). The specific transynaptic propagation of TMS effects will depend upon the concrete dynamics of the brain when stimulated (Feredoes et al., 2011; Higo et al., 2011).

iii. Site Localization

TMS can be applied mostly anywhere over the cortical surface, although most of the studies are localized in the primary motor area (M1). With introduction of frameless stereotaxic systems, TMS coil could be neuronavigated to target specific anatomical sites based on individual subjects' structural or functional brain images by creating a virtual link between Magnetic Resonance images and real anatomy, and allowing three-dimensional orientation by interactive visual navigation. That allowed improving target accuracy (outside M1 or visual areas) beyond rough methods such as EEG 10-20 system positioning (Herwig et al., 2003) or deviations from known landmarks (such as M1). Neuronavigation methods also allowed the optimized use of individual and groupal targeting protocols. Individual task activation (Sack et al., 2009), structural connectivity (Hannula et al., 2010) or resting-state connectivity

(Eldaief et al., 2011) has been used to optimize target selection. As an example, Sack (2009) showed that individually (f)MRI-guided neuronavigation yield a stronger behavioral effect showing better accuracy in target selection compared to other targeting methods.

iv. Safety

TMS is considered a safe technique but it is not exempt of some secondary effects. Safety guidelines are constantly reviewed and the latest avaliable have been published in 2009 (Rossi et al., 2009). Regarding studies investigating cognitive functions in normal subjects, as the benefits are only indirect, they only should be carried when the risk for the participants is low. All general ethical and safety consideration also apply to TMS studies. Regarding risks in TMS environment, the most severe consequence that may be provoked by stimulation on healthy participants is acute seizure induction although is an extremely rare event. In addition, a few of the reported episodes are dubious, and their clinical description matches with syncope more than a seizure ($p<0.01$ with high-frequency repetitive TMS [rTMS]). New TMS protocols and pro-epileptogenic drugs clearly increase individual vulnerability. Other consequences reported after TMS are pain, headache, discomfort and mood changes. All this changes are thought to be weak and transient.

v. Stimulation protocols

TMS is commonly applied in single, paired or repetitive trains. We will briefly review these protocols (**Figure 6**) with special interest in those able to produce plasticity. Initial applications of TMS focused on the motor system and enabled mapping of functional representations in primary motor cortex, with single-pulse TMS producing muscle contractions measured as motor-evoked potentials (MEPs). Furthermore, it has become standard practice to use these responses to inform the selection of appropriate stimulation parameters (Wassermann, 2002). Single-pulse TMS has also been extended to the visual system by applying suprathreshold stimulation of

occipital cortex and inducing phosphenes as well as to other brain areas in combination of neuroimaging techniques. Application of pulses during task execution can provide insights into the underlying neural substrates of cognitive task (Mottaghy et al., 2003) and motor learning (Pascual-Leone et al., 1995).

Paired-pulse TMS protocols enable further measurements of cortical physiology. We will refer the excellent review by Hallett (2007) for more information. Briefly, paired-pulse TMS can be used to study functional interactions within a single brain region or between two connected brain areas. Single-region paired-pulse TMS are typically limited to M1 and involve the application of both a subthreshold conditioning stimulus and suprathreshold test stimulus to the same region. Latency variations may results in intracortical inhibitory or intracortical facilitory effect that have been further related to GABA and Glutamate receptors activity. Paired-pulse TMS can also be used to investigate interactions between two spatially distinct brain. These protocols include afferent inhibition consisting in somatosensory stimulation of the hand and a conditioned pulse in the motor cortex, or cortico-cortical montages such as transcallosal and premotor inhibition protocols.

TMS applied in a repetitive fashion is usually applied offline (it can also be applied online in neurochronometric/lesional purposes such as Rossi and colleagues [2011]) thus generating effects that outlast the period of stimulation, giving insight into the role of the specific stimulated brain regions over cognition which implicates the engagement of plasticity mechanisms and that, ultimately, can also be exploited therapeutically. Offline mechanisms of actions, although again not clearly understood, show similar patterns of LTP and LTD phenomena; hence, TMS after-effects are generally classified as reflecting LTP/LTD-like plasticity effects (see Cárdenas-Morales et al., 2010; Huerta and Volpe, 2009; Thickbroom, 2007 for detailed discussions). TMS can be used in a variety of ways to induce plastic changes in the brain being the most popular Pair Associative Stimulation (PAS), rTMS and patterned stimulation protocols. PAS (Stefan et al., 2000; Wolters et al., 2003) consists in pairing stimulation of median nerve at the wrist with a TMS to the sensoriomotor cortex at specific latencies. Repeated PAS leads to corticospinal excitability changes that outlast for few minutes the period of stimulation. As a

simple motor learning task and PAS interact with each other, it does appear that PAS is a highly relevant model for brain plasticity (similar to Hebbian plasticity; Ziemann et al., 2004).

rTMS is also able to produce significant changes of excitability that outlast the period of stimulation, consistent with cooperativity and associativity aspects of the experimental LTP model. Traditionally, rate-dependent rTMS plasticity showed reduced excitability when applied at low frequency (1Hz or less) and increased excitability when applied at higher frequency (>5hz; Di Lazzaro et al., 2011). Duration of changes of excitability will depend on the total number of pulses, but it is relatively short-lasted.

Patterned rTMS protocols have recently appeared, such as quadripulse or theta-burst stimulation (TBS; Hamada et al., 2008; Huang et al., 2005), and applied in the motor cortex have been capable to induce longer, more efficient and more stable TMS after-effects. A typical theta-burst stimulation paradigm consists in the application of three stimuli at 50Hz, repeated at 5Hz. If given intermittently, typically 2s of stimulation every 10s, leads to increased excitability, while continuously applied leads to decreased excitability in human cortex (**Figure 6.** A) Schematic illustrations of TMS protocols showing how TMS can be applied. B) Graphical illustration of the main theta-burst stimulation paradigms used (from Sandrini et al., 2011). Each paradigm (intermittent, intermediate continuous) uses a theta burst stimulation pattern in which 3 pulses of stimulation are given at 50 Hz, repeated every 200 ms. They differed respect the interstimulus interval and the length of the trains (from Huang et al., 2005). C) Time course of changes in MEP amplitude following conditioning with iTBS , cTBS , or intermediate TBS (from Huang et al., 2005). Di Lazzaro et al., 2005; Huang et al., 2005). TBS mechanisms of action, again not fully understood, may differentially affect cortical circuits depending the protocol used as observed from its impact over corticospinal I-Waves (Di Lazzaro et al., 2005; Di Lazzaro et al., 2008) or activity-markers such as glutamic acid decarboxilase isoforms (Hoppenrath and Funke, 2013; Volz et al., 2013).

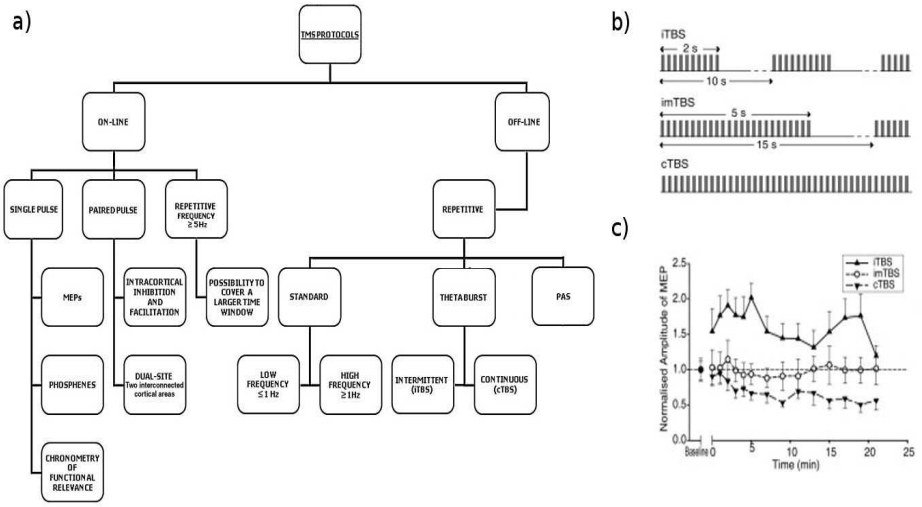

Figure 6. A) Schematic illustrations of TMS protocols showing how TMS can be applied. B) Graphical illustration of the main theta-burst stimulation paradigms used (from Sandrini et al., 2011). Each paradigm (intermittent, intermediate continuous) uses a theta burst stimulation pattern in which 3 pulses of stimulation are given at 50 Hz, repeated every 200 ms. They differed respect the interstimulus interval and the length of the trains (from Huang et al., 2005). C) Time course of changes in MEP amplitude following conditioning with iTBS , cTBS , or intermediate TBS (from Huang et al., 2005).

TMS after-effects may involve LTP and LTD-like processes as well as inhibitory mechanisms modulated by modification of GABAergic tonic activity (Stagg et al., 2009a). Some studies highlighted implication of glutamatergic system and NMDA receptors (Di Lazzaro et al., 2005; Huang et al., 2007) while other also suggested GABAergic implication (Stagg et al., 2009a; Thickbroom, 2007). Finally, another feasible mechanisms of action is modulation of cortex excitability by means of gene expression, and proteins (Aydin-Abidin et al., 2008; Benali et al., 2011). A number of variables such as gene polymorphisms, muscular contraction or metaplasticity has been reported as moderators of TBS effects (Ridding and Ziemann, 2010), similarly to other repetitive TMS techniques.

Change of MEP excitability after TBS protocols has usually been reported to last about 30 min after M1 stimulation. However applied in other areas and using other recording techniques (Grossheinrich et al., 2009) or alternatively selecting only

responders (López-Alonso et al., 2014), TBS effects can consistently last more than one hour.

TBS stimulation has been used to modify multiple cognitive and behavioral functions such as working memory, speech repetition, emotional control, top-down processing or motor learning (i.e. Lee and D'Esposito, 2012; Morgan et al., 2013; Restle et al., 2012; Volman et al., 2011). Therapeutic use of TBS has suggested for Amyotrophic Lateral Sclerosis, Multiple Sclerosis, Stroke, Dystonia, Pain Perception or Schizophrenia taking advantage of the capacity to induce plasticity changes in the brain.

vi. Combining TMS with other techniques

Given the obvious limitations of registering brain responses to TMS outside motor and visual areas and the impossibility to infer the full scale of neural network mechanisms affected by TMS, it is worth mentioning that progress in TMS (especially in cognitive neuroscience field) has taken obvious advances of the combination of stimulation with other neuroimaging approaches. Neuroimaging techniques can be acquired either with "off-line" or "on-line" TMS. The former is limited by the time window of TMS after-effects and should be acquired as soon as possible after stimulation. The latter entails complex experimental settings to avoid noise and interaction from TMS mechanical/physical signal. Most popular techniques that have been used in combination with TMS are EEG (Müller et al., 2013; Thut and Pascual-Leone, 2010), fractional near infrared spectroscopy (Kozel et al., 2009), PET (Paus et al., 1997), fMRI (Solé-Padullés et al., 2006) and Magnetic Resonance Spectroscopy (MRS; Stagg et al., 2009a). Pitfalls and advantages of each technique determine which aspects of brain response to TMS will be investigated (see Bestmann and Feredoes, 2013; Reithler et al., 2011; Stagg et al., 2010; Ziemann, 2011 reviews for detailed information).

Regarding "offline" rTMS in combinationwith neuroimaging techniques an important challenge concerns to the difficulty of teasing apart whether the obtained neuroimaging results truly reveals the underlying network connectivity or should

more accurately be interpreted as indications of the way in which the brain is trying to cope with the artificial disturbance. In the latter case, it may be interpreted as an engagement of compensatory mechanisms of plasticity in an attempt to maintain proper functioning after interference (O'Shea et al., 2007; Sack et al., 2005; Zanto et al., 2013). Next, the combination of fMRI and MRS neuroimaging techniques with TMS will be briefly reviewed.

fMRI-TMS:

Soon after successful combination of TMS with PET (Paus et al., 1997), Bohning (1998) reported the first combined TMS–fMRI study. The earlier TMS–fMRI studies measured the effects of focal brain stimulation while subjects were at rest and its most striking result probably was the observation that the neural consequences of focal TMS were not restricted underneath the site of stimulation but distal brain areas were clearly modulated by stimulation (**Figure 7**; Bestmann et al., 2003). Such studies also found that the impact of stimulation was dose-dependent, as stronger stimulation intensities evoked larger activity changes in those regions. Moreover, attention has moved towards the potential of this approach to investigate interregional interactions and their possible functional consequences for perception and cognition (Feredoes et al., 2011).

Alternatively "off-line" approaches have also shown its potential to study brain function, typically, by applying rTMS in-between two fMRI resonances. This combination have shown to be especially valuable to study either the spatio-temporal pattern of functional reorganization induced in the brain by TMS (i.e. O'Shea et al., 2007) or the lasting functional impact of TMS on task-related neural activity at the system level (i.e. Solé-Padullés et al., 2006). As example, O'Shea (2007) found that after a virtual lesion induced by TMS in the premotor cortex, a form of adaptive plasticity (increased BOLD signal) in the non-dominant hemisphere was observed supporting the hypothesis that interhemispheric compensation mediates functional recovery.

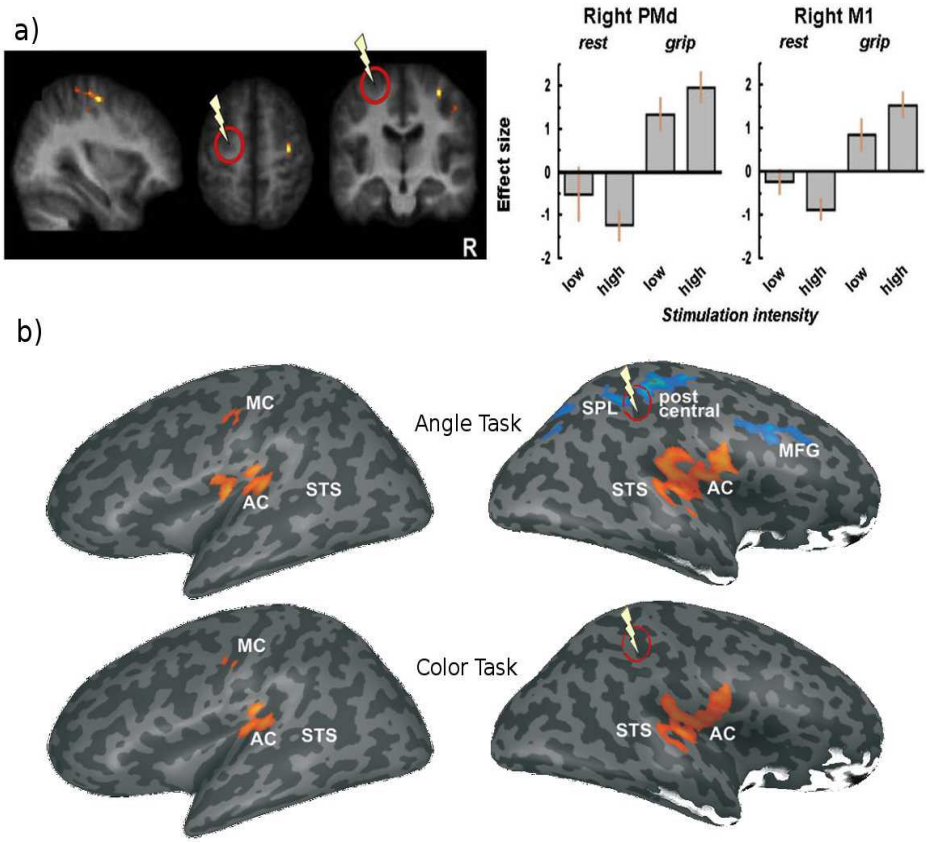

Figure 7. Two examples of both distal and state-dependent TMS effects by concurrent fMRI-TMS. A) State-dependence of inter-hemispheric influences of TMS in the motor system. Short bursts of TMS (high vs low intensity) were applied to left PMd during left-hand force production, concurrently with fMRI. Within the task-related right PMd and M1, top), TMS at high intensity leads to a relative activity increase, as compared to low intensity control TMS. The effect was reversed during non-motor rest (from Bestmann et al., 2008). Results of task execution with versus without right parietal TMS, separately for angle and color discrimination tasks. Blue/Redindicates areas with a significantly reduced/increased neural activity during task execution with TMS (from Sack et al., 2007).

Lastly, advances in fMRI methods and analysis have been rapidly adopted in this type of experiments. In the first case, the introduction of Arterial Spin Labeling (ASL) technique, which is a perfusion technique based in non-invasive magnetization-based labeling of blood, by using BOLD signal, should be highlighted (Moisa et al., 2010). In the second case, the introduction of graph-based fMRI analyses promise a

new progress towards understanding the effect of rTMS over brain networks (Polanía et al., 2011).

MRS-TMS:

MRS is a noninvasive imaging technique that allows accurate quantification of a number of neurochemicals, including GABA and glutamate. For technical reasons, the majority of MRS studies quantify the concentration of neurochemicals within one small region of the brain, termed the voxel of interest (VOI). VOI consist in a volume of brain tissue in the region of 8–27cm^3. The MRS signal is based on differences in the molecular structure of the neurochemicals. Every MR-visible neurochemical emits a signal at a specific set of frequencies, characteristic to that neurochemical. If MR signal is acquired over a range of frequencies, then peaks relating to a number of specific neurochemicals may be observed. The amplitude of the peak for a given neurochemical is related to the total number of molecules of that neurochemical within the voxel of interest, and therefore, refers to the total concentration of that neurochemical. MRS is therefore unable to distinguish between pools, limiting a straightforward interpretation of this measures, which often have to rely on extra evidence such as animal evidences. Concentrations of neurochemical are often given as ratios of external, water, or internal, N-acetylaspartate (NAA) or creatine, reference peaks, for reliability purposes.

There is increasing interest to detect GABA (and Glutamate) in the human brain, and MEGA-PRESS sequence have become the most popular MRS technique to quantify such metabolites (Mullins et al., 2014). Such sequence allows GABA signal to be separated from the stronger overlying signals of other larger metabolites resonance (creatine) by taking advantage of known couplings within the GABA molecule. Briefly, MEGA-PRESS involves the collection of two interleaved datasets which differ in their treatment of GABA spin system. In one dataset, an editing pulse is applied to GABA spins at 1.9 ppm in order to selectively refocus the evolution of J-coupling to the GABA spins at 3. In the other, the inversion pulse is applied elsewhere so that the J-coupling evolves freely throughout the echo. The majority of peaks in the spectrum are unaffected by the editing pulses, hence subtraction between both

spectrums removes all these peaks retaining only those peaks that are affected by the editing pulses such as GABA and Glutamate signals (see **Figure 8**; Mullins et al., 2014; Puts and Edden, 2012; Stagg, 2014 for more information).

It remains unclear what GABA and Glutamate (often as a Glx, a combinated measure of Glutamate+Glutamine) measures are assessing. GABA seems to reflect extra synaptic, tonic, GABA concentrations. Interpretation of Glutamate MRS quantification, as this molecule is involved in synaptic transmission as well as in a variety of metabolic roles, remains relatively obscure nowadays, though it has been repeatedly related to metabolic activity (for more information consult Rae [2014] and Stagg [2014] reviews). However, these neurochemicals have been strongly linked to neuroimaging techniques estimates. Importantly, GABA seems to be negatively related with BOLD signal and with gamma evoked related potentials when executing tasks (Donahue et al., 2010; Muthukumaraswamy et al., 2009) and with the magnitude of task-related deactivation (Hu et al., 2013; Northoff et al., 2007). At rest, GABA and Glutamate have been frequently associated with RSN integrity (Kapogiannis et al., 2013; Stagg et al., 2014) and between RSNs interactions (Anticevic et al., 2012b; Duncan et al., 2011; Falkenberg et al., 2012).

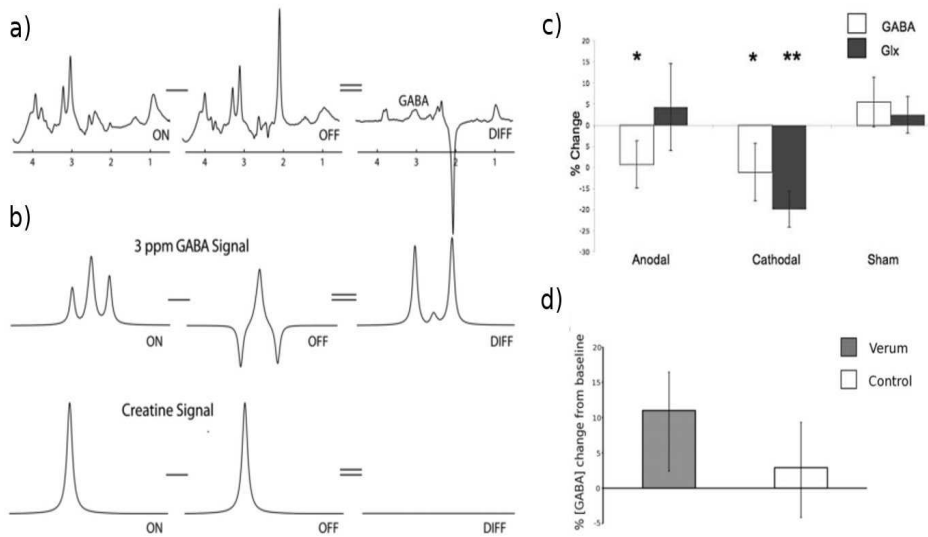

Figure 8. A) Schematic diagram of MEGA-PRESS editing for GABA (adpated from Mullins et al., 2014). B) Editing pulses applied at 1.9 ppm modulate the shape of the GABA signals at 3 ppm but not creatine signal (from Mullins et al., 2014). C) Changes in neurotransmitter-to-NAA ratios after tDCS stimulation in the motor cortex (from Stagg et al., 2009b). D) Percentage change in GABA:NAA ratio from baseline after cTBS in the motor cortex (from Stagg et al., 2009a)

Few studies have been performed investigating effects of NIBS over neurochemistry by means of 1HMRS (**Figure 8** ; Clark et al., 2011; Fregni et al., 2011; Luborzewski et al., 2007; Marjańska et al., 2013; Michael et al., 2003; Stagg et al., 2009a; Stagg et al., 2009b). One of these (Stagg et al., 2009a) explored the effects of inhibitory, continuous TBS applied to the M1 at rest and found increased GABA concentration after stimulation (compared to sham group). Marjańska (2013) applied 5-Hz rTMS over M1 in a group of dystonic patients and in a healthy control group. Neurochemical concentration modulation was found after rTMS, including Glutamate, GABA and tNAA levels. Additionally differential responses, hence activity-dependent neurochemical dynamics (plasticity responses), were observed according the clinical group. So far, results have been found to vary deeply according the protocol used highlighting the complex heterogeneity of mechanisms of synaptic plasticity within the neocrotex (Stagg et al., 2010a). Altogether, GABA-MRS it is considered a good technique to evaluate local plastic response due to NIBS or due to other plasticity-like interventions such as posterior midline responses to a pharmacological intervention in memory impaired elders (Friedman et al., 2013). MRS in combination with fMRI might be useful to evaluate the relationship between local and network plastic responses to TMS, though few evidence exist yet. A recent study (Stagg et al., 2014) demonstrated an inverse relationship between levels of the inhibitory neurotransmitter GABA within the M1 area and the strength of functional connectivity across the resting motor network. Also, this group found reduced GABA levels (Stagg et al., 2009b) and reduced integrity of motor network (Amadi et al., 2013) after cathodal M1 tDCS in separate studies.

d. Memory and Non invasive brain stimulation

NIBS have been used to study memory neuroanatomical correlates and its specific temporal window of implication from a causal approach (Innocenti et al., 2010; Machizawa et al., 2010; Rossi et al., 2001; Sandrini et al., 2013; Wais et al., 2012). Plasticity-induction capacity of NIBS, though, has been used in an attempt to improve performance in memory tasks. In the former case, stimulation is applied either "on-line" or "off-line" in an attempt to interfere with memory processes and consequently gain insight in brain-behaviour relationship (i.e. Rossi et al., 2001). When applied "on-line", knowledge about the temporal dynamics can also be obtained (Grafman et al., 1994; Rossi et al., 2011). In the latter case usually "off-line" NIBS is administered with the intention to modify cortical excitability in critical areas, thus temporarily enhancing cognitive capacities (see Manenti et al., 2012 for a review). Such approximation is seemingly interesting in the cognitive aging field as NIBS might become a valuable tool to partially reverse age-related decline in memory functions.

Utility of NIBS as a tool to causally explore brain-behavior relationship in episodic memory encoding and retrieval has been mostly focused in confirmation of different relevant topics and predictions mostly generated by neuroimaging. As MTL is directly unreachable by NIBS, interest has mostly focused on PFC (i.e. Blumenfeld et al., 2014; Innocenti et al., 2010; Machizawa et al., 2010; Rossi et al., 2011, 2004, 2001), but causal implication in episodic memory of other cortical regions such as parietal cortex (Manenti et al., 2013; Sestieri et al., 2013) and anterior temporal lobes (Chi et al., 2010) has also been demonstrated with transcranial stimulation. Thought a complete review of this field is out of the scope (see Blumenfeld et al., 2014; Machizawa et al., 2010; Manenti et al., 2012) two recurrent findings from this field of research might be highlighted. First, left PFC is currently involved in memory encoding (Blumenfeld et al., 2014; Machizawa et al., 2010; Rossi et al., 2004, 2001; Sandrini et al., 2003) and of right PFC during retrieval phases (Epstein et al., 2002; Rossi et al., 2011, 2004, 2001; Sandrini et al., 2003). This results are consistent with hemispheric encoding-retrieval asymmetry model (HERA; Tulving et al., 1994).

Whether such observations are guided by the type of material encoded (Floel et al., 2004) and the specific contributions of dorsolateral and ventrolateral frontal cortices (Blumenfeld et al., 2014) in encoding is still a matter of debate. On the other hand, other studies underscored the differential role of PFC areas as a function of encoding or retrieval strategies (Hawco et al., 2013; Innocenti et al., 2010; Manenti et al., 2010). As a paradigmatically example, Innocenti (Innocenti et al., 2010) use on-line TMS to demonstrate that left DLPFC region was determinant to deep (semantic) processing compared to shallow (perceptual) processing as TMS interference during encoding performance imparied subsequent retrieval. Indeed, after TMS, benefit of semantic encoding was abolished.

Regarding the use of NIBS to increase memory function several studies obtained better memory score in participants after application of plasticity induction NIBS protocols both in healthy subjects (Chi et al., 2010; Javadi et al., 2012)and in clinical populations (Ferrucci et al., 2008). Transcranial DCS, due to its characteristics, emerged however as the most important tool in this particular type of studies, though TMS has also resulted in improved memory function (Köhler et al., 2004; Solé-Padullés et al., 2006) in several studies.

e. Plasticity and Aging: assessment with NIBS techniques.

While plasticity mechanisms are continuously operational, the ability to change and adapt appears to peak around adulthood and may become increasingly less efficient with advancing age, where nonetheless, engagement of compensatory/maladaptative responses are promoted through plasticity. Several animal studies revealed that the neurobiological substrate of age-associated cognitive decline is associated with alterations in synaptic plasticity (Barnes, 1979; Rosenzweig and Barnes, 2003). In particular, deficits in the balance between LTP and LTD thresholds may lead to impaired learning and memory (Bliss, 2003; Larson and Lynch, 1986; Roman et al., 1987). Deficits in maintenance of hippocampal LTD in older rodents are characterized by a more rapid decay of LTP (Barnes, 2003, 1979;

Burke and Barnes, 2006) that, in turn, is associated with forgetfulness (Barnes and McNaughton, 1980; Kelly et al., 2006). Complementarily, exciting theories had recently appear from neuroimaging (Fjell et al., 2014) and NIBS fields (Freitas et al., 2013) linking age-related vulnerability with plasticity.

As stated above, neuroimaging techniques are able to reveal the consequences of plasticity mechanisms in aging, demonstrating that the ageing brain retains considerable plasticity of function but they often fail to reveal mechanisms per se (Oberman and Pascual-Leone, 2013) which, mostly assessed with fMRI, are described elsewhere (see **Neural networks and aging section**). NIBS, especially TMS, has been revealed as a good technique, not only for exploring consequences of plasticity in aging, but also to explore the state of such mechanisms and to make use of them to improve cognition and behavior.

Use of NIBS to study plasticity in aging shall be divided in three groups, although it has to be acknowledged some arbitrarity in such classifications (**Figure 9**). Studies are going to be clustered as: 1) those that try to evaluate the state of (local) plasticity mechanisms in aging by administering rTMS and evaluating the engagement of the plasticity mechanisms. Such studies are related to those assessing physiological characteristics of aging brain such as differences in cortical excitability or intracortical inhibition (i.e. Rossini et al., 2013; 2007); 2) those using NIBS to reveal consequences of engagement age-related plasticity such as compensatory recruitment (i.e. Rossi et al., 2004) using NIBS as a "physiological probe"; 3) those that use NIBS as a tool to induce plasticity with the aim to improve behavioral or cognitive performance (i.e. Solé-Padullés et al., 2006).

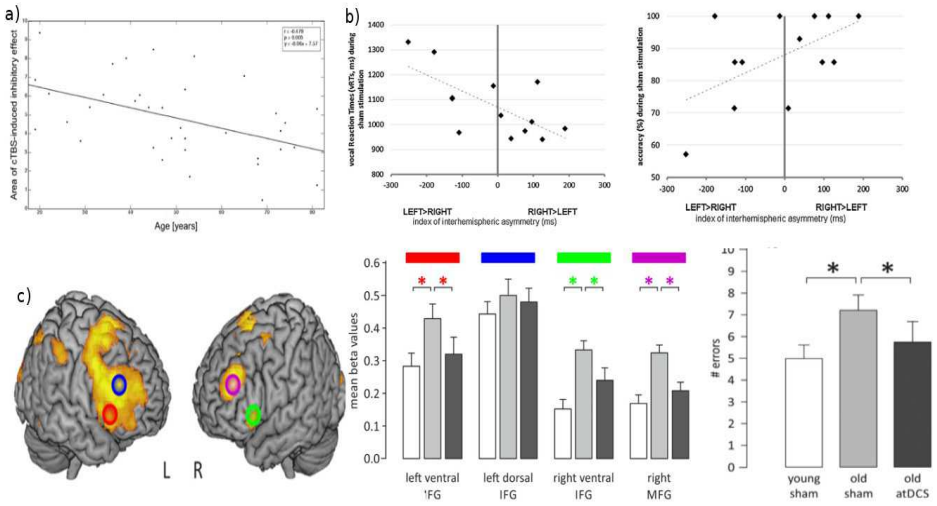

Figure 9. A) Correlation between age and area of inhibitory effects after cTBS in the motor cortex (from Freitas et al., 2011b). B) Correlations between the index of interhemispheric asymmetry (DLPFC-TMS assessed; reaction times during left-right DLPFC stimulation in action naming tasl) and the verbal reaction times or accuracy during sham conditions (from Manenti et al., 2013b). C) In the left side the main effect of a semantic word generation task and ROI locations. In the midlle, effect of anodal tDCS on fMRI activity resulting in reduced hyperactivity in several frontal areas. In the right side, reduce number of errors on the task due to anodal tDCS application (from Meinzer et al., 2013).

i. Evaluation of local plasticity mechanisms

In a similar fashion as single and paired pulsed TMS have been used to evaluate physiology of the human central nervous system, plasticity-inducing protocols can be used to evaluate the state of plasticity mechanisms in healthy aging and demonstrate whether plasticity mechanisms are preserved or not. In a clear example of this studies, Freitas et al. (2011) found that the duration and the magnitude of corticospinal excitability modulation by rTMS was inversely correlated with age, suggesting that LTD-like plasticity becomes increasingly less efficient with age (**Figure 9a**). Several other investigations compared TMS measures of plasticity in young vs. older adults (Fathi et al., 2010; Pellicciari et al., 2009; Tecchio et al., 2008; Todd et al., 2010), and overall, these studies have shown reductions in plasticity both in LTP and LTP-like mechanisms related to physiological aging in the sensoriomotor cortex. Tecchio (2008) and Fathi (2010) both found age-related reduction in plasticity using PAS as well as Müller-Dahlhaus (2008) that found a negative correlation between

LTP-like plasticity and age. An rTMS study also found reduced LTD-like plasticity in elders in the human cortex (Todd et al., 2010). Few studies have been carried studying plasticity mechansims in aging outside the motor cortex. A couple of investigations (Pellicciari et al., 2009; Wolters et al., 2005) found increased LTP-like plasticity in somatosensorial areas which was interpreted as the presence of compensatory mechanisms in response for diminished plasticity in motor cortex. Plasticity in high-order cortical areas can be evaluated by use of neuroimaging techniques in combination with NIBS (Pascual-Leone et al., 2011) though literature is scarce at this moment. Finally, study of plasticity mechanisms might be assessed via training (Cirillo et al., 2011; Rogasch et al., 2009) as it represents an intervention able to induce brain plasticity; however interpretation of behavioral differences as indices of plasticity might be challenging (see Freitas et al., 2013; Zimerman and Hummel, 2010 for reviews).

ii. Evaluation of consequences of plasticity

TMS in aging memory has also been used as a tool to causally assess the consequences of the engagement of plasticity mechanisms during lifespan. In this approach both single pulse TMS and plasticity inducing protocols have been used. Such approximation usually consists in a comparison of anatomical correlates of memory function in elders compared to the brain-behavior relationship from a younger group. Differences are often considered in a similar fashion as explained elsewhere (see **Neural networks and aging section**), generally as engagement of compensatory mechanisms. Such studies attempt to evaluate hypothesis and models generated by neuroimaging studies. Some of this studies targeted the motor cortex (Rowe et al., 2006), but it has been in the PFC, likewise in neuroimaging studies, and during execution of cognitive paradigms that this methodology obtained more consistent findings (Cotelli et al., 2012, 2010; Manenti et al., 2013b, 2011; Rossi et al., 2004). Regarding episodic memory the study by Rossi and colleagues (2004) must be highlighted. In these study either left or right rTMS was applied in two separate groups of participants either youngers or elders. The most striking results is that HERA pattern, left PFC implicated in encoding, right PFC involved in retrieval, was

abolished in elders during the retrieval suggesting a bilateral compensatory engagement of DLPFC during episodic memory performance. Another example is the study performed by Manenti and collaborators (2013a). They demonstrated asymmetrical involvement of the DLPFC during action naming in young subjects by means of rTMS. When elders performed in the same task, only low-performing elders showed asymmetric patterns of brain involvement, while high-performing elders showed a reduction in asymmetry consistent with HAROLD model in such phonemic fluency test (**Figure 9b**).

iii. Plasticity-inducing interventions

In this section, all studies taking advantage of NIBS capacity to produce experimentally-controlled induction of plasticity with the intention to improve cognitive (or behavioral) performance in aging should be included. Hence, the logic of these studies is similar to the ones performed with healthy subjects (Guse et al., 2010) which have shown the capacity of NIBS to induce temporarily modifications in cognitive functions. By targeting either in PFC, Parietal Cortices or Anterior Temporal lobes, few studies achieved modulation of several cognitive functions in aging such as memory (Flöel et al., 2012; Manenti et al., 2013a; Solé-Padullés et al., 2006), fluencies (Cotelli et al., 2010; Meinzer et al., 2013; Ross et al., 2011) or several executive functions (Berryhill and Jones, 2012; Harty et al., 2014; Kim et al., 2012). A handful of studies had also proved the feasibility to improve motor function in healthy elders (Hummel et al., 2010; Zimerman et al., 2013). Most of these studies have been performed with tDCS technique. One example of motor skill enhancement is the study conducted by Zimerman (2013). After application of M1 tDCS, old subjects experienced improved acquisition of novel skill which lasted at least 24 hours. A promising study enhancing cognitive function was published recently (Meinzer et al., 2013) and it consisted in a semantic word generator task. It was found that anodal tDCS in the left inferior frontal gyrus significantly improved performance in older adults up to the level of younger controls (**Figure 9c**). Further tDCS significantly reduces task-related hyperactivity in bilateral prefrontal cortices inducing a “youth-like” activity and connectivity pattern.

f. Memory and aging. Assessment with NIBS techinques

Few studies have studied memory domain in aging with NIBS. Of these, one of the most representatives is the seminal study by Rossi and colleagues (2004) partially described above. In this study, rTMS was used to expand neuroimaging evidence of HAROLD during an episodic memory task in aged subjects. While young subjects rTMS impaired memory in the right DLPFC during a visuospatial recognition task, aged subjects showed that both left and right rTMS stimulation impaired task performance (**Figure 10**). The authors concluded that such decrease in asymmetry had compensatory functions. Similarly, another study found similar results in an encoding task, as high-performing older adults showed less prefrontal asymmetry (more bilateral interference) when compared to low-performing elders (Manenti et al., 2011). As decline in memory is one of the hallmarks of cognitive aging, several groups attempted to modify memory performance through application of controlled induction of plasticity. In this sense, our group (Solé-Padullés et al., 2006) revealed that high-frequency PFC rTMS was able to improve associative memory to healthy elders with memory complaints when compared to placebo stimulation (**Figure 10**). Such changes in performance were accompanied by additional recruitment of right prefrontal and bilateral posterior cortical regions. TDCS was also used during a retrieval task and found that left parietal and left DLPFC anodal tDCS was able to increase verbal episodic memory retrieval in older subjects (Manenti et al., 2013b). Object-location associative learning has also been found to be susceptible of enhancement by NIBS in elders (Flöel et al., 2012). After anodal tDCS over the right temporoparietal cortex during an object-location task, elders showed improve delayed recall one week after the learning session. Though only a handful of studies used NIBS to characterize or modulate memory networks and function, these techniques have demonstrated to be useful tools to characterize and modulate memory networks and functions in aging.

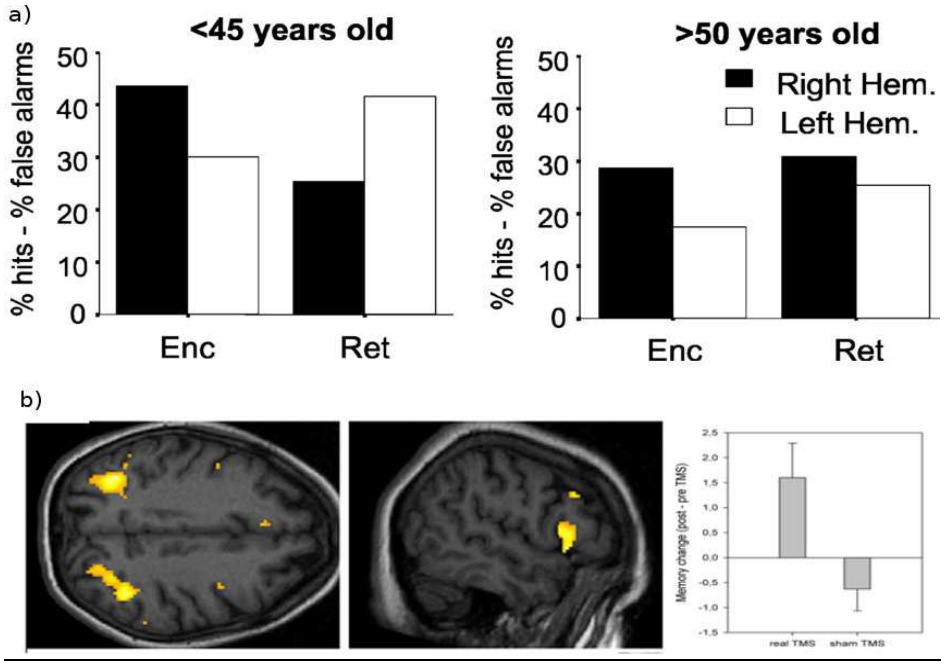

Figure 10. A) Hemispheric interaction of rTMS effects and age groups during retrieval, according to the HERA model. Young subjects show a clear hemispheric interaction while in older subjects the retrieval asymmetry is nolonger evident. Therefore, the HERA pattern is lost (from Rossi et al., 2004). B) Increased brain activationfollowing rTMS relative to baseline within the active group and changes in subsequent memory in both experimental groups (from Solé-Padullés et al., 2006)

It is worth mentioning that other methods capable to induce brain plasticity has been used in healthy elder populations such as cognitive training (Engvig et al., 2010) or exercise (Erickson et al., 2011) capable to increase in memory function and putative anatomofunctional correlates of mnesic functions.

g. Pathological aging, Plasticity and NIBS: a comment.

While this thesis is centered in healthy elders a comment has to be made regarding pathological aging, especially Alzheimer Disease and Mild Cognitive Impairment. As known, AD represents the most common neurodegenerative disorder, it is characterized by memory dysfunction and its best predictor is age. Also several

hypotheses sustain that plasticity mechanisms are highly involved in AD pathogenesis. Aß is involved in the pathogenesis of AD (Hardy and Higgins, 1992), and diffusible Aß42 were recently shown to directly inhibit LTP and induce synaptotoxicity (Shankar et al., 2007) as well as inhibitory circuitry (Ulfig, 1988). Conversely Aß is a normal product of brain activity (Kamenetz et al., 2003) involved in cell viability and survival (Pearson and Peers, 2006) and enhancement of learning and memory (Garcia-Osta and Alberini, 2009; Morley et al., 2010). Importantly, it appears that Aß-42 can impair or enhance synaptic function and memory depending on its concentration (inverted U-shape; (Puzzo et al., 2008). Abnormal levels of synaptic activity may lead to toxic Aβ secretion (Bero et al., 2011), suggesting that plasticity mechanisms and Aβ are part of a synaptic activity regulatory mechanism that is disrupted in early AD (Palop and Mucke, 2010).

In consequence, TMS has been widely used in this disease (see Freitas et al., 2011a) demonstrating alteration of plasticity mechanisms in AD (Battaglia et al., 2007; Inghilleri et al., 2006; Koch et al., 2012, 2011) and the feasibility to improve cognitive functions, though discrete results have been proved so far (i.e. Cotelli et al., 2011, 2008, 2006). TDCS application has also shown to improve specific cognitive features in AD subjects such as word (Ferrucci et al., 2008) and visual (Boggio et al., 2009) recognition. Together it seems that, at least, mild AD subjects (Cotelli et al., 2011; Ferrucci et al., 2008) can benefit from NIBS plasticity induction combined with training to transiently improve cognition. Such improvements have been proved to last for several weeks (Bentwich et al., 2011; Ferrucci et al., 2008).

An important number of studies exist using TMS alone or in combination with neuroimaging techniques focused in pathological aging, though a summary of them is out of the scope of this introduction. However, subjects with presence of small vascular disease, Aβ load and other known disease biomarkers are often masked in-between healthy subjects. Is still under discussion whether this subjects should or should not be considered healthy elders, yet, its special anatomofunctional features might jeopardize several designs and obscure some conclusions derived from aging studies (i.e. Oh and Jagust, 2013; Peña-Gomez et al., 2012) as this variables can exhert a relevant moderator effect. Thus, when explicitly referred, studies centered

in such populations have been discarded from this review as well as those referring to pathological aging.

Nevertheless, the study of healthy aging should not be considered as a topic completely unrelated to pathological aging. As age represents the major predictor for several neurodegenerative disorders, understanding those changes would be a keystone to better understand why neurodegenerative disorders preferably affects older brains. Understanding the dynamics of memory networks and function in aging, as both are highly affected by healthy and pathological aging, then, arises as a fundamental question which promises to significantly contribute in one of the most relevant problems of the neuroscience, and of the society, nowadays.

Chapter 2: Objectives and Hypothesis

1. General Objectives

The main objective of the present thesis is to characterize and modulate large-scale brain networks involved in long-term episodic memory through neuroimaging techniques and controlled induction of cortical plasticity mechanisms, focused in the implications towards the understanding of cognitive aging.

A second goal is to provide new insights into the relationship between plasticity mechanisms, studied through patterned TMS, and intrinsic brain connectivity patterns assessed with functional MRI.

2. Specific Objectives and Hypothesis

a. Study I

The goal of this study was to modulate episodic memory performance and task-related networks by means of patterned TMS. Episodic memory is one of the cognitive domains that is most affected by aging. However, when appropriate strategies are adopted, such as emphasizing semantic (deep) encoding, memory decline is partially reversed (Logan et al., 2002). Complementarily, NIBS proved to be able to modulate memory function in different populations, including elder subjects (Solé-Padullés et al., 2006), especially when applied to the PFC.

Another aim of this investigation was to obtain further knowledge about which variables modulate the impact of brain stimulation on behavioral and functional measures during an episodic memory task. In this regard, level of processing was identified as a significant variable as it was shown that PFC stimulation was able to

behaviorally disrupt deep memory processes while shallow encoding remained unaffected (Innocenti et al., 2010). Specifically, we pretended to evaluate the impact of the processing strategies and its connectivity pattern expression (task/strategy-dependent connectivity shifts) over the TMS effects.

In consequence we applied patterned *excitatory* TMS (iTBS) in combination with fMRI acquisitions at rest and during an encoding memory task with two levels of processing in a sample of elderly volunteers.

Given these objectives, we predicted the following:

1) Intermittent TBS compared to sham stimulation would result in a transient improvement of memory performance.
2) Intermittent TBS compared to sham stimulation will modulate the brain networks that support encoding processes.
3) State-dependent effects of iTBS will be observed; i.e. TMS applied over the left PFC will specifically affect deep encoding processes compared to shallow encoding. This state-dependent effect will be reflected both at behavioral and functional levels.

Study II

The main goal of **study II** was to assess the relationship between mPFC-PCU DMN connectivity and CBF, WM and GM correlates in elders as this measure is highly affected by age and related to memory processes. In the last few years it has been established that brain connectivity at rest is altered in ageing, specifically within posterior midline structure and medial prefrontal cortex DMN nodes (Ferreira and Busatto, 2013), which are central nodes of the brain connectome. DMN connectivity has been consistently related with cognition, usually with memory tasks (i.e Andrews-Hanna et al., 2007). Few studies have previously suggested that DMN connectivity is related with anatomical and perfusion measures (i.e. Khalsa et al.,

2013; Liang et al., 2014), though they were carried with few subjects, were limited to a single measure or restricted to specific bundles. In consequence, knowledge of causes and consequences of the DMN disconnection in aging remains limited. To gain further insight into the processes underlying age-related brain changes, the relationships between multiple neuroimaging measures need to be assessed, with the aim to broadening our understanding of the physiological mechanisms underlying age-related connectivity changes.

In order to accomplish the main goal of the study we analyzed a relatively large sample of old healthy subjects and an additional group comprising both young and old subjects. All subjects had multimodal neuroimaging data acquired including structural, diffusion, perfusion, and functional MRI sequences.

Given the main objective, the specific hypotheses were:

1) mPFC-PCU connectivity will be reduced in ageing.
2) mPFC-PCU connectivity will correlate with cognitive performance in elders, essentially with memory function.
3) In ageing, mPFC-PCU connectivity will correlate with grey and white matter integrity as well as with perfusion measures; these relationships will be found in, but not limited to, DMN areas and cingulum bundle (i.e. the major white tract connecting anterior and posterior medial components of this network).
4) Regions correlating with mPFC-PCU coupling strength will be located in areas of high age-related vulnerability.

Study III.

The main objective of this study was to produce by means of a single TMS session, a modulation of neurotransmitter concentration in both local and distal DMN nodes (left IPL and PCU), assessed with MRS. TMS is able to induce plasticity in a controlled manner which involve, amongst many other effects, local modification of GABA and

Glutamate concentrations, which can be assessed with MRS (Marjańska et al., 2013; Stagg et al., 2009a). On the other hand, DMN has proved to be relevant for memory processes (Andrews-Hanna et al., 2007) and affected both in healthy and pathological aging (in a normalcy-pathology homology; Jones et al., 2011). Similarly, plasticity, and GABA and Glutamate imbalances are also associated with these populations (Palop and Mucke, 2010). The possibility to induce and quantify neurochemicals modulation in the DMN might provide useful knowledge of plasticity dysfunction in certain conditions, as it might be a marker to track the state specific diseases.

Another goal of this study was to investigate whether individual differences in Default-Mode Network connectivity would be related to the neurochemical impact of TMS in the distal DMN node, as macrostructural properties such as connectivity and network responses to stimuli have repeatedly been linked to GABA and Glx concentrations (Kapogiannis et al., 2013). Complementarily distal effects of TMS are known to be modulated both by structural and functional integrity measures (Fox et al., 2012a; Hannula et al., 2010). To accomplish this objective, we applied individually guided neuronavigated sham, intermittent or continuous TBS stimulation in-between two MRI acquisitions.

Given these objectives, we predicted:

1) Active rTMS would induce neurotransmitter changes compared to sham stimulation in a similar fashion in both local and distal areas. Specifically it will increase GABA after cTBS and a reversed effect after iTBS.

The magnitude of neurotransmitter changes induced by rTMS would be related to the intrinsic connectivity between the stimulated area and the region where neurotransmitters were assessed.

Chapter 3: Methods

The current thesis consists on a total of three studies. Below, a brief description of the main methodological aspects of each study is provided. For further details, please refer to the corresponding publications, which can be found in the

Chapter 4: Results section. When the methods are common for several studies are described together in the top of the section. Otherwise, the methods are independently described. The studies are referred as **study I, II** and **III**.

1. Subjects

Subjects were independently recruited for **studies I, II and III**. In all studies, none of the participants had neurological or psychiatric disorder or any contraindications for MRI. All subjects gave informed consent and the protocol was in accordance with the Declaration of Helsinki and approved by the local ethics committee. In **studies I and III** none of the participants had any contraindications for TMS (Rossi et al., 2009).

a. Study I

Twenty-four healthy older adults were recruited in retirement homes and aging centers enrolled in the Institut Catala del Envelliment (ICN). Participants had a normal cognitive profile with Minimental State Examination (MMSE) scores≥24 and performances not below 1.5SD according to normative scores.

b. Study II

One–hundred sixteen healthy old subjects were included in this study. Subjects were recruited in retirement homes and aging centers enrolled in the ICN. Participants had a normal cognitive profile with MMSE scores≥24 and performances not below 1.5SD according to normative scores (sample 1). A second sample consisting of 25 young and 25 old healthy subjects was also analyzed. Data from the second sample (sample 2) has partially been used in studies I and III of the present thesis and partially published in other lab publications (Sala-Llonch et al., 2014a).

c. Study III

In **study III**, thirty-six healthy young subjects from the area of Barcelona were recruited for the study.

2. Neuropsychological evaluation

In **studies I and II** old participants underwent a neuropsychological evaluation that covered the major cognitive domains and included the following Spanish-adapted tests: MMSE, Test de Accentuación de Palabras (TAP), Rey auditory verbal learning test (RAVLT); Rey-Osterrieth complex figure (ROCF); Benton naming test (BNT); semantic and phonetic fluencies; forward and backward digits, symbol digits modalities test (SDMT), trail making test (TMT), Visual Object and Space Perception Battery (VOSP) incomplete letters and number locations tests and Stroop test.

a. Study I

Subjects enrolled in this study also realized WAIS-III vocabulary and London tower test, line orientation test and Popplereuter's embedded figures test.

b. Study II

Participants enrolled in this study realized the WAIS-III Block design; n-back test and a computerized version of Continuous Performance Test (CPT).

Individual psychometric tests were combined based on functional domains into four composite scales that assessed memory, speed processing, working memory.

3. MRI acquisitions

All subjects (from **studies I, II and III**) were examined in the same 3T MRI scanner (Magnetom Trio Tim, Siemens Medical Systems, Germany) located in Centre Diagnostic per la Imatge (CDIC) of Hospital Clínic de Barcelona.

a. Structural MRI acquisition

At least one high-resolution 3D structural dataset (T1-weighted magnetization prepared rapid gradient echo [MPRAGE], sagittal plane acquisition, TR=2300ms, TE=2.98ms, 240 slices, slice thickness=1mm, FOV=256mm, matrix size=256×256) was acquired in **studies I,II and III**.

Medium resolution 3D datasets (T1-weighted MPRAGE, sagittal plane acquisition, TR=1390ms, TE=2.86ms, 144 slices, slice thickness=0.625mm, FOV=240mm, matrix size=144x384) were acquired in **study III**.

b. Functional MRI

At least one resting state fMRI datasets (T2*-weighted GE-EPI sequence, TR=2000, TE=16 ms, 36 slices per volume, slice thickness=3 mm, interslice gap=25%, FOV=240 mm, matrix size=128x128, 150 volumes) were acquired in **studies I, II and III**.

Task related fMRI datasets (T2*-weighted GE-EPI sequence, TR=2000, TE=16 ms, 36 slices per volume, slice thickness=3 mm, interslice gap=25%, FOV=240 mm, matrix size=128x128, 312 volumes) was acquired in **study I**.

c. Magnetic Resonance Spectroscopy

Four ^{1}H-MRS spectra were acquired with a MEGA-PRESS sequence during the study either in the PCU or the left IPL in **study III**. MRS was acquired with the following parameters: MEGA-PRESS sequence; 256 spectral averages TR=1500 ms; TE=68 ms; voxel size=3.0*2*1.50 cm^3; approximate duration: 10 minutes. Voxel of interest (VOI) location was guided by functional and structural information. Double-banded pulses were applied at 4.7 ppm to suppress water and at 1.9 or 7.5 in alternate lines to edit the γ-CH_2 resonance of GABA at 3.0 ppm.

d. Diffusion Tensor Imaging

A diffusion tensor imaging (DTI) sequence (diffusion weighted echo-planar imaging sequence; 30 directions; TR= 7700ms; TE=89, 60 slices, slice thickness=2mm, FOV= 250mm and matrix sixe =122x122) was acquired in **study II** for all subjects.

e. Arterial Spin Labeling

A Pulsed-Arterial Spin labelling (PASL)-MRI perfusion acquisition (PICORE Q2T sequence, 50 tag-control scans, TR=2500ms TE=11.0ms, $T1_1$=700ms $T1_2$=1800ms, 16 slices; slice thickness=5mm, inteslice gap=25%, FOV=200mm) was acquired in **study II** for all subjects.

4. Experimental design

Studies **I** and **III** were design in an experimental manner; see (**Figure 11**Figure **12**) to consult for the experimental design timeline.

a. Study I

While all subjects previously underwent a neuropsychological assessment and a structural MRI, the main part of the study consisted of two MRI acquisitions, before and after subjects received real or sham iTBS Figure **11a**). In each MRI session subjects underwent an episodic memory encoding session in-between two rs-fMRI acquisitions Figure **11b**). After each scanning session, subjects performed a memory retrieval task outside the MRI.

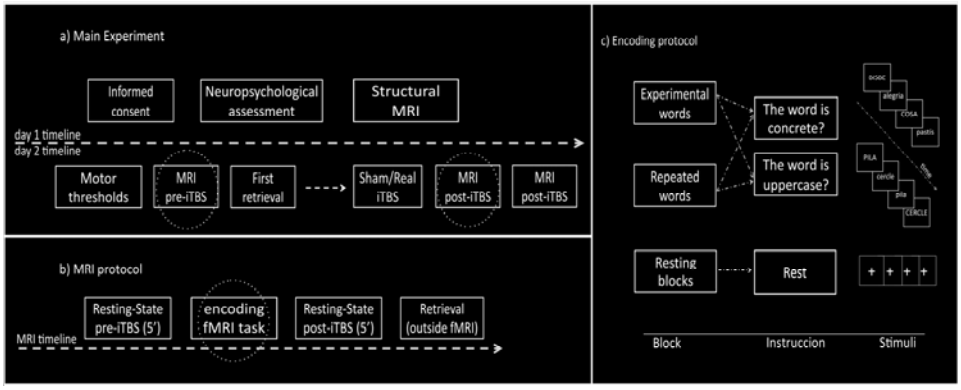

Figure 11. Schematic view of the study I experiment with A) timeline of the whole experiment, B) the MRI acquisition protocol and C) the encoding protocol realized inside the MRI. Circled boxes are detailed in the following section of the figure. For more information see

b. Study III

The main experimental protocol consisted of a TMS session in-between MRI acquisitions. In both MRI sessions, two MRS were acquired in the PCU and the left IPL, along with medium-resolution structural and rs-fMRI acquisitions (**Figure 12**). Prior to the main day of the experiment, subjects underwent an MRI acquisition with high-resolution 3D structural and rs-fMRI datasets.

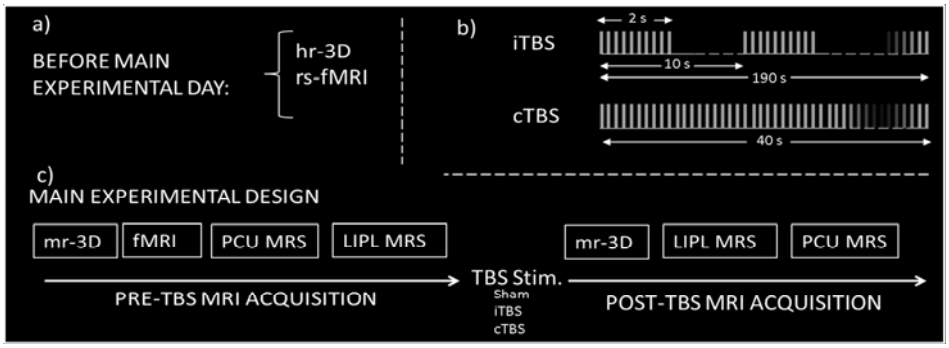

Figure 12. Protocol schema. A) Datasets acquired before the day of the experiment, B) schema of patterned repetitive transcranial magnetic stimulation used in the study and C) timeline schema of the main experimental design. For more information see

5. Behavioral task

In **study I**, each MRI session subjects completed a word encoding memory task followed by a recognition test designed with Presentation software (Presentation v10.1 Neurobehavioral Systems). Encoding batteries were generated with LEXESP (Gallés et al., 2000) and consisted of fixation, novel words and repeated words blocks. In word blocks, words were presented for 2s, half of them in uppercase and the other in lowercase. Instructions were presented asking subjects to rest or to answer according to either deep-semantic or shallow-perceptual judgments (**Figure 11.** Schematic view of the study I experiment with A) timeline of the whole

experiment, B) the MRI acquisition protocol and C) the encoding protocol realized inside the MRI. Circled boxes are detailed in the following section of the figure. For more information see

Chapter 4: Results section. The retrieval memory task started 15min after the encoding and consisted on a recognition task.

6. MRI Analyses

All MRI analyses in **studies I, II and III** were performed using tools either from FreeSurfer (http://surfer.nmr.mgh.harvard.edu, version 4.5), FSL (http://www.fmrib.ox.ac.uk/fsl/, version 4.1.5), AFNI (http://afni.nimh.nih.gov/) and inhouse scripts. Spectra data was analyzed with LCModel V.6.3-1a (Provencher, 1993).

a. Study I

i. fMRI data preprocessing

FMRI data preprocessing included removal of the first 5 volumes, motion correction, skull stripping, spatial smoothing, grand mean scaling and filtering. Task-related dataset was filtered with a high pass filter, while resting data was both high-pass and low-pass filtered. Registration of functional images to a standard template was performed using a two-step linear registration.

ii. Resting-state fMRI analyses

Independent Component Analysis and Dual Regression:

A data-driven temporal concatenation ICA approach as implemented in MELODIC followed by a dual-regression algorithm was used to study stimulation effects on RSN connectivity. Selected components were introduced into a dual-regression analysis involving a spatial and a temporal regression for each session. A subtraction between post and pre stimulation datasets was performed before entering maps into a group analysis. Comparisons were made using a voxel-wise nonparametric permutation testing and were Threshold-free cluster enhancement correction and family-wise error corrected (TFCE; FWE).

Amplitude of Low Frequency Fluctuation Analysis (ALFF):

Preprocessed datasets were transformed to frequency domain by a fast Fourier transform in order to calculate the power spectrum. Computation of the square root of the band-pass filtered power spectrum and the mean amplitude within the frequencies of interest is further performed. Fractional ALFF (fALFF), was computed by dividing the ALFF index for the sum of amplitude of the entire frequency range. Both ALFF and fALFF values were Z-standardized and introduced in the same pipeline as that described in the ICA section.

iii. Task-related fMRI analysis

Functional task-related images were analyzed with FEAT. After the preprocessing (see **fMRI data preprocessing**), a three-level general linear model (GLM)-based statistical analysis was performed for all the fMRI-task sessions. Corrections for multiple comparisons were made at cluster level using Gaussian random field theory.

Task-related iTBS changes:

For assessing task-related stimulation changes, we modeled the encoding deep (EncD), repeated deep (RepD), encoding shallow (EncS) and repeated shallow (RepS) task blocks as first-level regressors, which were convolved. The regressors, their temporal derivatives and realignment motion parameters were also entered into the GLM for each session. In the second-level fixed-effect analysis, pre-stimulation contrasts of interest were subtracted from post-stimulation contrasts. Finally, a third-level mixed-effect groupal analysis (real vs. sham stimulation) was performed.

Baseline memory networks:

Additionally baseline, pre-stimulation, patterns of task-related activation were analyzed to investigate the specific BOLD patterns of activation of every task. First-level pre-stimulation contrasts were entered in a matrix and mixed-effects analysis was performed.

Psychophysiological interaction:

A psychophysiological interaction analysis (PPI) was performed to test whether task-dependent changes in connectivity related to stimulation effects. We established five psychological regressors, consisting of the convolved task conditions plus a resting condition. In addition, a physiological BOLD signal regressor from a ROI was created in the stimulated area. Finally five PPI variables were created by multiplying a vector of each psychological condition with the physiological regressor. We generated PPI maps by testing the same contrasts as those mentioned in the task-related GLM analysis using instead the PPI regressors. A correlation between pre-stimulation PPI measures and posterior changes in BOLD activity induced by stimulation was performed.

b. Study II

i. MRI Preprocessing

Resting-state fMRI:

Data preprocessing was identical to **study I** (see **fMRI data preprocessing**). To obtain the DMN we performed an ICA with rs-fMRI preprocessed datasets as implemented in the MELODIC. From the resulting components, we selected DMN for ROI definition purposes. Preprocessed rs-fMRI data were further regressed with six rigid body realignment motion parameters, mean WM and mean CSF signal to extract rs-fMRI ROI-to-ROI connectivity.

Grey matter atrophy; Voxel-Based Morphometry (VBM):

Structural data were analyzed with FSL-VBM tool with a standard pipeline processing. Preprocessing included brain-extraction, tissue-type segmentation, non-linear registration to template, creation of a study-specific template and non-linearly re-registration to the study-specific template. Images were further modulated by dividing by the Jacobian of the warp field and smoothed.

Cortical thickness (CTh):

Cortical reconstruction was performed with the semi-automatic FreeSurfer image analysis suite with high-resolution 3D images. The procedures have been described thoroughly in Fischl and Dale (2000). Reconstructed and registered individual CTh maps were smoothed using a Gaussian kernel and introduced into a GLM-based analysis.

Grey-white matter contrast (GWC):

GWC was estimated with the reconstructed cortical surface by calculating the non-normalized T1-weighted image intensity contrast (100*[white–grey]/0.5*[white+grey]). Grey matter was taken at a 0.3 projection fraction from the boundary while white matter was assessed 1mm below the white matter surface. The resulting GWC was mapped to a common surface and smoothed with a Gaussian kernel.

White matter integrity:

Tract-Based Spatial Statistics (TBSS) analysis was carried out to assess WM microstructural integrity. Standard preprocessing included fitting to diffusion tensor model, nonlinear registration to standard space, creation of mean FA image and skeleton and alignment of subjects FA images to mean FA skeleton. Nonlinear warps and the skeleton projection were applied to MD data.

Perfusion imaging analysis:

ASL preprocessing was carried out with FSL-BASIL toolset to obtain CBF and CBF-GM (PVE corrected) maps. First we obtained a perfusion image by subtracting control from tag volumes. Next, kinetic model inversion was applied to the perfusion images. Spatial smoothing prior to and calculation of the equilibrium magnetization of arterial blood were also performed. CBF-GM measures were obtained by introducing partial volume estimates in native space into the kinetic model analysis. Finally, CBF images were transformed to standard space.

ii. Definition of mPFC-PCU DMN connectivity

To assess connectivity between mPFC and PCU, we created data-driven ROIs. After identifying the DMN we created 6mm-radius ROIs in peak coordinates of posterior midline and mPFC nodes. After thresholding ROIs with GM masks, the correlation between mPFC and PCU was obtained by calculating Pearson's (r) correlation between preprocessed and regressed rs-fMRI ROIs timeseries which werw further transformed to z-scores.

iii. DMN connectivity analysis

Group (young vs. old) comparisons between mPFC-PCU correlations were performed with the sample 2. To assess the relationship of structural and CBF correlates with anterior-posterior DMN connectivity, multiple voxelwise analyses were performed. Nonparametric testing as implemented in a randomized FSL tool was used for VBM, MD, FA, CBF and CBF-GM measures followed by TFCE and FWE corrections ($p<0.05$).

Relationships of CTh and GWC with connectivity were studied using linear modeling as implemented in FreeSurfer (FWE corrected using a Monte Carlo Null-Z simulation).

iv. Correspondence of mPFC-PCU DMN correlates and patterns of age-related decline

We extracted age-related patterns from the sample 2 and compared this brain patterns with those limited by the structural correlates of mPFC-PCU connectivity and those limited to DMN areas. First, we extracted maps of differences between old and young subjects using GLM-based analysis. Further, differences in the age-related effects on whole brain, connectivity correlates and DMN areas were compared using the raw statistics obtained in the group comparison and effect size calculations were performed.

v. Motion Correction Analysis

Mean relative translation and mean relative rotation parameters were calculated for every subject. Any subject with outlier values in relative movement was excluded from the analysis and all significant results were re-run using these measures as covariates.

5.3 Study III

i. Resting-state data preprocessing

Data preprocessing was identical to study I (see **fMRI data preprocessing**). Data was further regressed with six rigid body realignment motion parameters, mean white matter and the CSF signal and registered to an MNI standard space.

ii. Magnetic Resonance Spectroscopy analysis:

For both "edit OFF" and difference spectra, we calculated the best fit of the experimental spectrum as a linear combination of model spectra. Total creatine (tCR), total N-acetylaspartate (tNAA), GABA and Glx measures were extracted. Only Cramér-Rao lower bounds (CRLB)<15% were considered. Metabolites of interest were expressed as tNAA ratios, further corrected with a CSF partial volume factor.

iii. Structural analyses:

Medium resolution 3D images were co-registered with linear transformations to the previously acquired high resolution 3D images, which were segmented into tissue type to allow comparisons of the overlapping percentage between pre- and post-voxels and tissue type composition between experimental groups.

iv. Connectivity analyses:

To evaluate the relation between distal neurotransmitter modulation and connectivity, a seed-to-seed connectivity analysis was performed. PCU-ROI was created in the center of gravity of the spectroscopic VOIs, while Stim-ROI was placed in the coordinates used to guide stimulation. After GM masking, rs-fMRI preprocessed ROIs timeseries were extracted and correlated for each subject. Relationship between neurochemical modulation and connectivity was further studied (see **Data analysis section**).

<u>DMN resemblance:</u>

A seed-based correlation analysis was performed using ROIs created in the center of gravity of each spectroscopic VOI. Connectivity maps were later transformed to MNI standard space and voxelwise testing (TFCE and FWE corrected) was performed to determinate which areas were functionally connected to left IPL and PCU ROIs. Subject-specific connectivity maps were further compared to previously reported RSN (Smith et al., 2009) with one-sample t-test.

Specificity of left IPL-to-PCU connectivity as a predictor of neurotransmitter changes in PCU:

We correlated neurochemical changes due to stimulation and seed-to-brain connectivity maps, from PCU and Stimulation ROI timeseries. For each experimental group, four analyses were performed, as Stim-to-brain and PCU-to-brain connectivity maps were independently regressed for GABA and Glx change. Nonparametric permutation testing was performed (TFCE corrected).

v. Movement-control analysis

Mean relative translation and mean relative rotation parameters were calculated and any subject with outlier values relative movement was excluded from the analysis. Also, a correlation between mean relative rotation and translation and connectivity between the stimulation site and PCU-ROI was performed. Regarding MRS, we carried a 3x2 ANOVA with spectral FWHM as a measure of movement and group (Sham, cTBS, iTBS) and time (pre- post-TBS) as independent variables.

7. Data analysis

Non voxelwise tests were performed using the PASW/SPSS (v.18-20; Statistical Package for Social Science, USA) using the appropriate tests. The main analyses are briefly outlined next:

a. Study I

To examine any possible effect of iTBS on memory recognition, 2*2*2 repeated measures ANOVA for accuracy was performed, including Group (sham/real iTBS),

Time (pre/post stimulation) and Level of Processing (deep/shallow) as independent variables. Similarly, in neuroimaging data equivalent 2*2*2 ANOVAs were performed as main analyses.

b. Study II

In the sample 1 all the analyses pretended to explore correlations between DMN and other neuroimaging measures, as explain above. Additionally, the influence of DMN connectivity on cognition was studied. To evaluate the influence of mPFC-PCU connectivity to cognition we performed a multivariate GLM-based analysis in the main sample, where connectivity, age, years of education and gender was introduced in a model predicting the four cognitive factors. In the sample 2 all the analyses consisted in group (old vs. young) comparisons assessing age-differences in specific neuroimaging modalities. In the sample 1, age, gender, and years of education were used as covariates for all analyses while in only gender was used in sample 2.

c. Study III

The main hypothesis which assessed TBS effects on metabolite concentrations (Glx and GABA) was tested using 3x2 repeated measures ANOVA with group (sham*cTBS*iTBS) and time (pre*post) as independent variables. To study GABA and Glx covariation and the relationship between connectivity and neurotransmitter modulations, both bivariate Pearson's and nonparametric Spearman's correlations were performed.

8. Transcranial Magnetic Stimulation

Transcranial Magnetic Stimulation was applied in **studies I** and **III**. Magnetic pulses were delivered using a MagPro x100 (MagVenture A|S, Denmark) with an eight-figure coil. Resting and active motor threshold (RMT; AMT) were determined for the right first dorsal interosseous (FDI) muscle over the left M1 hand knob. RMT was defined as the lowest intensity of the TMS required to elicit MEPs of at least 50 µv peak-to-peak amplitude in at least 6 of the 10 consecutive trials (Rothwell et al., 1999). AMT was defined as the minimum stimulus intensity that elicited at least 6 of 10 consecutive MEPs of 200-µv during FDI isometric contraction. Neuronavigated stimulation with stereotactic registration was used (eXimia Navigated Brain Stimulation, Nexstim, Finland) while sham device was employed for sham stimulation. Immediately after TBS stimulation the subjects did the second MRI acquisition.

a. Study I

Either sham or real patterned iTBS was applied according to the protocol described by Huang end colleagues (Huang et al., 2005). Bursts consisted of 3 pulses of 50 Hz at an intensity of 80% AMT, repeated every 200ms during 2". These trains were repeated once every 10" for a total of 20 repetitions (600 pulses-190"). Based on a fMRI verbal encoding meta-analysis we selected a region (MNI(x,y,z): -42,14,30) in the left inferior frontal gyrus as stimulation target (Kim, 2011).

b. Study III

Sham, cTBS and iTBS was applied between two MRI sessions, according to previously described protocols (Huang et al., 2005). iTBS was identic to **study I** (see **Study I**).

cTBS consisted in 3 pulses at a 50 Hz pattern repeated every 200 ms, which were applied (600 pulses - 40”).

The stimulation target was individually defined as the area in the left IPL that showed highest functional correlation within the DMN based on the rs-fMRI dataset acquired before the main day of the experiment. We operationalized this concept in a similar way as described in the literature (Eldaief et al., 2011; Halko et al., 2010). We created spherical ROIS in the mPFC, the PCU/PCC and the right IPL which were transformed to functional space to perform a seed-based connectivity analysis within the left IPL. The targeted point was defined as the voxel with maximum conjoint correlation of the abovementioned connectivity maps.

Chapter 4: Results

1. Study I

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Task-dependent Activity and Connectivity Predict Episodic Memory Network-based Responses to Brain Stimulation in Healthy Aging

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ABSTRACT

Background: Transcranial magnetic stimulation (TMS) can affect episodic memory, one of the main cognitive hallmarks of aging, but the mechanisms of action remain unclear.
Objectives: To evaluate the behavioral and functional impact of excitatory TMS in a group of healthy elders.
Methods: We applied a paradigm of repetitive TMS – intermittent theta-burst stimulation – over left inferior frontal gyrus in healthy elders ($n = 24$) and evaluated its impact on the performance of an episodic memory task with two levels of processing and the associated brain activity as captured by a pre and post fMRI scans.
Results: In the post-TMS fMRI we found TMS-related activity increases in left prefrontal and cerebellum-occipital areas specifically during deep encoding but not during shallow encoding or at rest. Furthermore, we found a task-dependent change in connectivity during the encoding task between cerebellum-occipital areas and the TMS-targeted left inferior frontal region. This connectivity change correlated with the TMS effects over brain networks.
Conclusions: The results suggest that the aged brain responds to brain stimulation in a state-dependent manner as engaged by different tasks components and that TMS effect is related to inter-individual connectivity changes measures. These findings reveal fundamental insights into brain network dynamics in aging and the capacity to probe them with combined behavioral and stimulation approaches.

Introduction

Episodic memory is one of the cognitive domains that is most affected by aging [1], and is accompanied by volumetric changes in brain structures, white and gray matter changes and dopamine receptors depletion [2].

Repetitive transcranial magnetic stimulation (rTMS) is able to modulate cortical excitability and produce cognitive [3] and motor [4] changes. Previously, we observed improvements in a face-name memory task after prefrontal rTMS applied to older subjects which was accompanied by increased recruitment of right prefrontal and bilateral posterior areas [5]. Cognitive improvements after transcranial stimulation have also been shown in mild cognitive impairment and Alzheimer's disease populations [6–8]. However,

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Conflicts of interest: Dr. Pascual-Leone serves on the scientific advisory boards for Nexstim, Neuronix, Starlab Neuroscience, Neuroelectrics, and Neosync; and is listed as an inventor on several issued and pending patents on the real-time integration of transcranial magnetic stimulation (TMS) with electroencephalography (EEG) and magnetic resonance imaging (MRI).

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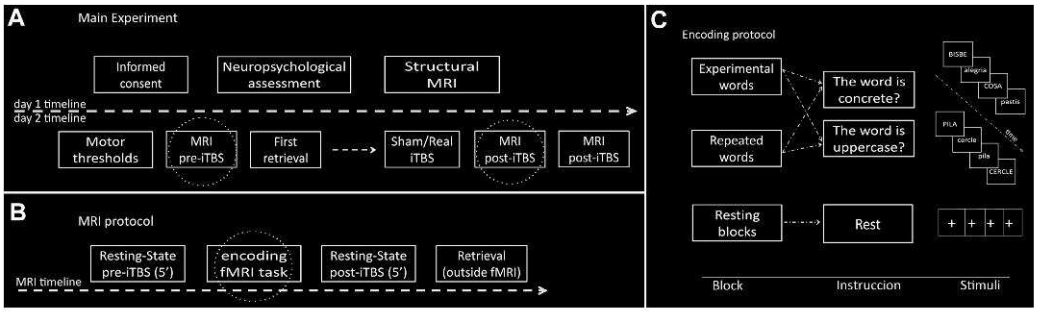

Figure 1. Schematic view of the experiment with A) timeline of the whole experiment, B) the MRI acquisition protocol, and C) the encoding protocol realized inside the MRI. Circled boxes in section A) are detailed in section B); circled boxes in section B) are detailed in section C).

mechanisms underlying cerebral and behavioral responses to rTMS remain unclear.

A mechanism that modulates TMS effects is the state-dependent phenomenon [9–11]. That is, TMS can induce changes revealing the potential to interact with ongoing cognitive processing or physiological states. At a functional level, state-dependency has shown to be related to both, regional activity [11], and connectivity [12–14], therefore representing relevant variables that can help to understand TMS variability, together with other factors such as age [15], genetics [16], technical aspects [17] or anatomical characteristics [18]. Neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), have become a powerful tool to reveal shifts in connectivity and regional activity and it is increasingly being used together with TMS [19,20].

Recently, new patterned protocols of stimulation have emerged from animal studies, such as theta-burst stimulation (TBS) [21]. Applied in an intermittent fashion (iTBS), it enhances cortical excitability, while continuous TBS (cTBS) produces inhibitory post-effects. When applied to prefrontal areas, TBS has been shown to affect various cognitive functions, such as working memory [12,22], speech repetition [23] and emotional control [24].

The left prefrontal cortex (PFC) is a region that has consistently been implicated in the encoding of verbal material. TMS studies have causally shown the involvement of the PFC during episodic memory formation, both in the left dorsolateral prefrontal cortex [25–27] (DLPFC) and left inferior frontal gyrus [28–30] (IFG). Neuroimaging evidence also supports left PFC involvement in semantic encoding, compared to shallower encodings [31–33]. These findings can be contextualized into a classical psychological theory of level of processing (LoP). That is, different LoP at encoding, such as semantic or perceptual analysis of the incoming information, result in differentially durable traces, therefore affecting the probability of a successful retrieval [34,35]. Although there is some evidence of age affecting LoP [36–38], this phenomenon has not been thoroughly investigated in aging. Importantly, it seems that if appropriate support is given during encoding phases (i.e. semantic elaboration), aging effects on memory performance can be minimized [36,39].

In the present study, we applied excitatory TMS (iTBS) in combination with fMRI acquisitions at rest and during an encoding memory task with two levels of processing in a sample of elderly volunteers. The main objectives of the study were: 1) to investigate whether iTBS compared to sham stimulation could result in a transient improvement in memory performance, 2) to study the brain networks that support encoding processes and TMS effects on them and 3) to study state-dependent effects of iTBS.

Material and methods

Subjects

Twenty-four healthy older adults, between 61 and 80 years, were recruited (mean age = 71.75 y.o.; standard deviation [SD] = 6.81). Participants had a normal cognitive profile with MMSE scores ≥24 and performances not below 1.5 SD according to normative scores (adjusted for age, gender and education [40,41]) on a neuropsychological evaluation that covered the major cognitive domains (including verbal memory: Rey auditory verbal learning test; visual memory: Rey–Osterrieth complex figure; language: Benton naming test; semantic and phonetic fluencies; frontal/executive functions: direct and inverse digits, symbol digits modalities test, trail making test, Stroop test, London tower test; visuospatial: line orientation, and visuoperceptive: Popplereuter's embedded figures test). All participants were right-handed and none of them had any neurological or psychiatric disorder or any contraindications for TMS [42]. All subjects gave informed consent and the protocol was approved by local ethical committee. Subjects were randomly assigned to either the sham or experimental group as described below, although neuroimaging analysis was carried out with 10 subjects in the sham group, due to MRI acquisition problems.

Design and procedure

All subjects previously underwent a neuropsychological assessment and a structural MRI acquisition for subsequent TMS neuronavigation. The main part of the study consisted of two MRI acquisitions, before and after subjects received a real or sham iTBS session (Fig. 1A). In each MRI session subjects underwent an episodic memory encoding session in-between two resting-state fMRI acquisitions (Fig. 1B). After a wash-out period (≈1 h), subjects received real or sham iTBS and performed an equivalent fMRI encoding session. After each scanning session, subjects performed a memory retrieval task outside the MRI.

MRI acquisition

All subjects were examined on a 3T MRI scanner (Magnetom Trio Tim, Siemens Medical Systems, Germany). A high-resolution 3D structural dataset (T1-weighted magnetization prepared rapid gradient echo [MPRAGE], sagittal plane acquisition, TR = 2300 ms, TE = 2.98 ms, 240 slices, slice thickness = 1 mm, FOV = 256 mm, matrix size = 256 × 256) was acquired before the main

experimental day. fMRI sets of images consisting of 150 and 312 volumes for resting and task runs were also acquired (T2*-weighted GE-EPI sequence, TR = 2000, TE = 16 ms, 40 slices per volume, slice thickness = 3 mm, interslice gap = 25%, FOV = 240 mm, matrix size = 128 × 128).

Memory task

In each MRI session subjects completed a word encoding memory task followed by a recognition test designed with Presentation software (Presentation v10.1 Neurobehavioral Systems). Four block-designed encoding batteries were generated with LEXESP [43], although subjects completed two encoding batteries (words from the non-presented batteries were used as new words during the retrieval). The presentation of batteries and the button response were counterbalanced. Each encoding battery consisted of 8 fixation, 8 novel words and 8 repeated words 24s-blocks, in which 4 words were constantly repeated. In word blocks, 12 words were presented for 2s, half of them in uppercase and the other in lowercase, while in fixation blocks a white cross was presented. Instructions were presented for 2s asking subjects to rest or to answer according to either deep-semantic (concrete/abstract) or shallow-perceptual judgments (uppercase/lowercase; Fig. 1C). Both batteries and within-battery blocks were equivalent according LEXESP normative data. The retrieval memory task started 15 min after the encoding and consisted on a recognition task (old/new decision) in which all the encoded words were presented mixed with new words from a non-presented battery (half new).

Transcranial magnetic stimulation

Magnetic pulses were delivered using a MagPro X100 (MagVenture, Denmark) with an eight-figure coil. Resting and active motor threshold (RMT; AMT) were determined for the right first dorsal interosseous (FDI) muscle over the left M1. RMT was acquired according to published protocols [44] while AMT was defined as the minimum stimulus intensity that elicited at least 6 of 10 consecutive MEPs of 200-μv during FDI contraction.

iTBS was applied according to the protocol described by Huang et al. [21]. Bursts consisted of 3 pulses of 50 Hz at an intensity of 80% AMT, repeated every 200 ms during 2″. These trains were repeated once every 10″ for a total of 20 repetitions (600 pulses - 190″). Based on a fMRI verbal encoding meta-analysis we selected a region (MNI (*x*,*y*,*z*): −42,14,30) in the left IFG as stimulation point [45]. Neuronavigated stimulation with stereotactic registration was used while sham device was employed for sham iTBS. iTBS was applied in a room next to the scanner and immediately after the stimulation, subjects did the second MRI acquisition.

Data analysis

Behavioral data

To examine any possible effect of iTBS on memory recognition, 2*2*2 repeated-measures ANOVA for accuracy was performed, including Group (sham/real iTBS), Time (pre/post stimulation) and Level of Processing (deep/shallow) as independent variables. Accuracy was defined as ([Hits-False Alarms]/∑encoding words) and the range of the index was between −1 and 1, where 1 was an optimal encoding.

Sociodemographic and neuropsychological data

Unpaired *t*-tests and Chi-square tests were performed when appropriate both for behavioral and sociodemographic data. Tests were performed using the PASW 18.0 (Statistical Package for Social Science, USA). When no specified data is presented as mean (SD); error bars represent standard errors of mean.

MRI analyses

MRI analysis was performed using tools from FSL (http://www.fmrib.ok.ac.uk/fsl/) and AFNI (http://afni.nimh.nih.gov/). Registration of functional images to a 3 mm standard template was performed always immediately before group analyses using a two-step linear registration [46].

fMRI data preprocessing included removal of the first 5 volumes, motion correction, skull stripping, spatial smoothing (full width at half maximum [FWHM] = 6 mm), grand mean scaling and filtering. Task-related dataset was filtered with a high-pass filter of 150″, while resting data were both high-pass and low-pass filtered (0.1 and 0.01 Hz).

Resting-state analyses

From the resting preprocessed dataset, both an independent component (ICA) and an amplitude of low frequency fluctuation (ALFF) analysis were performed to assess any iTBS-related changes produced either in the connectivity of resting-state networks (RSN) or in spontaneous brain activity respectively.

Independent component analysis

A data-driven temporal concatenation ICA approach [47] as implemented in MELODIC followed by a dual-regression algorithm [48] was used to study iTBS effects on RSN connectivity. MELODIC uses probabilistic ICA to decompose 4D data into an estimated number of spatial and temporal components [49,50]. Eight components were selected as reflecting spontaneous brain fluctuation while the remaining ones were considered artifactuals after comparison with existing RSN reported in the literature (Biswal, 2010; Smith, 2009): the anterior default mode network (DMN), the posterior DMN, the cerebellar network, the frontal executive network, the left and right frontoparietal networks, the sensorimotor network and a visual network [51,52].

Selected components were introduced into a dual-regression analysis involving a spatial and a temporal regression for each session. A subtraction between post-iTBS and pre-iTBS was performed to obtain one map reflecting differences between sessions. Maps were registered to a template and entered into a group analysis. Comparisons were made using a voxel-wise nonparametric permutation testing [53] (5000 permutations). Threshold-free cluster enhancement correction [54] was used and maps were thresholded at $P < 0.05$ family-wise error (FWE).

ALFF

An analysis based on the ALFF, thought to be a measure of spontaneous intrinsic brain activity [55], was performed with resting data. Preprocessed datasets [56] were transformed to frequency domain by a fast Fourier transform in order to calculate the power spectrum. ALFF is then computed as the square root of the band-pass filtered power spectrum, and the index arises from the mean amplitude within the frequencies of interest for each voxel. Fractional ALFF [57] (fALFF), was computed by dividing the ALFF index for the sum of amplitude of the entire frequency range (0–0.25 Hz). Both ALFF and fALFF values were *Z*-standardized and introduced in the same two-step pipeline as that described in the Independent component analysis section.

Task-related fMRI analysis

Functional task-related images were analyzed with FEAT. After the abovementioned preprocessing, a three-level general linear

model (GLM)-based statistical analysis was performed for all the fMRI-task sessions.

For assessing task-related iTBS changes, we modeled the encoding deep (EncD), repeated deep (RepD), encoding shallow (EncS) and repeated shallow (RepS) task blocks as first-level regressors, which were convolved. The regressors, their temporal derivatives and realignment motion parameters were also entered into the GLM for each session [58]. In the second-level fixed-effect analysis, pre-iTBS contrasts of interest resulting from the first-level analysis (Memory Effect [Mem > Rep], LoP [Deep > Shallow] and Interaction [(EncD > RepD)>(EncsS > RepS)]) were subtracted from post-iTBS contrasts for every subject. Finally, a third-level mixed-effect groupal analysis was performed [59]. This analysis allowed the study of an iTBS state-dependent effect over Memory, Level of processing, and the interaction of Memory*LoP, focused on assessing the rTMS effect over Deep Encoding. As we found iTBS effect over Interaction contrast, the model was re-run to explore the iTBS effect on the EncD > EncS and EncD > RepD first-level contrasts, that is, separating the Interaction contrast into two simpler first-level contrasts to gain further insight in the results.

Additionally baseline, pre-stimulation, patterns of task-related activation were analyzed to investigate the specific BOLD patterns of activation of every task. First-level pre-stimulation contrasts were entered in a matrix and mixed-effects analysis was performed. In both analyses, corrections for multiple comparisons were made at cluster level using Gaussian random field theory (min $Z > 2.3$; cluster significance $P < 0.05$).

PPI

A post-hoc connectivity analysis was performed to test whether there was a task-dependent change in connectivity between the stimulation coordinates and the regions in which iTBS effect was found. We hypothesized that state-dependent iTBS-induced effects on deep encoding would be related to a task-dependent shift in connectivity in the pre-stimulation encoding. Psychophysiological interaction (PPI) [60] allows the study of regions that differ in connectivity by context or condition. In this regard, applying this technique, we expected increased connectivity between posterior and IFG regions during (pre-stimulation) semantic encoding as well as a correlation of the connectivity shift (PPI measure) with distal rTMS effects. For the PPI, we established five psychological regressors, consisting of the convolved task conditions plus a resting condition spanning all the experimental time [61]. In addition, a physiological BOLD signal regressor from a ROI was created in the stimulated area (5 mm-radius sphere). Finally five PPI variables were created by multiplying a vector of each psychological condition with the physiological regressor. We generated PPI maps by testing the same contrasts as those mentioned in the task-related GLM analysis using instead the PPI regressors. We created a 5 mm-ROI around the peak activation of the task-related Interaction contrast (MNI (*x,y,z*): −6,−82,−16) and examined ROI-to-ROI task-dependent change in connectivity. Finally, a correlation between pre-stimulation PPI measures and iTBS posterior changes in BOLD activity was performed to establish a relationship between both measures.

Table 1
Mean sociodemographic variables (standard deviations), motor threshold intensities and neuropsychological memory test scores (Rey Auditory Verbal Learning Test (RAVLT) and Rey–Osterrieth Complex Figure Test (ROCF) both assessed at 30 min for real and sham iTBS group.

	Real iTBS ($n = 12$)	Sham iTBS ($n = 12$)	t/χ (P)
Age	73.00 (6.28)	70.08 (7.15)	1.06 (0.300)
Education	0:5:5:2	0:4:2:6	3.40 (0.183)
Sex	6:6	6:6	0.00 (1.000)
RMT	57.75 (7.45)	53.08 (6.95)	1.59 (0.127)
AMT	52.83 (7.23)	48.80 (6.06)	1.47 (0.156)
RAVLT	7.67 (3.80)	8.08 (3.55)	−0.28 (0.784)
ROCF-30′	17.68 (5.27)	18.18 (3.55)	0.29 (0.776)

Education and Sex had been tested by Chi-square tests while for the other measures unpaired *t*-tests were used. Education levels are classified as basic:primary:secondary:superior whereas Sex levels are men:woman.

Table 2
Behavioral descriptive results from the recognition test assessed after each MRI encoding session.

	Real iTBS ($n = 12$)	Sham iTBS ($n = 12$)
Pre-stimulation retrieval		
Deep accuracy	0.32 (0.19)	0.38 (0.16)
Shallow accuracy	0.10 (0.09)	0.12 (0.11)
Post-stimulation retrieval		
Deep accuracy	0.31 (0.14)	0.32 (0.14)
Shallow accuracy	0.04 (0.09)	0.14 (0.15)

Statistical comparisons are given in the main text.

Results

The sham and real iTBS groups did not differ statistically in age, gender, education, motor thresholds or performance on neuropsychological memory tests ($P > 0.05$; Table 1).

Behavioral analysis

Mean memory performance was 0.21 (0.11). The three-way repeated-measures ANOVA to assess effects on retrieval accuracy only revealed main effects of LoP ($F = 143.78$, $P < 0.001$) being the semantic encoded material more easily recognized than the perceptual one. No main effects of Time nor Group were found ($F = 1.37$, $P = 0.258$; $F = 1.22$, $P = 0.281$). Concerning iTBS effects, neither Time*Group ($F = 0.83$, $P = 0.776$) nor Time*Group*LoP interaction ($F = 3.22$, $P = 0.087$) effects were found, indicating that iTBS had no impact on the main behavioral memory measures (Table 2; Fig. 2).

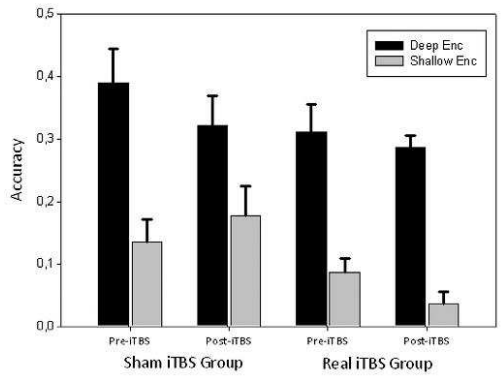

Figure 2. Accuracy in the recognition memory task. Only significant main effect of level of processing was found significant (Deep > Shallow accuracy; $F = 143.780$, $P < 0.001$). Error bars represent standard error of mean.

Table 3
Baseline task-related activations for the main contrasts investigated and the whole sample of subjects ($N = 22$; see also Fig. 3).

Task	Region	Location (*x,y,z*)	mm^3	*Z*-max
Mem > Rep	Cerebellum/OC	14,−98,0	115,748	5.02 (25.80)
	Cerebellum/OC	−44,28,−14	21,856	4.97 (7.53)
Rep > Mem	Right AG	36,−68,38	4596	4.02 (1.66)
Deep > Shallow	Left PFC/PAC	−55,22,0	42,984	5.39 (13.70)
	Right cerebellum	34,−78,−40	10,084	4.41 (4.30)
	Left TOC	−60,−52,−2	3940	4.19 (1.62)
(EncD > RepD)>	Left IFG	10,10,60	5068	3.58 (1.73)
(EncS > RepS)	SMA/SFG	−52,16,18	4264	3.67 (1.40)

OC = occipital cortex; AG = angular gyrus; PFC = prefrontal cortex; PAC = paracingulate cortex; TOC = temporoccipital cortex; IFG = inferior frontal gyrus; SMA = supplementary motor area; SFG = superior frontal gyrus. *Z*-max = (−log10(*P*))
Coordinates are referred in MNI coordinates system.

Baseline fMRI task-related analysis

The Memory network (Mem > Rep) included activation of cerebellum, occipital cortex (OC), orbitofrontal cortex and left IFG and deactivation of right angular gyrus. LoP (Deep > Shallow) activation was mostly localized in the left PFC including the IFG, the middle and the superior frontal gyrus (SFG). The paracingulate cortex (PAC) and right cerebellum were also found to be implicated in the network. The Interaction contrast ([EncD > RepD] > [EncS > RepS]); also referred as deep encoding contrast) resulted in activation patterns in the left IFG and the dorsomedial PFC, encompassing the supplementary motor area and the SFG (Table 3; Fig. 3).

Pre- vs. post-iTBS analyses

The following analyses were carried to test iTBS impact over neuronal networks both in task and rest context.

Resting fMRI, ICA and ALFF analysis

In the eight RSN networks extracted from ICA, the FWE-corrected analysis did not reveal any significant clusters that indicated iTBS effects on the functional architecture of the brain. Even at an uncorrected level ($P < 0.001$) no relevant clusters emerged from the analysis. Results of the ALFF analyses did not demonstrate any effect of iTBS on FWE-corrected maps neither at an uncorrected level ($P < 0.001$).

Task-related fMRI analysis

Task-related analysis was initially focused on studying the effect of iTBS on Memory, LoP and Interaction BOLD contrasts. Results from these analyses would indicate a state-dependent effect of iTBS for the specific contrast (i.e. results on memory contrast would reflect a specific effect of iTBS during memory compared to repeated condition). Only in the Interaction contrast a cluster emerged encompassing primary visual areas, lateral OC, ventral occipitotemporal areas and the cerebellum (Table 4, Fig. 4). In other words, iTBS compared to sham, induced increased activation specifically for deep encoding (LoP*Memory Interaction).

We additionally explored iTBS effect over EncD > RepD and EncD > EncS contrasts to confirm a more specific involvement of EncD condition in the interaction contrast (LoP*Memory) as opposite of an effect related to RepS condition. Congruently, similar clusters emerged in posterior areas for both contrasts. Additionally, the EncD > EncS contrast showed an additional cluster in the left IFG, the area underlying stimulation (Table 4, Fig. 4). These results indicate that iTBS had an effect on brain networks specifically when subjects were performing a deep encoding task, both in local and distal areas that belong to memory encoding network. As we wanted to gain some insight regarding the mechanisms underlying rTMS modulation over posterior areas, we performed a post-hoc connectivity analysis.

Table 4
Brain regions where iTBS effects (Time*Group interaction) were found (see also Fig. 4).

Task	Region	Location (*x,y,z*)	mm^3	*Z*-max
((EncD > RepD) > (EncS > RepS))	Cerebellum/OC	−6,−82,−16	15,532	3.87 (5.44)
EncD > RepD	Cerebellum/OC	−20,−82,−16	15,720	3.98 (6.53)
EncD > EncS	Cerebellum/OC	6,−88,−30	22,692	3.84 (8.13)
	Left IFG	−46,34,10	6668	3.42 (2.80)

OC = occipital cortex; IFG = inferior frontal gyrus. *Z*-max = (−log10(*P*))
Coordinates are referred in MNI coordinates system.

PPI – relationship between baseline task-dependent connectivity and iTBS effects

PPI ROI-to-ROI analysis revealed significant changes in connectivity during the pre-iTBS encoding, in a task-dependent fashion ((EncD > RepD) > (EncS > RepS)), between the stimulated region and the region in which maximum effects of iTBS during deep encoding were observed ($t = 2.53$, $P = 0.029$). Hence, coupling between both areas was greater when subjects performed a deep encoding than in the other 'psychological' states. To note, during pre-stimulation encoding, posterior ROI was more coupled with left IFG ROI in semantic condition even when no BOLD changes were observed in posterior areas for this specific contrast in the former area (see Fig. 3). Finally, PPI changes were associated with an iTBS effect on the BOLD signal in this posterior ROI ($r = 0.63$, $P = 0.028$) in the active group (Fig. 5), but not in the sham group ($r = -0.22$, $P = 0.551$). In other terms, greater task-dependent shifts in coupling between both areas in the pre-stimulation encoding correlated with greater state-dependent iTBS BOLD modulation in distal areas after stimulation.

Discussion

We applied real or sham iTBS over the left IFG in healthy elders in order to study behavioral and brain functional changes during an episodic memory task. Main conclusions could be summarized as: 1) iTBS did not produce changes in performance in the memory task. 2) Functional brain TMS-induced changes were found specifically when subjects were performing a deep encoding task in local (left IFG) and distal areas (OC and cerebellum). 3) Areas where TMS effects were found belong to memory encoding networks. 4) TMS effects correlated with task-related connectivity shifting measures at pre-iTBS encoding task. Finally, 5) when subjects were at "resting-state" there were no stimulation changes in connectivity or in spontaneous brain activity measures.

iTBS does not produce behavioral changes in memory retrieval

Although behavioral changes were hypothesized in accordance with existing literature, lack of cognitive effect of the stimulation in our study can be explained by a number of factors. First, most of the TMS protocols reporting effects on memory employed on-line protocols [30], while off-line protocols usually involve stimulation over a number of days [3]. Second, elderly people may be more resistant to show behavioral effects. Bihemispheric [5] or contralateral stimulation may be needed in the elderly to induce behavioral effects in accordance with literature on cognitive aging compensation processes [2,62,63]. Third, functional increases in OC and cerebellum may be unrelated to behavioral improvements

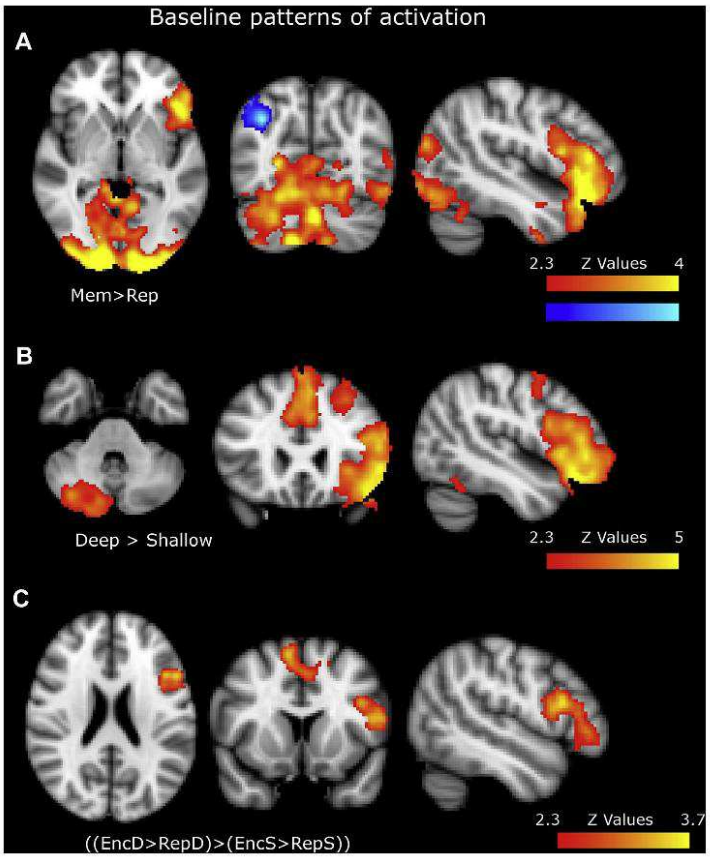

Figure 3. Baseline patterns of activation for A) Memory, B) LoP, and C) Interaction contrast task contrasts (images shown in radiological convention). For precise anatomical localizations, see Table 3.

since magnitude of activation is not fundamental in remembered items, compared to forgotten ones [45,63]. Four, mechanisms modulated by rTMS in the left IFG may be more related to encoding processes that do not strongly influence subsequent retrieval in terms of recognition accuracy [29,30]. Although unexpected, absence of behavioral changes allows us to interpret brain function avoiding complications of interpreting neural changes associated to behavioral modifications [10,64,65].

iTBS did not induce resting-state brain network changes in connectivity or in spontaneous brain activity measures

Our results show that the functional architecture of the brain as measured by resting fMRI was not modified by iTBS in contrast with published literature [66–69]. However, only two previous studies examined TMS effects over large-scale RSN [67,69] both modulating DMN connectivity. As the stimulated area in the present study was not clearly part of DMN or any well-established large-scale RSN, it is possible that effect of stimulation could be quite inconsistent due to subjects variability in its connectivity patterns and, thus, difficult to assess with groupal analysis. Other methodological approximations, though, such as graph-theory methods [70], and seed-based analyses [71] could be more sensitive to reveal connectivity changes in such conditions. ALFF analyses also failed to detect TMS effects on spontaneous activity during the resting state. Similarly, other studies that used MRI techniques failed to detect changes of activity at rest after PFC TBS [72], suggesting that TBS may have a greater impact during a task than during resting-state conditions.

iTBS induced specific changes in BOLD signal during deep encoding task in local and distal related areas

Results strongly indicate that iTBS modulated brain network expression in a task-dependent manner. Specifically, we found that stimulation only produced differences in BOLD activity when subjects were performing a semantic encoding task while brain networks remained unaltered when subjects were at rest or doing a perceptual encoding task. Hence, LoP modulated LIFG stimulation effects in a state-dependent fashion similarly to other paradigms in TMS literature [10,73]. This task-dependent effect might be related

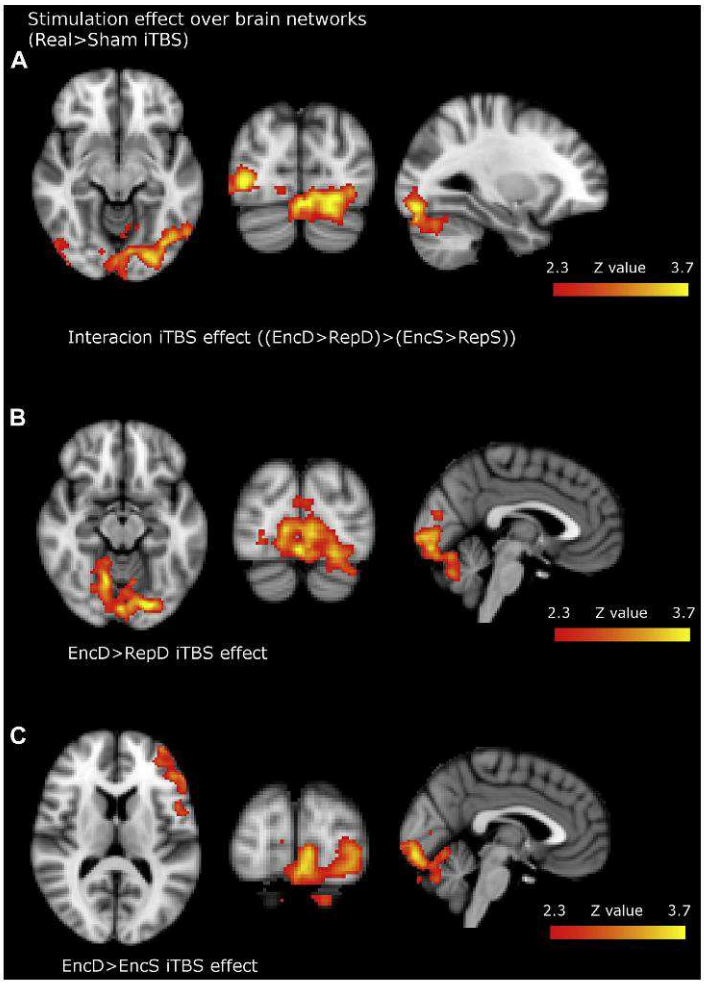

Figure 4. Brain regions where iTBS effects (Time*Group interaction) were found after unpaired two sample *t*-tests. All contrasts showed increased BOLD activity of real compared to sham group. A) Depicts increased activity for Interaction (Memory*LoP) first-level contrast, namely increased activity specifically for deep encoding. B) and C) show increased activity after real iTBS for EncD > RepD and EncD > EncS task contrasts respectively (images shown in radiological convention). For precise anatomical localizations, see Table 4.

to the regional state [31–33] of the targeted area and its functional implication during semantic encoding tasks [10,72–74] and it can be linked with recent TMS findings on the LoP theory [27]. Innocenti et al. found that deep encoding processes were interfered by the application of on-line interfering TMS over left PFC while shallow encoding processes remained unaffected during a memory task. This suggested that left PFC is a node engaged during long-term memory and crucial for semantic processing, and that TMS methods can selectively interact with semantic encoding networks. However the targeted area differed in both studies, being left DLPFC in the previous study and left IFG in ours. It has been proposed that DLPFC may be implicated in executive-monitoring processes [75] while left IFG seems to be specifically involved in phonological and semantic control [76] and semantic elaboration processes [30].

Our results are in good agreement with a role of semantic control of left IFG, suggesting that this area may selectively exert top-down modulation over posterior areas. rTMS modulations were observed in the ventral visual stream, from primary visual areas to the ventral occipitotemporal cortex (see Fig. 4A), while areas included in the semantic network, such as the anterior temporal lobe [76,77], remained unaffected. Moreover, modulation was observed in relatively unspecific areas, hierarchically located before visual areas that are selectively involved in word and semantic recognition [78,79] such as the visual word form area. However, early visual activity has been shown to be modulated by semantic

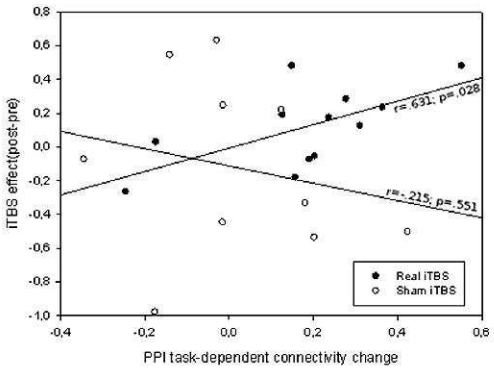

Figure 5. Correlation between PPI task-dependent connectivity changes at baseline and the iTBS-induced effects. PPI values reflect a deep encoding specific increased connectivity (positive values) between stimulated area and a ROI localized in the area where maximum iTBS effects were found. iTBS effect reflects relative change of signal in this area induced by stimulation (arbitrary units). See main text for ROI and contrast definitions.

properties such as non-predictive semantic cues [80], semantic priming paradigms [81], bilingual literacy [82], age of acquisition [83], or shifting in attention to semantic category [84]. Since these areas do not specifically respond to semantic properties of the stimuli, results were generally interpreted as evidences of top-down modulations. Further, EEG studies reported early sensitivity of posterior areas to semantics [85,86] before the widely described N400 event-related potential linked to semantic processing emerges [87]. However, off-line TMS and fMRI does not allow extracting conclusions of such temporal resolution, so we cannot ascertain whether IFG modulation over posterior regions reflects an early attentional shift toward facilitating deep encoding, such as the ones proposed by cuing paradigms or instead it reveals a continuous feed forward network that leads to enhanced recognition during semantic encoding [83]. Involvement of left IFG in encoding during a wide temporal window [29,30,88] in chronometric TMS approaches suggest a continuous involvement of prefrontal areas during semantic processing.

Interindividual changes in network connectivity with increasing task demands at baseline predict iTBS modulation of deep memory encoding networks

Our results showed a correlation of IFG-posterior coupling shifts toward deep encoding condition and task-dependent rTMS modulation. This result strengthens the idea of a semantic-selective top-down modulation of IFG to posterior areas probably through inferior fronto-occipital fascicle [89,90], a bundle that connects both areas. Relevance of connectivity parameters as a factor of variability in rTMS has been observed in several studies [14]. Both, connectivity from targeted region to rTMS distal modulations and brain intrinsic connectivity dynamics have been shown to modulate stimulation responses [10,12,13,91]. In our study, the PPI analyses revealed that the amount of posterior modulation is mediated by individual's subject capacity to shift between psychological contexts. The influence of IFG to posterior areas is not static and fixed but dynamically changes with different task conditions (in our study, depending on semantic and encoding demands) as suggested in other elegant studies that modulated activity in visual processing areas after IFG stimulation [65,92,93] in a content and context specific manner. PPI analysis does not measure coupling but instead examines connectivity shifts in interaction with different psychological contexts. Although "absolute" connectivity patterns have been usually used in conjunction with rTMS, PPI measures may represent an appropriate approximation when considering task-dependent modulations of TMS. However, more research is needed in order to know whether absolute or relative patterns better predict TMS effect [36,39,94].

Regarding aging, the recruitment of networks were highly constrained by the specific task-demands on each condition which probably eliminated a differential engagement (compared to young subjects) of effective learning strategies which are known to be affected during intentional encoding in aging [36,39]. Experimentally establishing encoding strategies, has shown to minimize memory decrements in aging. Also, age-related functional changes seem to consist on task-related decreased deactivations and over-recruitment of additional areas while role of primary cognitive networks (those engaged in young subjects) usually remains partially preserved. Still, it is not possible to rule-out the possibility that age-related changes over networks are conditioning our results, similarly to other paradigms [26,95]. In the future, a young group and an intentional encoding condition should be considered, as well as stimulation over overrecruited areas, to fully explore the role of left IFG over memory encoding and its subsequence age-related changes. Our results, in conjunction with Innocenti et al. [27], highlight the relevance of combining stimulation within a specific strategy of encoding in order to obtain functional modifications of memory networks. These results have to be seriously taken into account in future studies involving both cognitive and transcranial stimulation. In conclusion, these results highlight the role of the left IFG in semantic encoding, probably by exerting top-down modulation effects, enforce the idea of task-dependency for higher cognitive functions, and emphasize the role of connectivity in order to understand TMS-induced changes.

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2. Study II

frontiers in AGING NEUROSCIENCE

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Decreased Default Mode Network connectivity correlates with age-associated structural and cognitive changes

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Ageing entails cognitive and motor decline as well as brain changes such as loss of gray (GM) and white matter (WM) integrity, neurovascular and functional connectivity alterations. Regarding connectivity, reduced resting-state fMRI connectivity between anterior and posterior nodes of the Default Mode Network (DMN) relates to cognitive function and has been postulated to be a hallmark of ageing. However, the relationship between age-related connectivity changes and other neuroimaging-based measures in ageing is fragmentarily investigated. In a sample of 116 healthy elders we aimed to study the relationship between antero-posterior DMN connectivity and measures of WM integrity, GM integrity and cerebral blood flow (CBF), assessed with an arterial spin labeling sequence. First, we replicated previous findings demonstrating DMN connectivity decreases in ageing and an association between antero-posterior DMN connectivity and memory scores. The results showed that the functional connectivity between posterior midline structures and the medial prefrontal cortex was related to measures of WM and GM integrity but not to CBF. Gray and WM correlates of anterio-posterior DMN connectivity included, but were not limited to, DMN areas and cingulum bundle. These results resembled patterns of age-related vulnerability which was studied by comparing the correlates of antero-posterior DMN with age-effect maps. These age-effect maps were obtained after performing an independent analysis with a second sample including both young and old subjects. We argue that antero-posterior connectivity might be a sensitive measure of brain ageing over the brain. By using a comprehensive approach, the results provide valuable knowledge that may shed further light on DMN connectivity dysfunctions in ageing.

Keywords: Default Mode Network, resting-state fmri, memory, aging, connectivity, gray matter, white matter, arterial spin labeling

INTRODUCTION

As demographic changes in developed countries push up the proportion of elderly adults in the population (Cohen, 2003), age-related cognitive decline is emerging as a major concern. Ageing entails cognitive and motor decline and is the main risk factor for neurodegenerative disorders, especially Alzheimer's Disease (AD; Hebert et al., 2003). Cognitive domains affected by age include speed of processing, working memory capacity, inhibitory function and long-term episodic memory (Park and Reuter-Lorenz, 2009; Salthouse, 2010). Neuroimaging studies have contributed to the understanding of the ageing brain and have classically associated the elderly with gray mater (GM) shrinkage (Good et al., 2001; Salat et al., 2004; Fjell et al., 2009a,b), ventricular expansion (Walhovd et al., 2011), decreased WM (WM) integrity (Davis et al., 2009; Bennett et al., 2010; Westlye et al., 2010; Sala et al., 2012) and neurotransmitter depletion, particularly of the dopaminergic system (Reeves et al., 2002).

GM atrophy in both cortical and subcortical structures, evident as early as middle age (Salat et al., 2004), has been demonstrated in both cross-sectional (Good et al., 2001; Fjell et al., 2009b) and longitudinal evidences (Raz et al., 2004; Fjell et al., 2009a). Grey matter atrophy analyses, carried out either with Cortical Thickness (CTh) or with Voxel-based morphometry (VBM) approaches, consistently indicate that prefrontal cortices and most subcortical structures are regions of high age-related vulnerability (Good et al., 2001; Fjell et al., 2009b). Lateral and medial temporal lobes and posterior midline structures are also significantly affected by age-related atrophy (Fjell et al., 2014a). Most structures show a linear decline, though specific structures

such as the hippocampus may exhibit an increased rate of atrophy with ageing (Fjell et al., 2013).

Both macrostructural and microstructural WM alterations that includes damage in the myelin sheath and reduction in the total number of nerve fibers have been widely reported in aged humans (Tang et al., 1997; Bartzokis et al., 2004; Davis et al., 2009; Westlye et al., 2010; Sala et al., 2012). These changes are believed to impair the efficiency of communication between neural regions and to contribute to the functional decline in elders (Bartzokis et al., 2004). Diffusion Tensor Imaging (DTI) techniques are sensitive to the degree and direction of water molecule permeability and are able to characterize microstructural properties of WM *in vivo*. Increased Fractional Anisotropy (FA) and reduced Mean Diffusivity (MD) are the most frequently used DTI measures associated with WM integrity, as they are able to provide summarized information on the state of WM. Diffusion Tensor Imaging measures are altered over the lifespan, presenting an inverted U-shape curve and accelerated decreases during senescence (Westlye et al., 2010; Sala et al., 2012). Regarding the pattern of age-related differences in DTI measures across brain regions, a variety of models haven been proposed that include frontal, anterior to posterior gradient and retrogenesis, last-in first-out, models (Bennett and Madden, 2013). It has also been suggested that thin myelinated fibers, near the brain surface or on the periphery of fasciculi may be more vulnerable to age-related degradation than deeper structures (Tang et al., 1997; Bartzokis et al., 2004). In addition, a measure that might provide information on both GM and WM atrophy near the cortical surface is the GM/WM contrast (GWC). Several studies have found striking reductions in GWC during ageing (Magnaldi et al., 1993; Salat et al., 2009; Westlye et al., 2009) indicating that GM and WM tissue becomes less differentiated as the brain ages. Gray/white matter contrast alteration is particularly notable in medial and lateral prefrontal areas, inferior and posterior midline parietal areas and lateral temporal areas. A microstructural basis for the blurring of the GM/WM boundary might reflect several processes in ageing, among them alterations in the structure and density of myelin sheaths (Cho et al., 1997), changes in iron concentration (Ogg and Steen, 1998) and/or increased content of water in WM (Magnaldi et al., 1993).

It is widely accepted that ageing and vascular processes interact to disrupt cerebral hemodynamics (de la Torre, 2012). Additionally, neurovascular unit (involving endothelial cells, myocites, neurons and its processes and astrocytes amongst others) is involved in cerebral hemodynamics while preservation of this hemodynamics processes is vital for neural activity and cognitive function (Popa-Wagner et al., 2013). Cerebral blood flow (CBF), classically assessed with radio-ligand based neuroimaging techniques and closely correlated with brain metabolism is a cerebral hemodynamic process known to be reduced in ageing (Martin et al., 1991), vital for optimal neural function and it has been identified as a contributor to cognitive impairment in older adults (Brown and Thore, 2011; Gorelick, 2011). Arterial Spin labeling (ASL) is a noninvasive technique able to assess tissue perfusion by magnetic labeling of arterial blood water and have aroused considerable interest in recent years. Arterial Spin labeling measures are affected by ageing (Asllani et al., 2009), even when corrected for partial volume effects (PVE), however systematic studies are needed to fully characterize CBF values over the lifespan and to determine its topological pattern.

More recently, though, brain connectivity at rest has consistently been found to be altered in ageing using the fMRI technique (rs-fMRI; Hafkemeijer et al., 2012; Ferreira and Busatto, 2013). rs-fMRI is able to detect interregional correlations in low-frequency spontaneous BOLD fluctuations (Biswal et al., 1995) which have shown to be a key feature in healthy brain functioning and are altered in multiple neuropsychiatric pathologies (Broyd et al., 2009). A significant finding in rs-fMRI ageing literature is the observation of decreased long-range functional connectivity in elders (Meunier et al., 2009; Tomasi and Volkow, 2012) usually complemented by increased local clustering (Tomasi and Volkow, 2012; Sala-Llonch et al., 2014). Age-related decreases in rs-fMRI mainly affect the Default Mode Network (DMN; Andrews-Hanna et al., 2007; Bluhm et al., 2008; Damoiseaux et al., 2008; Razlighi et al., 2014), a resting state network (RSN) that comprises several structures including posterior midline structures (precuneus/posterior cingulate cortex [PCU/PCC]), medial prefrontal cortex (mPFC), inferior parietal lobule (IPL), and middle and medial (entorhinal/hippocampus) temporal cortex (Laird et al., 2009). Default Mode Network recruitment has been directly associated with episodic memory retrieval, prospective memory, self-referential processes, social cognition or mind wandering and it is usually deactivated during external-oriented tasks (Anticevic et al., 2012), a process strongly affected in ageing (Miller et al., 2008). The interest in the DMN, though, also arises in pathological ageing as connectivity within this network is strongly decreased in Alzheimer's Disease (AD; Jones et al., 2011) and most of its nodes are key cores of the pathology (Buckner et al., 2009).

The DMN appears to be more susceptible to the effects of ageing (Damoiseaux et al., 2008) than other RSN and this susceptibility may be evident even in middle-age healthy subjects (Bluhm et al., 2008; Biswal et al., 2010; Evers et al., 2012). Moreover, age-related reductions in DMN nodes seems to involve coupling between mPFC and posterior midline structures in particular, which are the key nodes of the DMN (Andrews-Hanna et al., 2007; Bluhm et al., 2008; Biswal et al., 2010; Campbell et al., 2013; Mevel et al., 2013). Default Mode Network rs-fMRI connectivity predicts cognitive and behavioral measures, especially memory function, both in different populations including healthy young subjects (Sala-Llonch et al., 2012) and healthy elders (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Wang et al., 2010; He et al., 2012; Mevel et al., 2013; Razlighi et al., 2014). Alterations in the DMN, a core brain network, have been attributed to inefficient reallocation of resources, though the exact causes of decreased DMN are still unknown.

While preserved cerebral connectivity patterns seem to sustain healthy brain functioning, other physiological brain measures appear to modulate connectivity and may provide useful insights into age-related connectivity changes. Perfusion and connectivity have been linked in young adults (Liang et al., 2013), while, during ageing, Aβ deposition (Hedden et al., 2009) and the dopaminergic

system (Achard and Bullmore, 2007) have been related to brain functional connectivity. In contrast, local GM atrophy does not seem to fully explain rs-fMRI connectivity changes occurring in ageing (Damoiseaux et al., 2008). However GM functional and structural covariance patterns are highly related (Segall et al., 2012) and relationship between connectivity patterns and GM topology (Seeley et al., 2009) and integrity (Pujol et al., 2013; Baggio et al., 2014) have been shown in several disorders. In contrast it is increasingly accepted that anatomical connectivity supports functional connectivity, which, at rest, is especially tangible in key brain nodes such as those belonging to the DMN (Skudlarski et al., 2008; Honey et al., 2009; Horn et al., 2013). Indeed, relationships between WM integrity indices and functional connectivity measures have been reported in several populations (van den Heuvel et al., 2008; Khalsa et al., 2013) including healthy elderly adults (Andrews-Hanna et al., 2007; Teipel et al., 2010) emerging in the late childhood (Supekar et al., 2010; Gordon et al., 2011). The coupling strength between DMN nodes seems to be supported by the cingulum bundle integrity in ageing which connects posterior to anterior and temporal DMN areas (Greicius et al., 2009).

To gain further insight into the processes underlying age-related brain, the relationships between multiple neuroimaging measures need to be assessed. These experimental settings do not allow any inference of causality between the changes evaluated using different modalities, but can broaden our understanding of the physiological mechanisms underlying age-related changes (Chételat et al., 2013b). Studying the relationship of DMN with other neuroimaging modalities may help to unify disparate findings of the neuroimaging literature and provide a more comprehensive description of altered connectivity in ageing. So far, despite almost unequivocal evidence that DMN connectivity is highly vulnerable to the effects of aging, our understanding of the causes and consequences of decreased antero-posterior DMN connectivity remains limited. Studies investigating structural correlates of DMN integrity in ageing have been limited to specific bundles and to local GM integrity, and little is known about how age-related connectivity changes relate to brain patterns of reduced grey and WM integrity and CBF in ageing.

The main objective of this study was to assess the relationship between mPFC-PCU DMN connectivity and CBF, WM and GM correlates in elders. The specific hypotheses were: (1) mPFC-PCU connectivity will be reduced in ageing; (2) mPFC-PCU connectivity will correlate with cognitive performance in elders, essentially with memory function; (3) in ageing, mPFC-PCU connectivity will correlate with grey and WM integrity as well as with perfusion measures; these relationships will be found in, but not limited to, DMN areas and cingulum bundle; (4) regions correlating with mPFC-PCU coupling strength will be located in areas of high age-related vulnerability. To test these hypotheses we used two independent samples. One large sample of healthy elders with small age-related variability was used to extract structural, perfusion and cognitive correlates of mPFC-PCU coupling strength, while a second sample including young and old subjects was used for purposes of group (age-related) comparisons.

MATERIALS AND METHODS

PARTICIPANTS

One hundred and sixteen healthy old subjects (age = 68.25 (3.053); range: 63–78, 37 males) were included in this study. Mean years of education (YoE) was 11.09 (4.149). Subjects were recruited in retirement homes and centers for the elderly registered with the Institut Català del Envelliment (ICN) in the area of Barcelona. All participants had normal cognitive profile with Mini-mental State Examination test (MMSE) scores $\geq$25 and performances not below 1.5SD according to normative scores adjusted for age, gender and education in a neuropsychological evaluation that covered the major cognitive domains. Informed consent was obtained from all participants. The study was approved by the Hospital Clinic de Barcelona ethical committee which follows the guidelines of the Declaration of Helsinki. All subjects underwent MRI acquisition (DTI data were available for 100 subjects). This aged sample was used to assess the relationship between rs-fMRI connectivity on the one hand, and cognition, structural and perfusion indices on the other and will be referred as *sample 1*.

A second, independent, sample (referred as *sample 2*) consisting of 25 (8 males) young and 25 (8 males) old healthy subjects was used for direct age-groups comparison purposes. Magnetic resonance imaging data from this sample was obtained using exactly the same scanner and sequences as in the primary sample. Mean age was 23.08 (2.02; range 19–28) and 68.92 (3.67; range 64–76) for the young and old group respectively. Mean YoE was 19.64 (1.77) and 11.36 (4.02) for the young and old group respectively. Neither age, nor gender and YoE differences between the *sample 1* and the old group from the *sample 2* were found ($p > 0.3$). However YoE between old and young groups in the *sample 2* differed ($t = 9.40$, $p < 0.001$) as young subjects were recruited in an academic environment. When not specified, methods employed with both samples are assumed to be equivalent. Data from sample 2 has been partially published in previous studies of our group (Sala-Llonch et al., 2014; Vidal-Piñeiro et al., 2014).

NEUROPSYCHOLOGICAL ASSESSMENT

The neuropsychological battery used comprised the major cognitive domains and included the following spanish-adapted tests (see **Table 2**): Mini-mental State Examination test, Rey auditory verbal learning test (RAVLT); Test de accentuación de palabras (TAP; Spanish analog of the National Adult Reading Test); WAIS-III Block design; Rey-Osterrieth complex figure (ROCF); Benton naming test (BNT); semantic and phonetic fluencies; forward and backward digits; symbol digits modalities test (SDMT), a mean d'-score of a 2 and 3-back working memory test (as in Sala-Llonch et al., 2012), Trail Making Test (TMT), Stroop test, Visual Object and Space Perception Battery (VOSP) Incomplete letters and Number locations tests and a computerized version of the Continuous Performance Test (CPT). Psychometric tests were further combined into different composite scores representing different cognitive domains (see below). Old subjects from the *sample 2* completed thorough neuropsychological batteries which are described elsewhere (Sala-Llonch et al., 2014; Vidal-Piñeiro et al., 2014).

MRI ACQUISITION

All participants were examined on a 3T MRI scanner (Magnetom Trio Tim, Siemens Medical Systems, Germany) at the Center Diagnostic per la Imatge in the Hospital Clínic of Barcelona. Magnetic resonance imaging acquisition included the following sequences: a high-resolution 3D structural dataset (T1-weighted magnetization prepared rapid gradient echo [MPRAGE], sagittal plane acquisition, TR = 2300 ms, TE = 2.98 ms, 240 slices, slice thickness = 1 mm, FOV = 256 mm, matrix size = 256 × 256); a rs-fMRI sequence (T2*-weighted GE-EPI sequence, TR = 2000, TE = 26 ms, 40 slices per volume, slice thickness = 3 mm, interslice gap = 25%, FOV = 220 mm, matrix size = 128 × 128) that lasted 5 min (150 volumes); a DTI, sequence (diffusion weighted echo-planar imaging sequence; 30 directions; TR = 7700 ms; TE = 89, 60 slices, slice thickness = 2 mm, FOV = 250 mm and matrix sixe = 122 × 122) and an Pulsed-Arterial Spin labeling (PASL)-MRI perfusion acquisition (PICORE Q6T sequence, 50 tag-control scans, TR = 2500 ms, TE = 11.0 ms, T11 = 700 ms, T12 = 1800 ms, 16 slices; slice thickness = 5 mm, inteslice gap = 25%, FOV = 200 mm, matrix size = 64 × 64).

MRI PREPROCESSING

Magnetic resonance imaging analysis was performed using tools from FreeSurfer[1], FSL[2] and AFNI[3].

Resting-state fMRI: Data preprocessing included removal of the first five volumes, motion correction, skull stripping, spatial smoothing (full width at half maximum [FWHM] = 7 mm), grand mean scaling, high and low pass filtering (0.1–0.1 Hz) and normalization with two-step linear transformations (Jenkinson and Smith, 2001) to a standard template. Preprocessed rs-fMRI data were further regressed with six rigid body realignment motion parameters, mean WM and mean ventricular signal. Preprocessed rs-fMRI signal was used to extract main RSNs through independent component analysis (ICA) analysis while regressed data was analyzed for calculating rs-fMRI ROI-to-ROI connectivity indices.

Gray matter atrophy; Voxel-Based Morphometry (VBM): Structural data were analyzed with FSL-VBM tool (Good et al., 2001) with a standard pipeline processing (as in Balasa et al., 2012). Preprocessing included brain-extraction, tissue-type segmentation, non-linear registration to template, creation of a study-specific template and non-linearly re-registration to the study-specific template. Images were further modulated by dividing by the Jacobian of the warp field and smoothed (FWHM ≈9 mm).

Cortical thickness: Cortical reconstruction was performed with the semi-automatic FreeSurfer image analysis suite with high-resolution 3D images. The procedures have been described thoroughly in Fischl and Dale (2000). Reconstructed and registered individual CTh maps were smoothed using a Gaussian kernel of 15 mm FWHM and introduced into a GLM-based analysis. Two subjects were removed from further comparisons due to problems during reconstruction ($n = 114$).

Gray-white matter contrast: GWC was estimated with the reconstructed cortical surface by calculating the non-normalized T1-weighted image intensity contrast (100*[white–grey]/0.5*[white+grey]). Values close to 0 indicate less contrast and thus more blurring of the GM/WM boundary. GM was taken at a 0.3 projection fraction from the boundary while WM was assessed 1 mm below the WM surface. Before performing statistical analyses, the resulting GWC was mapped to a common surface and smoothed with a 15 mm FHWM Gaussian kernel. Two subjects were removed due to problems during cortical reconstruction ($n = 114$).

White matter integrity: Tract-Based Spatial Statistics (TBSS; Smith et al., 2006) analysis was carried out to assess WM microstructural integrity. Standard preprocessing (as in Bosch et al., 2012) included fitting to diffusion tensor model, nonlinear registration to standard space, creation of mean FA image and skeleton (thresholded at >0.2) and alignment of subjects FA images to mean FA skeleton. Nonlinear warps and the skeleton projection were applied to MD data. Four subjects were further removed due to machine artifacts ($n = 96$). The model was re-run, but this time aligning subjects to the standard skeleton instead of using the mean derived skeleton which, allowed comparisons of the results between the two samples. The results did not qualitatively change between the two procedures (data not shown).

Perfusion imaging analysis: Arterial Spin labeling preprocessing was carried out with FSL-BASIL toolset (Chappell et al., 2009) to obtain CBF and CBF-GM (PVE corrected) maps in absolute ml/100 g/min units. First we obtained a perfusion image by subtracting control from tag volumes and taking the average of these "difference" images. Next, kinetic model inversion (Buxton et al., 1998) was applied to the perfusion images. Constant relaxation times for blood and tissue were set at 1.6 and 1.3, inversion efficiency to 0.98 and bolus arrival time was fixed at 0.7. Spatial smoothing was applied prior to the estimated CBF images and a calculation of the equilibrium magnetization of arterial blood was then performed using WM as reference tissue. Cerebral blood flow-gray matter measures were obtained by introducing partial volume estimates in native space into the kinetic model analysis, which were calculated from native-transformed structural segmentation. The method proposed, described in Chappell et al. (2011), is able to exploit both partial volume estimates and the different kinetics of the ASL signal arising from GM and WM. Finally, CBF images were transformed to standard space.

DATA ANALYSIS

The appropriate tests were carried out with SPSS 20.0 (Statistical Package for Social Science, Chicago, IL, USA) software to handle non-neuroimaging data. Significance was set at $p < 0.05$ (Bonferroni corrected when necessary). When not specified, data is presented as mean (SD), error bars represent standard error of mean (SEM) and coordinates are reported in MNI space. In the sample 1 age, gender, and YoE were used as covariates for all analyses. Cases were excluded pairwise in the different analyses. In the sample 2 only gender was used as covariate. The rationale for not covariating for education is the overrepresentation of YoE effects due to group differences which biased the results. However, the results were not qualitatively modified when YoE was added as an additional covariate (data not shown).

[1]http://surfer.nmr.mgh.harvard.edu, version 5.3
[2]http://www.fmrib.ox.ac.uk/fsl/, version 5.0.6
[3]http://afni.nimh.nih.gov/

Cognitive Domain Factorialization

In *sample 1*, individual psychometric tests were combined based on functional domains into four composite scales that assessed memory, speed processing, working memory and inhibition in accordance with the ageing literature (Park and Reuter-Lorenz, 2009). To compute composite scores, raw scores were converted to z-scores for all tests and then underwent factor analysis (Principal Component Analysis). Missing values (<2%) were replaced with subjects' mean for the specified factor. The various tests that comprised each cognitive factor are shown in **Table 2**.

Definition of mPFC-PCU DMN connectivity

The measure of mPFC-PCU DMN connectivity were obtained with a two-steps pipeline that involved extraction of DMN through ICA which allow extraction of ROI coordinates for posterior ROI-to-ROI connectivity analysis. This pipeline was independently performed in both samples.

To obtain the DMN we performed an ICA with rs-fMRI preprocessed datasets (Beckmann et al., 2005) as implemented in the MELODIC tool which decomposes data into a number of spatial and temporal components (fixed to 20 components). From the resulting components, we identified typical components described in the literature such as right and left frontoparietal networks, a visual network, a sensoriomotor network, a cerebellum network, an executive network and the DMN, all of them common in both samples. This latter network was used for Region-of-Interest (ROI) definition. After identifying the DMN in the respective samples, which included posterior midline structures, mPFC, bilateral IPL and bilateral medial temporal gyrus (MTG) components (**Figure 1**), we assessed connectivity between mPFC and PCU in a ROI-to-ROI analysis that involved the definition of 6 mm-radius ROIs in peak coordinates of posterior midline and mPFC nodes. After thresholding ROIs with GM masks, the correlation between mPFC and PCU was obtained by calculating Pearson's (r) correlation between preprocessed and regressed rs-fMRI ROIs time series. Next, correlations were transformed to z-scores. For sake of completeness bilateral IPL and bilateral MTG DMN ROIs were also extracted. The Euclidian distance between posterior midline structures and mPFC ROIs from both samples was small (PCU = 6 mm; mPFC = 3 mm) and mPFC-PCU correlation did not differ between old adults from the sample 2 and sample 1 population ($t = -1.533$, $p = 0.128$; see **Table 1** for ROI coordinates).

Table 1 | Peak voxels of main DMN nodes. Region-of-Interests were centered in this coordinates.

DMN node	MNI (x,y,z) sample 1	MNI (x,y,z) sample 2	Euclidian distance (mm)
PCU/PCC	0,−63,30	0,−63,36	6
mPFC	0,63,−3	0,63,−6	3
LIPL	−51,69,27	−48,69,30	4.24
RIPL	54,−60,27	51,−63,27	4.24
LMTG	−63,−12,−18	−66,−15,−15	5.2
RMTG	63,−9,−15	63,−6,−18	4.24

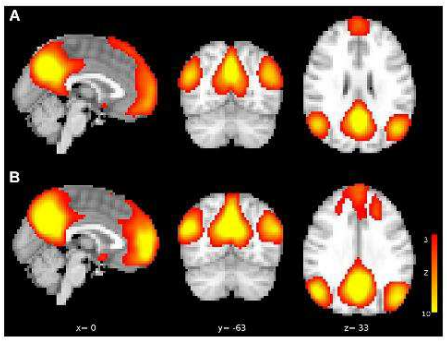

FIGURE 1 | Independent components corresponding to represent DMN from (A) the first sample and (B) the second sample. Both components are arbitrarily thresholded at z = 3 and red-yellow scale indicates greater connectivity to DMN.

Correlations are reported as r (before z-transformations) to facilitate interpretation.

DMN connectivity analysis

Effects of age on DMN connectivity: Firstly, we studied whether mPFC-PCU DMN connectivity was reduced in elders. Group (young vs. old) comparisons between mPFC-PCU Z-transformed correlations were performed with the sample 2. For the sake of completeness, correlations between other DMN nodes were also performed.

Table 2 | Sociodemographic characteristics and comorbidities factors.

Sociodemographics and comorbidities factors			
	Distribution	**Range**	**t/r(p)**
Participants	116		
Gender (male:female)	37:79	–	−1.316(0.19)
Handedness	111:0:5	–	−0.637(0.53)
Age range	68.25 (3.05)	63–76	−0.041(0.67)
Years of School	11.09 (4.15)	2–21	−0.018(0.86)
Hypertension	61:55	–	0.225(0.82)
Diastolic pressure	76.11 (9.71)	51–103	−0.017(0.85)
Systolic pressure	123.74 (17.34)	74–170	−0.023(0.81)
Dyslipemia	61:55	–	−1.549(0.12)
Total cholesterol	209.95 (35.62)	118–381	−0.150(0.11)
Diabetes	111:5	–	−0.629(0.629)

In the right side of the table the relationship of these variables with mPFC-PCU DMN connectivity measures is evaluated. Gender: male:female. Handedness: Right:Left:Ambidextrous. Hypertension: Yes:No; Systolic/diastolic pressure ≥ 140/90 mmHg or antihypertensive medication. Dyslipemia: Yes:No; At least one of: (a) LDL-cholesterol ≥ 160 mg/dl, (b) HDL-cholesterol ≤ 40 mg/dl in men ≤ 50 mg/dl in women, (c) triglycerides > 150. Diabetes Yes:No; At least one of: (1) Current treatment with insulin or oral hypoglycemic drugs, (2) Fasting blood glucose > 127 mg/dl, (3) Glycated hemoglobin > 6.5%.

Influence of DMN connectivity on cognition: To evaluate the influence of mPFC-PCU connectivity to cognition we performed a multivariate GLM-based analysis in the main sample, where connectivity, age, YoE and gender were introduced in a model predicting the four cognitive factors.

Relation of CTh, VBM, FA, MD, CBF and CBF-GM maps to DMN connectivity: To assess the relationship of structural and CBF correlates with anterior-posterior DMN connectivity, multiple voxelwise nonparametric analyses were performed. Nonparametric testing with 5000 permutations as implemented in a randomized FSL tool was used for VBM, MD, FA, CBF and CBF-GM measures followed by threshold-free cluster enhancement (TFCE) and familywise error (FWE) multiple comparison correction ($p < 0.05$). Relationships of CTh and GWC with connectivity were studied using linear modeling as implemented in FreeSurfer. After thresholding results at $p < 0.01$, FWE correction for multiple comparisons using a Monte Carlo Null-Z simulation (10.000 iterations; $p < 0.05$) was performed.

Correspondence of mPFC-PCU DMN correlates and patterns of age-related decline

To gain further insight into the structural correlates of mPFC-PCU DMN connectivity, we studied whether they overlapped areas of high age-related vulnerability. Consequently, these analyses were only carried out in the modalities in which significant mPFC-PCU connectivity correlations were found (MD, GWC and VBM). To do so, we extracted age-related patterns from the *sample 2* (group comparisons) and compared age-related patterns in the brain with those limited by the structural correlates of mPFC-PCU connectivity and those limited to DMN areas (and the cingulum bundle). First, we extracted maps of differences between old and young subjects using GLM-based analysis with group and gender as regressors. Further, differences in the age-related effects on whole brain, connectivity correlates and DMN areas were compared using the raw statistics obtained in the group comparison (thus age-effects) and effect size calculations were performed (Cohen's D; Cohen, 1988). It is important to note that these tests were not inferential in nature but descriptive. Default Mode Network (mPFC-PCU) areas for the different modalities were defined as follows: for MD, the mask was defined as the conjunction of JHU white-matter tractography atlas cingulum bundle (Hua et al., 2008) and the mean sample skeleton; for VBM and GWC modalities, the DMN mask was defined as those voxels belonging to mPFC and posterior midline structure DMN areas (from the study specific DMN; $z > 4$; see **Figure 2**). These areas have been previously reported to be regions of high age-related vulnerability.

Motion Correction Analysis

As motion correction may affect fMRI correlations (Power et al., 2012; Van Dijk et al., 2012), mean relative translation and mean relative rotation parameters were calculated for every subject. Any subject with outlier values relative movement (>0.60 mm/degrees) was excluded from the analysis. Additionally, all significant results were re-run using these measures as covariates.

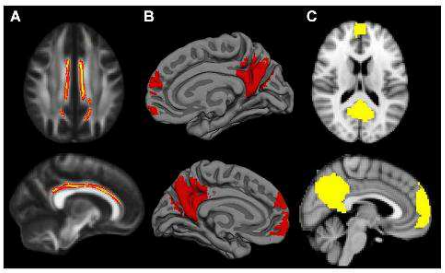

FIGURE 2 | Default Mode Network masks for the different neuroimaging modalities: (A) cingulum bundle for MD modality; (B) mPFC and posterior midline areas for GWC; (C) mPFC and posterior midline areas for VBM. When necessary masks were resampled to match templated characteristics. Cingulum mask is inflated for visual purposes.

RESULTS

NEUROPSYCHOLOGICAL PERFORMANCE

Neuropsychological performance, comorbidity factors and sociodemographic characteristics of the main sample are detailed in **Tables 2**, **3**.

mPFC-PCU DMN CONNECTIVITY IS DECREASED IN AGED SUBJECTS

First, in the between-group comparison (*sample 2*), we found reduced DMN connectivity in ageing, specifically between mPFC and PCU areas in which connectivity was clearly lower in the old group 0.17 (0.27) compared with the young group 0.46 (0.21). Inferential comparisons yielded significant differences in mPFC-PCU coupling strength ($F = 17.858$, $p < 0.001$) as a function of age. Complementarily mPFC-MTG ($F = 12.380$, $p = 0.001$) and mPFC-IPL ($F = 7.558$, $p = 0.008$) couplings also differed in both groups. PCU-MTG ($F = 4.63$, $p = 0.037$) and IPL-MTG ($F = 4.493$, $p = 0.039$) also showed significant age-related difference though they did not survive Bonferroni correction ($p < 0.05/6 = 0.0083$). No age-related differences were observed between IPL and PCU ($F = 0.366$, $p = 0.548$) ROIs (**Figure 3**).

INFLUENCE OF mPFC-PCU CONNECTIVITY ON COGNITION

mPFC-PCU coupling was able to predict cognitive performance as it was significantly associated with memory factor ($F = 9.047$, $p = 0.003$; **Figure 5**), and with speed processing domain ($F = 3.165$, $p = 0.023$), though this last comparison did not survive Bonferroni corrections ($p < 0.05/4 = 0.0125$). The working memory and the inhibitory factor were unrelated to connectivity. Years of education was associated with all cognitive factors ($F > 13$, $p < 0.001$) while age was only significantly associated with the speed processing factor ($F = 6.482$, $p = 0.012$); this was expected, given the small standard deviation in this variable.

RELATIONSHIP BETWEEN STRUCTURAL AND PERFUSION INDICES AND ANTERO-POSTERIOR DMN CONNECTIVITY

Voxel-Based Morphometry: Regional GM intensity was positively correlated with mPFC-PCU connectivity in several areas. Most

Table 3 | Neuropsychological measures for sample 1.

Neuropsychological measures (n = 116)			
Memory component		**Other tests**	
ROCF 3′	19.68 (6.37)	MMSE	29.22 (1.13)
ROCF 30′	19.49 (6.23)	TAP[a]	23.04 (5.83)
RAVLT total learning	48.35 (7.40)	CPT detectability	47.10 (10.49)
RAVL delayed recall	10.37 (2.49)	CPT omissions	59.60 (25.92)
Working Memory		Forward digits	8.80 (2.43)
d′ prime n-back (2 & 3-back)	2.12 (0.60)	Phonetic fluency (FAS)	33.97 (10.45)
Backward digits	6.40 (2.37)	TMT B[b]	104.5 (60.05)
TMTB-TMTA[b]	63.52 (45.76)	BNT[c]	54.21 (3.75)
Speed Processing		Semantic fluency (animals)	20.84 (5.48)
TMTA[b]	41.34 (18.30)	Incomplete letters	19.65 (0.56)
SDMT	39.62 (12.54)	Number location	9.13 (0.88)
CPT RT	60.58 (11.34)	Block design	30.92 (12.48)
Inhibition		FCR copy	34.33 (2.80)
CPT Commissions	46.64 (8.41)		
Stroop interference	10.84 (2.30)		

[a] One-subject data is missing. [b] Two-subjects data is missing. [c] BNT scores with semantic clues.

A

		PCU	mPFC	IPL	MTG
PCU	Yng	1	**.46(.21)****	.62(.13)	**.37(.23)□**
	Old	1	**.17(.27)**	.64(.13)	**.24(.19)**
mPFC	Yng		1	**.40(.24) ****	**.42(.23)***
	Old		1	**.17(.34)**	**.19(.23)**
IPL	Yng			1	**.46(.22)□**
	Old			1	**.32(.23)**
MTG	Yng				1
	Old				1

B

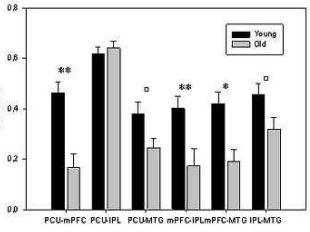

FIGURE 3 | (A) Mean correlations between DMN ROIs. **(B)** Bar charts displaying mean connectivity between DMN ROIs in both old and young groups. ** = $p <$ 0.01 and * = $p <$ 0.05 after Bonferroni correction; □ = $p <$ 0.05 before Bonferroni correction but no longer significant when multiple comparison were applied.

of the significant clusters were located in posterior areas of the brain. Regional GM correlations were found in the PCU, extending to the lateral occipital cortex and superior parietal lobe. Another cluster was found encompassing the right supramarginal gyrus. Other areas related to mPFC-PCU connectivity were found in temporo-occipital and temporo-parietal junctions and in the cerebellum (see **Figures 4**, **5**, **Table 3**).

Cortical Thickness: No relationship was found between DMN connectivity and cortical thickness. However, when the threshold was less stringent ($p < 0.05, p < 0.05$ FWE corrected) correlations were found between mPFC-PCU connectivity and CTh in bilateral parietal and right superior frontal areas.

Gray-White Matter contrast: GWC correlated with mPFC-PCU coupling in a widely distributed set of regions including frontal, temporal and parietal lobes bilaterally. In the left hemisphere three clusters were found: one encompassing the middle and inferior temporal gyrus; another extending towards the PCU, isthmus and posterior cingulate; and a third cluster covered lateral prefrontal areas. In the right hemisphere, a large cluster was found covering medial and lateral parietal lobes, the lateral temporal lobe and the prefrontal cortex with peaks of significance located in pars opercularis, supramarginal and PCU regions (see **Figures 4**, **5**, **Table 3**).

Mean diffusivity: Mean diffusivity was negatively related to mPFC-PCU connectivity thus revealing a correlation between mPFC-PCU functional connectivity and increased WM integrity. Significant clusters were found bilaterally, mainly in anterior areas of the brain and in long-range antero-posterior WM tracts. Anterior thalamic radiation, cingulate bundle, inferior fronto-occipital fascicle, uncinated fasciculus, superior longitudinal fasciculus and forceps minor were bilaterally correlated to mPFC-PCU connectivity (see **Figures 4**, **5**, **Table 3**).

Fractional Anisotropy: In contrast to MD, no relationship was found between FA and mPFC-PCU coupling measures after multiple comparison corrections. Relaxed thresholds ($p < 0.01$) did not show relationship with mPFC-PCU DMN connectivity.

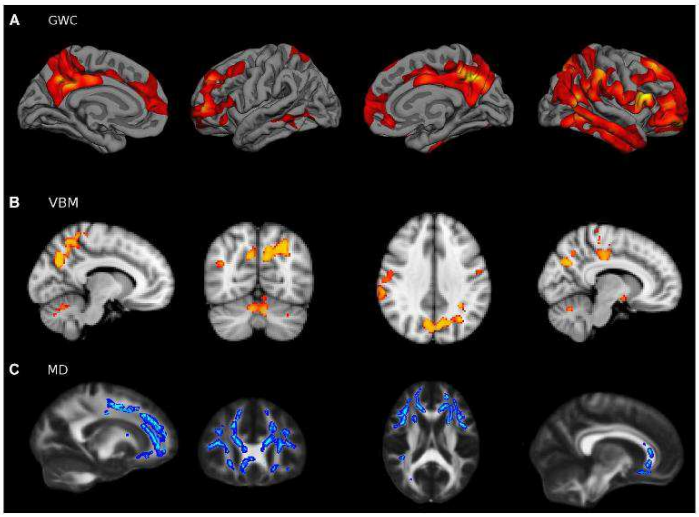

FIGURE 4 | Relationship ($p < 0.05$; FWE corrected) between mPFC-PCU DMN connectivity and (A) GWC (B) VBM and (C) MD. Positive relationships are shown on a Red-Yellow scale and negative relationship on a blue-lightblue scale. Both VBM and CTh showed a positive correlation with DMN connectivity, thus indicating preserved GM integrity with higher connectivity values. Mean Diffusivity showed a negative relationship with mPFC-PCU connectivity thus reflecting preserved MD integrity. For visual purposes, voxel significance is depicted in clusters that survived multiple comparisons. Gray-white matter contrast image is displayed by lateral and medial views of an inflated brain. Voxel-based morphometry slices are shown at $x = -10,10$; $y = -66$ and $z = 34$ and Mean Diffusivity slices are obtained in $x = -18,20$; $y = 28$, $z = 18$.

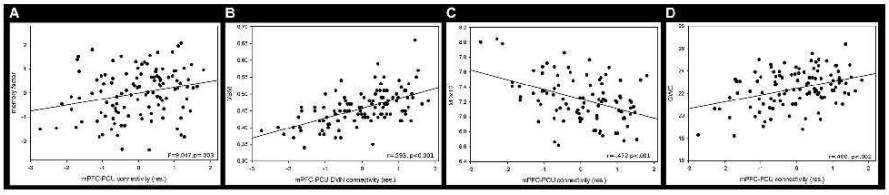

FIGURE 5 | Mean correlation between mPFC-PCU DMN connectivity and (A) memory factor, (B) VBM, (C) MD and (D) GWC. Neuroimaging measures were obtained by calculating the mean value of the largest significant cluster (see **Table 3**, **Figure 4**).

Cerebral Blood Flow: No relationship was found between perfusion fMRI measures and DMN connectivity. Neither uncorrected ($p < 0.01$) nor ROI analyses revealed any consistent differences.

Gray Matter Cerebral Blood Flow: When corrected for PVE estimates, CBF-GM estimates did not correlate with connectivity measures. No significant results were found at an uncorrected level ($p < 0.01$) or with ROI analyses.

Summarizing, both GM and WM measures were related to mPFC-PCU DMN connectivity. The topological pattern was not limited either to DMN nodes or to the main tract connecting DMN structures but was distributed across the brain in areas typically characterized as highly affected by ageing. Cerebral blood flow measures were unrelated to antero-posterior DMN connectivity while GWC, a measure of GM/WM blurring, was found to be related to connectivity in distributed brain areas. To gain further insight into these results, mPFC-PCU structural correlates were compared to the age-related patterns of decline extracted with the second sample.

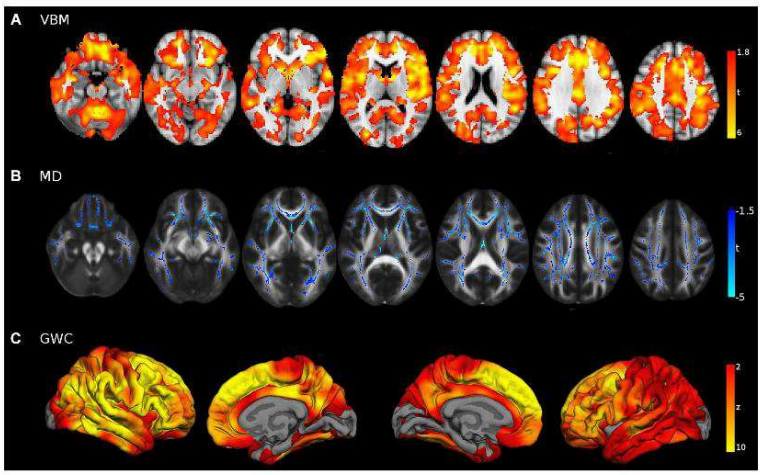

FIGURE 6 | Young vs. Old group comparisons (FWE corrected $p < 0.05$) in (A) VBM, (B) MD and (C) GWC neuroimaging modalities, performed with sample 2. For visual purposes voxel significance is overlaid in those areas that survived multiple corrections. Bilateral medial and lateral surfaces are displayed in GWC while axial slices ($z = [-20:40:10]$) are displayed both for VBM and MD results.

AGE DIFFERENCES IN STRUCTURAL MODALITIES

Group comparisons with sample 2 yielded evident age-related effects in wide brain areas (**Figure 6**). Voxel-Based Morphometry showed a widespread pattern of decreased GM intensity in the ageing group with the exception of some regions in the occipital lobe. Areas with greatest group differences were found in the cingulate, inferior parietal and prefrontal cortices. Increased MD was also found in widespread areas of the WM skeleton in older adults, with the exception of some corticospinal tract regions which remained relatively unaffected. Maximum effects of age were identified in forceps minor, cingulum, uncinate and inferior fronto-occipital tracts. Finally, the GWC age-related pattern was strikingly significant, showing age differences in the entire cortical mantle except in primary visual areas. The greatest vulnerability to age was found in the superior and medial frontal cortices. However, lateral PFC and posterior midline structures were also highly affected, in a bilateral fashion. Overall, age-related patterns of decline showed great similitude to those reported in the literature.

CORRESPONDENCE BETWEEN mPFC-PCU CONNECTIVITY CORRELATES AND YOUNG VS. OLD DIFFERENCES

Structural correlates of mPFC-PCU within the primary old sample were not limited to the DMN (i.e., the underlying cortical region) or to the main tract connecting them, but extended beyond those nodes and were distributed along the cortical mantle. Interestingly, this distribution resembled the impact of age on the brain in each specific neuroimaging modality. To quantify this correspondence, mean effects of age were computed for areas related to mPFC-PCU connectivity and compared to both the overall ageing pattern and the effects of ageing in DMN areas through effect size tests.

Unequivocally, voxels that exhibited a relationship with mPFC-PCU DMN connectivity also tended to be more susceptible to the effects of age. Effect sizes were medium for VBM and right hemisphere GWC and high for MD and left hemisphere GWC according to criteria described elsewhere (Cohen, 1988). That is, for the different structural modalities, areas significantly related to mPFC-PCU DMN connectivity presented above average susceptibility to the effects of age compared to the overall pattern in their respective modality (**Figure 7**).

In contrast, DMN areas did not show increased susceptibility (compared to whole brain) to age either in VBM, MD or right hemisphere GWC. In left hemisphere GWC, DMN voxels presented small effects size. Finally, effect size analyses comparing age-vulnerability of DMN areas with areas related to mPFC-PCU connectivity showed, in all the modalities, an increased susceptibility to age effects in the latter regions (which showed medium effect size; **Table 4**). That is, structural correlates of mPFC-PCU DMN connectivity were located in areas that were more susceptible to age than DMN areas. Significantly, a proportion of voxels belonged to both areas (DMN areas and mPFC-PCU connectivity correlates, though the exclusion of these voxels did not alter effect sizes analyses). These analyses, though only descriptive, suggest that areas related to mPFC-PCU DMN connectivity are located in areas of increased vulnerability to ageing effects.

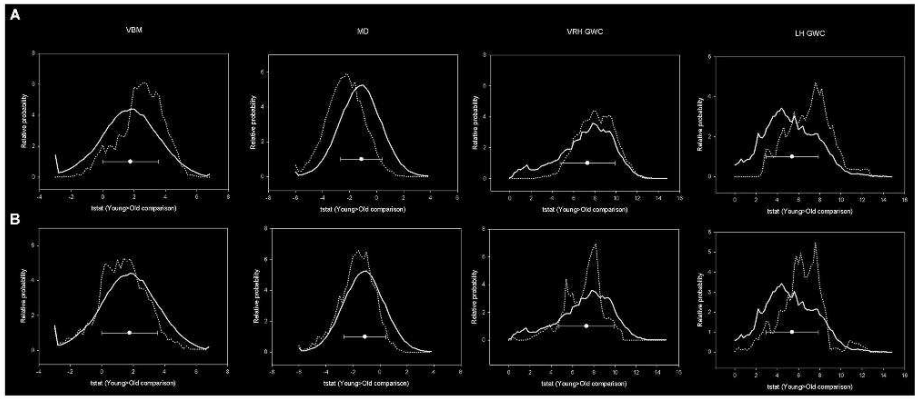

FIGURE 7 | Histograms showing distribution of age-related effects across predefined masks. In the upper row, the continuous line represents the distribution of the age-effect across all brain voxels while dotted line represents the voxels included within mPFC-PCU correlates mask. In the lower images the dotted line represents the age-related distribution within predefined DMN areas. Bins have 0.2 width while the Y axis has been scaled to represent a relative distribution. Circles represent the means and error bars the standard deviations of age-related effects of all brain voxels.

Table 4 | Clusters (>100 voxels) showing relationship between GWC MD and VBM brain regions and mPFC-PCU connectivity measures.

Modality	*Size*	Max	X	Y	Z	Area
GWC	35741	<0.001	48.5	−27.7	38.0	Right supramarginal
	7370	<0.001	−32.8	49.8	−0.8	Left rostral middle frontal
	5239	<0.001	−7.0	−41.5	30.6	Left isthmus cingulate
	866	0.008	−50.2	−60.7	1.1	Left inferior temporal
MD	4139	0.025	−26	29	16	Forceps minor
	3710	0.025	36	39	−2	Right inferior fronto-occipital fasciculus
	2769	0.037	19	44	21	Forceps minor
	215	0.047	−18	−3	45	Left superior longitudinal fasciculus
	158	0.045	−11	24	50	Left uncinate fasciculus
VBM	2998	0.007	−28	−38	54	Left superior parietal lobule
	1149	0.007	−38	−52	−28	Cerebellum
	302	0.017	60	−2	20	Right precentral cortex
	293	0.016	62	−32	30	Right supramarginal gyrus
	226	0.010	−50	−6	24	Left precentral gyrus
	221	0.016	14	2	−10	Pallidum
	198	0.019	68	−18	10	Right superior temporal gyrus
	125	0.035	20	−38	56	Right precentral gyrus

See also ***Figure 4***.

MOTION CORRECTION

No outliers were found. Mean relative movement was negatively associated with mPFC-PCU connectivity in both samples ($r = -0.26$, $p = 0.006$; $r = -0.39$, $p = 0.004$). However, no significant changes were found in the tests after this variable was added in the statistical models. The relationship of mPFC-PCU with memory and with age (group comparisons) remained significant ($p < 0.05$), while the relationship of mPFC-PCU connectivity with anatomical correlates was still highly significant ($p < 0.001$) in all significant clusters. While motion correction impacts connectivity, it does not significantly alter our results.

DISCUSSION

Main results can be summarized as follows: (1) mPFC-PCU DMN connectivity is highly reduced in ageing; (2) in ageing, this coupling is related to cognition, specifically to memory performance; (3) mPFC-PCU DMN connectivity is related to measures of GM and WM integrity in several brain areas not restricted to DMN; (4) perfusion measures are unrelated to DMN connectivity; (5)

Table 5 | Mean effect of age and effect sizes comparisons between brain mask, mPFC-PCU correlates mask and DMN mask.

Measure	Whole-brain age-effect	mPFC-PCU correlates age-effect	DMN age-effect	Brain vs. connectivity effect size	Brain vs. DMN effect size	Connectivity vs. DMN effect size
VBM	1.77 (1.89)	2.51 (1.42)	1.37 (1.65)	0.39	(−)0.21	0.73
MD	−1.06 (1.53)	−2.22 (1.36)	−1.42 (1.21)	(−)0.76	(−)0.24	(−)0.60
LH-GWC	5.38 (2.52)	7.32 (2.10)	6.49 (2.15)	0.92	0.44	0.39
RH-GWC	7.28 (2.52)	8.30 (1.73)	7.17 (1.68)	0.40	0.04	0.65

Age-effects are assessed through t/z statistics and effect sizes are evaluated with Cohen's d'. Greater age-effects represent greater differences between young and old groups.

mPFC-PCU DMN structural correlates appear to be located in areas of high age vulnerability.

DMN CONNECTIVITY IS REDUCED IN AGEING

Firstly, this study adds new evidence of reduced DMN connectivity in ageing assessed by rs-fMRI (Ferreira and Busatto, 2013). Reduced connectivity of mPFC node from temporal and posterior parts of DMN in aged subjects was evident even with a relatively low number of subjects (in *sample 2*). Group comparisons suggested that the most significant effect of age was present in the coupling between mPFC and posterior midline structures, in agreement with other studies (Andrews-Hanna et al., 2007; Tomasi and Volkow, 2012; Campbell et al., 2013; Mevel et al., 2013). These areas form the nucleus of DMN which is composed by other several subsystems (Laird et al., 2009) involving temporal, superior frontal and lateral parietal cortices. These results also suggest that decreases in mPFC-PCU connectivity are probably not associated with compensatory mechanisms; rather, this disconnection is likely to be a reflection of brain ageing and inefficient processing though the exact causes of age-related functional connectivity abnormalities in healthy elders are mostly unknown.

REDUCED mPFC-PCU DMN CONNECTIVITY RELATES TO WORSE MEMORY FUNCTION

We add further evidence highlighting the relevance of DMN resting-state connectivity over cognition in ageing. Specifically, we found a relationship between antero-posterior connectivity and a memory factor composed by visual and verbal memory scores. Significantly, in the analysis, age was used as covariate; that is, elders display a relationship between memory and mPFC-PCU coupling despite the known effect of biological age on both measures. Several other studies have investigated the effect of DMN connectivity measures and cognition in different populations, including healthy elders (Hafkemeijer et al., 2012; Ferreira and Busatto, 2013). While speed of processing (Andrews-Hanna et al., 2007) or executive (Damoiseaux et al., 2008) scores have been related to decreased DMN integrity in ageing, it is the memory domain that has been most consistently associated with DMN connectivity (Andrews-Hanna et al., 2007; Wang et al., 2010; He et al., 2012). A recent study by our group in an independent sample also found a relationship between functional DMN connectivity measures and memory using an independent sample of middle-aged and aged subjects and graph-based analysis (Sala-Llonch et al., 2014). Regional cluster coefficients were the main measure used in that study, a measure of segregation which reflects the prevalence of clustered connectivity around individual nodes. Regional cluster coefficients were significantly increased with age in numerous cortical regions, including main DMN nodes, which were negatively related to visual and verbal memory scores. This relationship persisted in posterior midline areas after regressing the effects of age, while, in addition, greater age-related connectivity changes were found between parietal and frontal areas (which include DMN and dorsal attentional network intra-connections). Despite their major methodological differences, both studies support the hypothesis that DMN rs-fMRI integrity is a key element for preserved memory encoding in ageing. In addition, the observation of decreased DMN connectivity and the pattern of decline in episodic memory on a population basis broadly coincide over the lifespan.

The specificity of DMN connectivity regarding memory performance remains to be fully elucidated though it may be related to the fact that medial temporal lobes, essential for memory processes, are often considered part of the DMN. Default Mode Network core nodes have also been strongly linked to prospective and autobiographical memory (Spreng and Grady, 2010). During rest, medial temporal lobe structures tend to be coupled with main DMN nodes; however, during episodic encoding these structures are highly activated and are disconnected respect to other DMN nodes, which are deactivated. These processes are crucial to correctly encode stimuli. Deficient coupling at rest might reflect inefficient allocation of neural resources necessary for correct performance during episodic memory tasks (Stevens et al., 2008). Surprisingly, though, most studies relating DMN with cognition highlights the role of parieto-frontal interactions (Andrews-Hanna et al., 2007; He et al., 2012; Sala-Llonch et al., 2014), and the medial temporal lobe DMN subsystem does not seem highly affected by ageing (Campbell et al., 2013), suggesting a more complex explanation linking decreases in memory and DMN connectivity. An alternative hypothesis would be to consider the antero-posterior DMN disconnection as an unspecific measure of ageing, which is altered at an early stages, probably related to the centrality of these areas regarding the connectome (de Pasquale et al., 2012), and memory as a process highly vulnerable both to age-related disruption and to cortico-cortical and cortico-limbic dysfunctions.

mPFC-PCU CONNECTIVITY IS RELATED TO WM AND GM INTEGRITY INDICES AND NOT TO CEREBRAL BLOOD FLOW

WM microstructure: Relationships between WM structure and DMN connectivity were evident for MD in frontal areas and in long range anterior-posterior connections which may be more sensitive to ageing effects than FA measure. In addition, GWC, thought to partially reflect WM atrophy near the GM boundary, was related to DMN connectivity.

The notion that structural networks represent the physical substrate of functional connectivity patterns in the human brain has received considerable attention over the last few years (Honey et al., 2009; van den Heuvel and Sporns, 2013). This literature suggests that the structural connectome plays a key role in the neural synchronization patterns in the human brain. However, spontaneous neural activity is not limited by the underlying anatomy, as polysynaptic connections also contribute to functional connectivity patterns (Honey et al., 2009). Several studies (Skudlarski et al., 2008; Honey et al., 2009; Horn et al., 2013) have studied brain structural-functional connectivity relationships and have demonstrated that several brain regions show similitudes between functional and structural connectivity patterns, especially those belonging to DMN areas. Regarding DMN areas, it is well established that the cingulum tract supports DMN connectivity from posterior to temporal and frontal areas (Greicius et al., 2009). Accordingly, several groups (Andrews-Hanna et al., 2007; van den Heuvel et al., 2008; Teipel et al., 2010; Khalsa et al., 2013) have reported associations between cingulum integrity and DMN functional connectivity. In particular, Andrews-Hanna (2007) reported a correlation between WM integrity (including the cingulum and adjacent areas) and mPFC-PCU functional connectivity in elders. Complementarily, Teipel (2010) studied the relationship between WM microstructure and posterior midline-hippocampus connectivity in aged subjects, finding that large areas of WM including the cingulum bundle were associated with DMN functional integrity. These findings have generally been discussed in terms of structural connectivity supporting functional connectivity. Nevertheless in these previous studies cingulum-DMN connectivity correlations were not especially strong after adjusting for age ($r \approx 0.2$–0.3) and, to some extent, are comparable to those found in our study (cingulum MD—mPFC-PCU connectivity: $r = 0.20$, $p = 0.060$, data not shown). While age-related mechanisms affecting structural and functional connectivity might be partially related, the results also suggest that other mechanisms are involved in the maintenance of resting-state DMN connectivity besides those assessed with diffusion-based imaging. In development literature the link between functional and structural anteroposterior DMN connectivity seems to emerge in the late childhood ($\approx$10 years; Supekar et al., 2010; Gordon et al., 2011). Supekar and colleagues found a DMN-like RSN in children (7–9 years) and observe an immature PCC-mPFC coupling that was unrelated to a diffusivity measure of the cingulum bundle. However, young adults displayed functional-structural connectivity relationships which lead to the suggestion that the maturation of mPFC-PCC functional connectivity depends on the maturation of WM tracts within the cingulum bundle. The emergence of DMN during development is still discussed (Power et al., 2010) but several studies suggest that late childhood is a critical period for its development. A moderating effect of age in the relationship between WM microstructure and functional communication it is probable being present in critical lifespan periods (Andrews-Hanna et al., 2007; Supekar et al., 2010) such as during development or in late adulthood where increased WM insults might critically evidence the structural-functional relationship within the DMN.

We found that DTI correlates of mPFC-PCU connectivity were not limited to the cingulum bundle, which was only related to function in its anterior portions, but extended to other WM regions associated with long-range antero-posterior tracts and frontal interhemispheric tracts. These tracts are highly affected by age (Westlye et al., 2010; Sala et al., 2012) and have been linked to cognitive processes (Bennett and Madden, 2013). In a group of young subjects Luo et al. (2012) studied structural networks associated with DMN by examining regions that covariated with those usually reported to belong to this network. Our results resemble to a great degree the structural WM networks associated with covariance networks reported in that study. Those authors suggested that long association fibers connected the GM regions that made up the DMN, suggesting a role for these fibers in forming the skeleton of the structural network underlying the DMN. Similarly, Teipel (2010) found that the WM microstructure correlating with DMN functional connectivity extended to larger areas beyond the cingulum tract. As functional connectivity may be maintained in the absence of direct structural connection, a role for long-range antero-posterior fibers partially contributing to the maintenance of DMN connectivity in ageing is plausible. However none of these studies allow inferences of causality, so caution is required when discussing these relationships. Another possibility is that mPFC-PCU connectivity and WM microstructure are not strictly related by a direct structure-function relationship but may also be linked by an indirect relationship in which structural and functional connectivity are mediated by similar age-related vulnerability mechanisms.

Gray/white matter contrast: The rationale for including GWC contrast in our study was the strong impact of age on this measure (Magnaldi et al., 1993; Salat et al., 2009; Westlye et al., 2009) and the fact that it partially reflects WM integrity close to the brain surface. Westlye et al. (2009) suggested that, during ageing, this measure was more affected by WM values than by GM values while several authors stress that this measure reflects reduced density of myelin sheaths (Cho et al., 1997) and increased water content in WM (Magnaldi et al., 1993). It also seems that the age-related impact on WM is greater in peripheral, thinly myelinated WM (Tang et al., 1997; Bartzokis et al., 2004). This interpretation is coherent with our results, as GWC correlates of mPFC-PCU DMN largely overlapped the DMN network. Consequently, WM near GM boundaries may be closely associated with the maintenance of the synchronous function among neural networks, upon which optimal cognition would depend. These novel results are highly interesting, as might better reflect WM ageing changes, and further studies should seek to replicate them and expand them to other networks and samples. However, caution is in order, as no clear interpretation of the GWC is available as yet.

Gray matter: Since fMRI is generally interpreted as an indirect measure of neuronal activity, GM intensity may have a significant impact on patterns of fMRI activity (Kalpouzos et al., 2012). However, age-related decreases in DMN connectivity have been found to persist after correction for GM volume (Damoiseaux et al., 2008), thus showing that decreased resting-state activity in ageing cannot be attributed to local GM atrophy alone. Nonetheless, these results do not imply an absence of a relationship between GM and connectivity measures, as this issue has not been explicitly assessed.

Analyses of GM covariance, which allow the study of structural networks, have reported that structural GM networks mimic main RSN networks, including DMN (Supekar et al., 2010; Segall et al., 2012). Structural covariance topological patterns are thought to change during the lifespan. Specifically in the DMN, elders show a reduced pattern of GM covariance limited to posterior areas in comparison to young subjects in whom it extends to mPFC areas (Chen et al., 2011; Li et al., 2013) which is in agreement with functional connectivity studies showing deficits in antero-posterior DMN couplings. Our results showing correlations between mPFC-PCU connectivity and GM integrity limited to posterior brain areas can be explained by changes in the pattern of GM covariance with age. Posterior midline GM atrophy has usually been related to pathological ageing (Buckner et al., 2009); however, a decline in this structure is prominent in healthy aged subjects with very-low risk of dementia (Fjell et al., 2009b). Additionally, the results suggest that age-related decreases in DMN connectivity are mediated by posterior structures. This interpretation is in agreement with the assumption that the DMN primary core is located in posterior midline regions. It is plausible that the declining GM trajectories of DMN nodes become disengaged as the brain ages and the antero-posterior disconnection arises. Thus, mPFC atrophy may be unrelated to posterior midline atrophy due to both functional and structural disconnection.

Cerebral blood flow: In the present report, functional connectivity and CBF indices were unrelated even at uncorrected level. To our knowledge, no directly comparable studies have been published. BOLD signal changes, though, are not independent from CBF, as the ratio of oxygenated and deoxygenated hemoglobin is primarily affected by an influx of oxygenated blood in response to the increased metabolic demands of neuronal activation. Indeed, relationships with static indices of CBF and BOLD signal (Davis et al., 1998) as well as with dynamic indices have been observed (Tak et al., 2014). Evidence linking regional CBF with connectivity is limited; however, in relation to the DMN, two studies with healthy young subjects should be mentioned (Liang et al., 2013; Khalili-Mahani et al., 2014). Liang (2013) linked CBF and functional connectivity strength which was stronger in DMN areas; this relationship was further discussed in terms of larger energy demands to maintain long-range connections. Khalili-Mahani et al. (2014) also found a relationship between CBF measures and DMN strength. However, that study also found that alterations in BOLD signal and rCBF differ considerably after pharmacological intervention, suggesting that these measures may provide complementary information about the dynamics of the brain's hemodynamic responses. Studying left inferior frontal junction in ageing, Chételat et al. (2013) did not find an association between connectivity and regional metabolism, to which CBF is closely related. Similarly, our results showed that, although both CBF and DMN connectivity are known to decline in ageing, the patterns may be unrelated.

TOPOLOGY OF STRUCTURAL mPFC-PCU CONNECTIVITY CORRELATES CORRESPONDS TO AREAS OF HIGH-AGE RELATED VULNERABILITY

In our study, structural correlates of mPFC-PCU DMN connectivity extended to several brain areas which were highly vulnerable to ageing effects. Importantly, mPFC-PCU connectivity correlates showed greater age-related vulnerability than DMN areas or the cingulum bundle, even though these areas have been reported to be highly vulnerable with advanced age (GM: Fjell et al., 2014a; WM: Westlye et al., 2010; Sala et al., 2012; GWC: Salat et al., 2009; Westlye et al., 2009). While these results are only descriptive and should be interpreted with caution, they highlight the value of the single measure of mPFC-PCU DMN connectivity as a metric closely related to brain ageing by linking it to structural hallmarks of lifespan brain changes.

It is possible that DMN functional connectivity alterations in elders are greater than WM microstructure and GM atrophy changes within the DMN, at least until a certain age. In this regard, some studies demonstrate clear alterations in DMN connectivity in middle-aged subjects (Bluhm et al., 2008; Biswal et al., 2010; Evers et al., 2012). In addition, although the evidence is not conclusive, it appears that GM levels in several DMN areas, especially medial temporal structures but also posterior and anterior DMN nodes (Thambisetty et al., 2010; Taki et al., 2013; Fjell et al., 2014b) as well as cingulum integrity WM indices (Westlye et al., 2010; Sala et al., 2012) may show an accelerated decline in late adulthood. While there is no doubt that brain activity is partially supported by structure integrity, it is highly feasible that, to some extent at least, functionality is affecting structure in ageing, through Hebbian principles. While plastic changes are easily assessed using rs-fMRI techniques (Albert et al., 2009), it is well known that plastic interventions, such as those produced by cognitive or behavioral demands, are able to impact both WM (Engvig et al., 2012) and GM (Engvig et al., 2010) structure assessed with MRI techniques. The suggestion that regions characterized by a high degree of life-long plasticity are vulnerable to the detrimental effects of normal ageing (and that this age-vulnerability also renders them more susceptible to additional, pathological AD-related changes, Mesulam, 1999; Fjell et al., 2014a) is a provocative hypothesis that is gaining attention and is coherent by our results. The relevance of plasticity has been both considered as an adaptive mechanism and as a factor of risk that may lead to pathology (Oberman and Pascual-Leone, 2013). Default Mode Network areas are among the ones with the highest neuroplasticity in the cerebral cortex, playing a central role in brain functioning as well as being highly involved in learning and memory processes, which implies greater demands of plasticity. This increased demand of activity and/or life-long plasticity mechanisms may thus make this system especially vulnerable during the lifespan (Fjell et al., 2014a). Within this context, and through its association with GM and WM areas showing high age-related changes, dysfunctional DMN

connectivity may represent an early marker of brain ageing, which could emerge from maladaptive plasticity mechanisms during the lifespan.

LIMITATIONS

The study has a number of limitations. First, the results may be partially explained by factors that were not assessed such as a proportion of cases fulfilling preclinical AD criteria by virtue of an altered early biomarker (i.e., high levels of amyloid-β (Aβ) deposition), the presence of the APOE ε4 allele or dopamine depletion. Around 20% of older subjects without cognitive decline have significant Aβ deposition, an early AD biomarker (Chételat et al., 2013a). Amyloid-β has been hypothesized as a potential cause of rs-fMRI abnormalities (Ferreira and Busatto, 2013), such as reductions in DMN connectivity (Hedden et al., 2009), further diminished in AD (Jones et al., 2011), in a normalcy-pathology homology. However, decreases in functional connectivity exist without evidence of Aβ deposition (Andrews-Hanna et al., 2007) as well as it is evident in middle-aged subjects which have a small percentage of Aβ+ subjects. Also, most mPFC-PCU connectivity correlates results were unrelated to areas proposed as AD biomarkers. While it is unlikely that the results can be explained by Aβ deposition, partial contamination of the results cannot be ruled out. Further studies might assess a possible moderator or mediator effect of Aβ deposition in the reported relationships or alternately might use samples of subjects with low probability of dementia. Similarly, dopamine depletion and genetic polymorphisms such as APOE ε4 may influence anteroposterior DMN connectivity (Achard and Bullmore, 2007; Sheline et al., 2010) and further interact with multimodal and cognitive correlations. However, the effect of the dopamine system on the DMN seems weak (Achard and Bullmore, 2007) in contrast to fronto-striatal circuits, while the exact effect and direction of the APOE ε4 allele on DMN connectivity is still debated (Reinvang et al., 2013).

Another potential limitation is the effect of motion over rs-fMRI measures, however, *post hoc* tests did not qualitatively change any of the main results reported here. *Post hoc* testing was used because no standardized procedures exist to deal with this potential confounding. A third limitation refers to the analyses performed to assess age-related decline, since relatively small group comparisons were carried out. Using this methodology instead of cohorts encompassing the entire life-span or longitudinal approaches may have altered age-related patterns. Still, the patterns were topologically similar to those reported previously in the literature and we preferred to exploit the advantages of using a sample acquired in the same scanner machine. Lastly, the exploratory and correlational character of the study limits the explanatory power of the results.

CONCLUSIONS

This study provides valuable new information of DMN connectivity in healthy ageing. The main finding is that mPFC-PCU DMN connectivity is related to GM and WM indices of integrity, not only in DMN areas but also in areas of high age-related vulnerability. This coupling is affected by ageing and is related to cognitive performance in elders. Elucidating the basis of disconnection in ageing is of the utmost importance for understanding healthy brain function and cognition as well as the development of brain pathology.

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3. Study III

Neurochemical modulation in default-mode network nodes induced by transcranial magnetic stimulation

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Abstract

Glutamate and GABA are the major excitatory and inhibitory brain neurotransmitters respectively, and constitute the basic microcircuit in the brain. These neurotransmitters have consistently been related to large-scale network expression, such as the default mode network (DMN). The DMN is deactivated during task-related processes and it is severely compromised in several psychiatric and neurodegenerative disorders. To investigate whether glutamate and GABA could be

modulated within the DMN, we applied individually guided, neuronavigated, patterned repetitive transcranial magnetic stimulation (rTMS) to the left inferior parietal lobe (IPL), in-between two magnetic resonance spectroscopy (MRS) acquisitions from 36 young subjects. After rTMS, modulations in GABA and Glx (glutamate + glutamine measure) were observed in both local and distal DMN nodes. Intermittent theta-burst stimulation increased GABA in the precuneus (PCU), while continuous theta-burst stimulation increased Glx in the left IPL. No changes were detected after sham stimulation. Neurotransmitter modulation in the distal areas (PCU) was found to be correlated with baseline fMRI connectivity between this region and the TMS-targeted area (left IPL). The prediction of neurotransmitter modulation by connectivity highlights the relevance of connectivity patterns to predict brain responses to protocols inducing plasticity-like changes, and encourages the use of connectivity-based methods of rTMS targeting. Finally, the ability to modulate major inhibitory and excitatory neurotransmitters within distal core nodes of the DMN by means of rTMS may open new avenues to interventional studies in major neurodegenerative and psychiatric conditions.

Introduction

In the last two decades, resting-state functional imaging studies have identified the presence of certain components that fluctuate synchronously(1, 2), which are thought to reflect the intrinsic functional architecture of the brain. One of the resting-state networks (RSN), the default mode network (DMN), has been intensively investigated(3). This network is comprised of posterior medial midline structures (precuneus/posterior cingulate cortex [PCU/PCC]), medial prefrontal cortex (mPFC), lateral inferior parietal lobes (IPL) and temporal nodes. The DMN has been shown to be consistently activated during rest, while its nodes exhibit decreased engagement during externally oriented tasks(4). This network is compromised in several neurodegenerative and psychiatric disorders, such as schizophrenia(5, 6), autism(7), Alzheimer's disease(8, 9), and even healthy aging(10). Alterations in the DMN are often reflected by reductions in connectivity between nodes, reduced resting-state metabolism, and a lack of task-induced deactivation.

Glutamatergic and GABAergic cells are the main excitatory and inhibitory constituents of the canonical microcircuit(11), and hence mediate neuronal activity. Glutamate and GABA neurotransmitters can be non-invasively quantified in humans by MR spectroscopy(12), and have consistently been associated with neuroimaging technique estimates(13, 14). Specifically, in the DMN, GABA concentration has been shown to predict task-induced deactivations(15), while glutamate showed an inverse pattern, and hence predicts reduced deactivations(16). Connectivity within DMN nodes and the interconnectivity of this system with other RSN has also been shown to be mediated by GABA and glutamate(14, 17).

In accordance with their fundamental role in mechanisms of neuroplasticity, GABA and glutamate imbalances(18, 19) have been reported in the abovementioned conditions characterized by DMN dysfunction. In this regard, in the Alzheimer's disease (AD) neuropathological pathway, neuroplasticity is altered in association with a glutamate/GABA microcircuit dysfunction, which shows complex interactions with Aβ deposition(20). Therefore, glutamate and GABA modulation within the DMN after the application of plasticity-inducing procedures may offer a unique opportunity to assess the state of excitatory/inhibitory circuitry and its relationship with cognition and macronetwork expression. Ultimately, these measures may also be useful to monitor disease progression or response to treatments.

The use of non-invasive brain stimulation techniques (NIBS), such as repetitive transcranial magnetic stimulation (rTMS), has emerged as an optimal methodological approach in humans to modulate the neurochemical substrate of the DMN nodes in a controlled manner. Patterned protocols of stimulation such as theta-burst stimulation(21) (TBS), can modulate cortical excitability. This produces long-term potentiation (LTP) and long-term depression (LTD)-like phenomena, up to an hour after stimulation cessation. Physiological and pharmacological investigations revealed glutamatergic(22) and GABAergic(23) involvement in TBS-induced plasticity effects. According to the pattern, intermittent TBS (iTBS) enhances cortical excitability, while continuous TBS (cTBS) produces inhibitory post-effects(21). However, NIBS after-effects, both in the underlying stimulated tissue and in distal interconnected areas(24), are highly dependent on a variety of parameters(25). In this sense, functional brain connectivity patterns have emerged as relevant measures to predict behavioral(26) and BOLD(27) rTMS after-effects.

rTMS can directly modulate DMN functionality(28) by targeting an accessible cortical node of this network in the left IPL. So far, it has been shown that stimulating this area produces connectivity changes between DMN nodes assessed by rs-fMRI(29), and alpha rhythm enhancement assessed by electroencephalography(30). In addition, magnetic resonance spectroscopy (MRS) has been proved a sensitive technique to capture NIBS after-effects(31–33), such as revealing increased GABA concentration in the motor cortex following cTBS(23). These neurotransmitter changes after stimulation have been shown to be functionally relevant, as GABA responsiveness to transcranial direct current stimulation was related to greater changes in reaction times and task-related fMRI signal decrements(34).

Although NIBS can modulate neurotransmitter concentration, and these neurotransmitters proved to be relevant for understanding interindividual differences in DMN properties, no study has so far addressed whether GABA or glutamate measures can be modulated by TMS within the DMN. The main objective of this investigation was to study whether a single TMS session would result in the modulation of neurotransmitter concentration in both local and distal DMN nodes (left IPL and PCU), assessed with MRS. To accomplish this objective, we applied individually guided neuronavigated sham, intermittent or continuous TBS stimulation in-between two MRI acquisitions. We hypothesized that active rTMS would induce neurotransmitter changes compared to sham stimulation in a similar fashion in both local and distal areas. We expected GABA increases after cTBS (as ref. 26) and a reversed effect after iTBS. We also hypothesized that the magnitude

of neurotransmitter changes induced by rTMS would be related to the intrinsic connectivity between the stimulated area and the region where neurotransmitters were assessed (Stim-to-PCU connectivity).

Results

Participants completed the experimental protocol detailed in Figure 1. Specifically, subjects underwent TBS stimulation in-between two MRI acquisitions and were assigned to one of three experimental groups that differed in the stimulation protocol: Sham, iTBS or cTBS. TBS targets were defined individually, based on an rs-fMRI dataset acquired before the main day of the experiment. Stimulation trains were delivered over the left IPL area, following a method previously described to identify the peak of maximum connectivity within the default mode network(28, 29) (see SI). On the main day of the experiment, pre- and post-TBS MRI acquisitions included two ^{1}H-MRS sequences whose voxels of interest (VOIs) were placed: 1) 'locally', on the area underlying the TBS stimulation in the left IPL, and 2) 'distally' in the PCU (Figure 2c). Additionally, an rs-fMRI was acquired during the pre-TBS MRI acquisition, to study the relation of neurotransmitter modulation and brain intrinsic dynamics. The main analysis, which assessed TBS effects on metabolite concentrations (Glx and GABA), consisted of repeated-measures analysis of variance (ANOVA) accounting for group (sham*cTBS*iTBS) and time (pre*post). Hence, a total of 4 ANOVAS were performed, one for each spectra location (PCU and left IPL) and neurotransmitter (Glx and GABA). Spectroscopy was acquired with a MEGA-PRESS J-difference editing pulse sequence (Figure 2a,b, and Methods). Glx and GABA quantifications, from difference spectra, were expressed as tNAA ratios and multiplied by the CSF correction factor. For simplicity in the text we refer to such ratios as GABA and Glx.

The descriptive results of neurotransmitter concentration are summarized in Table 1. Left IPL Glx ANOVA showed a significant interaction ($F=4.776$, $p=0.017$), with a higher Glx concentration after cTBS than in the sham group. No main effects of group ($F=2.785$, $p=0.08$) or time ($F=2.117$, $p=.157$) were found. No main or interaction effects for GABA were detected in the left IPL ($p>0.2$). In the PCU, a significant interaction was found in the GABA ANOVA ($F=5.041$, $p=0.013$), with higher GABA concentration after iTBS stimulation than in the other two groups. No main effects were detected (Group:[$F=.216$, $p=.807$], Time: [$F=.246$, $p=.624$]). No main or interaction effects for Glx were detected in the PCU ($p>0.1$; Figure 3, Figure S2). The main results were not dependent on internal

reference or on the application of the CSF partial volume correction, as a complementary analysis in which these variables were changed replicated the main results (SI).

Next, we investigated the covariation between Glx and GABA modulation due to stimulation in every experimental group (iTBS, cTBS and Sham) and location (left IPL and PCU). A correlation between GABA and Glx changes was found in those conditions where TMS produced a significant modulation in a neurotransmitter. Hence, a negative correlation between GABA and Glx changes was found in the left IPL for the cTBS group ($r=-0.73$, $p=0.027$) and a trend towards significance in the PCU was observed in the iTBS group ($r=-0.62$, $p=0.055$) (Figure 4). No other conditions showed a significant effect ($p>0.05$).

Finally, we studied the influence of connectivity patterns on neurochemical changes in distal regions produced by TBS stimulation; as such patterns are increasingly considered a key factor to understand stimulation effects. Consequently, we correlated Glx and GABA changes (post- and pre-TBS ^{1}H-MRS) for each experimental group with rs-fMRI connectivity between stimulation (Stim-ROI) and the PCU VOI (PCU-ROI) in a seed-to-seed approach using the functional sequence acquired during the main day of the experiment, minutes before stimulation. The functional connectivity measure was obtained by correlating preprocessed rs-fMRI time series of both regions of interest (ROIs) in the functional space. ROIs only included GM voxels and were individually defined as 6 mm-radius spherical masks, centered over stimulation coordinates and over the center of gravity of the PCU VOI (see Methods). The distal GABA increase after stimulation was positively predicted by Stim-to-PCU connectivity. In other terms, positive changes in GABA concentration in PCU after iTBS were strongly related to baseline functional connectivity between the stimulated area and the spectroscopic VOI (Figure 5). Additionally, Glx variations for cTBS and iTBS groups were negatively correlated with rs-fMRI connectivity. GABA and Glx changes after sham stimulation were unrelated to rs-fMRI functional connectivity (Table 1). An additional exploratory seed-to-brain connectivity analysis showed relatively high specificity of the Stim-to-PCU connectivity measure as a predictor of distal neurotransmitter change; especially for neurotransmitter changes in the iTBS group (see SI and Figure S3).

To confirm the correct location of spectra VOIs in-between DMN nodes, a control analysis was performed to study the resemblance of VOI connectivity to DMN. Subject-specific voxelwise connectivity maps were obtained by regressing left IPL and PCU ROIs timeseries, which were centered in VOIs center of gravity. After transformation to standard space, individual connectivity

maps were compared to reported large-scale RSN(2), and group connectivity maps were obtained by non-parametrical permutation testing. Both results confirmed that voxels were correctly acquired within DMN (Figure S1). Next, the test-retest reproducibility of neurotransmitter ratios was assessed by the intraclass correlation coefficient (ICC) in the sham group for left IPL (n=10) and PCU (n=11) ^{1}H-MRS acquisitions. Glx reproducibility indices were ICC=0.83 and ICC=0.36, while GABA indices were ICC=0.75 and ICC=0.79 for left IPL and PCU VOIs respectively. All reproducibility coefficients were high(35) (>0.72), except for the PCU Glx quantification. Finally analyses were performed to rule-out the possibility that different location or tissue composition variables of the spectra could explain the main results. A high-resolution 3D image (hr-3D) acquired prior to the main day of the experiment was further segmented into tissue type (Table S1). Co-registration of medium-resolution 3D images (mr-3D), acquired during the main day of the experiment, to the hr-3D images allowed an overlapping percentage and spectra tissue type composition analysis. No differences were found for the GM/(WM+GM) ratio or for overlapping percentages in any statistical test ($p>0.05$). We found an interaction effect ($F=4.105$, $p=0.028$) of CSF percentage in the left IPL, which suggests that CSF values were higher after iTBS. Although it is unlikely that this difference affects previously described results, main analyses were performed with and without CSF partial volume correction. For the detailed control results see SI.

Discussion

The present study provides the first evidence of controlled neurotransmitter modulation within the DMN, by means of plasticity-inducing stimulation protocols. Two main findings were observed: first, rTMS applied to the left IPL induced brain dynamic neurotransmitter changes both locally and distally in a modality-dependent fashion; and second, the magnitude of the induced neurotransmitter modulation in the PCU was strongly associated with the strength of baseline functional connectivity between the TMS-targeted area and this distal region.

After-effects of rTMS on cortical excitability outlast the stimulation period by a few tens of minutes, and are supported by changes in synaptic strength(36). Our observation of changes in the metabolite concentrations during an MRS acquisition performed several minutes after the TBS cessation are consistent with glutamate and GABAergic implication in TBS after-effects(21, 23), as well as with current evidence that NIBS techniques can modify regional metabolite

concentrations(23, 31, 33). Novel observation of the distal modulation of neurotransmitter concentrations is in accordance with established knowledge that shows propagation of rTMS after-effects to distal interconnected areas(24). In our study, the observation that TBS applied to the IPL modulates the neurochemical characteristics of the PCU is likely to be related to the fact that these regions exhibit strong patterns of functional connectivity, possibly mediated by direct structural connections(37).

Previous studies employing TBS in the motor cortex observed a modulation of motor evoked potentials, with iTBS resulting in prolonged increases of excitability and cTBS producing decreases(21). However, TBS modality may affect distinct neuron populations and intracortical circuits(38). Although precise mechanisms of brain responses to rTMS remain to be fully elucidated, when compared to previous literature, the observed direction of spectroscopic changes after both stimulations in our reports seems counterintuitive(23, 32, 33). Hence, in the most comparable study, Stagg and colleagues(23) found GABA increases in M1 following cTBS over this region, while we observed local Glx increases in the locally stimulated area (IPL) after the same type of stimulation. Discrepancies between both findings may be related to the distinct characteristics of the targeted networks. Neuroimaging techniques have revealed DMN idiosyncratic properties, such as deactivation during goal-directed external tasks(4), increased metabolism at rest(39), a high correlation between functional and structural connectivity(37), or that DMN is active in the most stable state of the brain(40). Intracranial recordings(41, 42) further confirmed that DMN has a unique pattern of synaptic activity, which has been postulated as one of the several factors modulating the rTMS brain response(43). Notably, the only pair of studies that stimulated left IPL and explicitly studied rTMS modulation over DMN are compatible with our results(29, 30). Both investigations found increased coupling between DMN nodes after low-frequency TMS, while one of them also found reduced connectivity after high-frequency TMS(29). Our results that reflect increased GABA related to iTBS and increased Glx to cTBS may show some parallelism with these latter findings, as neuroimaging investigations demonstrated associations between higher glutamate-MRS measured concentrations and greater integrity of the DMN, and the opposite pattern for GABA(14, 16). However, it should be noted that discrepancies with previous MRS results can be related to other possibilities such as differential cito- and neurotransmitter architectonics(44, 45), coil orientation with gyral morphometry or a differential modulating role of other neurotransmitters (46).

The feasibility of distally increasing GABA in the PCU may be particularly relevant, considering that this region represents a core connectivity hub of the brain(47). We showed novel evidence that distal neurochemical modulation is predicted, with high specificity, by rs-fMRI connectivity between the stimulation targeted area and the PCU region. Intrinsic functional connectivity has proved to be capable of predicting behavioral rTMS post-effects(26), to interrogate the status of specific networks of interest in distinct conditions(48), and is emerging as an effective strategy to guide stimulation(28, 29). Increased baseline connectivity between the TMS target, the PCU site and the reported GABA concentration changes in response to stimulation may suggest direct propagation of stimulation effects. However, it is likely that network dynamics and their responses to stimulation interact in a more complex fashion, reflecting a multiplicity of changes that occur in the cortex, beyond those associated with GABA and glutamate-MRS. Changes may also be understood through adaptive compensatory processes that arise at different system levels. Such changes are known to emerge after stimulation and represent the plastic efforts of the brain to maintain the networks' functional role(49). Reciprocal relationships between task-positive and DMN systems(50), which are known to be mediated by glutamatergic and GABAergic measures(17, 51), and intra-DMN node relationships(29) may interact with the observed regional concentration modulation of neurochemicals. In this regard, ICC indices in the left IPL for both experimental groups were systematically lower than in the sham stimulation, which supports the idea of engagement of plasticity mechanisms even when no significant neurochemical modulation is detected. Altogether, the results suggest that connectivity must be taken into account when investigating TMS-induced distal changes, and a reciprocal relationship with neurotransmitters' MRS measures and network properties.

Our study provides further evidence that healthy brains respond to stimulation in a complex, dynamic manner, in order to maintain effective neuronal processing(52), thus revealing unique (adaptive) mechanisms of plasticity. Brain plasticity is critically tied to brain health across the lifespan. Local cortical and network plasticity might keep a fine-tuned balance within mechanisms that promote change and others that promote stability(53), optimizing functionality. Conversely, a dysfunction in plasticity underlies the symptoms of major neuropsychiatric disorders. Therefore, normalizing plasticity mechanisms may represent novel and effective therapeutic interventions(53). In AD, the impairment of neuroplasticity mechanisms is related to metabolism of the Aβ amyloid protein, which follows a pattern of aggregation closely overlapping the topography of the DMN(54). Aβ oligomers, which are highly involved in AD pathogenesis, disrupt

glutamatergic transmission(55) and inhibitory circuitry(56). Conversely, neuronal activity regulates Aβ concentrations(57). Abnormal levels of synaptic activity may lead to toxic Aβ secretion(58), suggesting that plasticity mechanisms and Aβ are part of a synaptic activity regulatory mechanism that is disrupted in AD(20). In human motor cortex, TMS studies in AD patients consistently revealed alteration of plasticity mechanisms(59, 60). As discussed above, the distal GABA responses to TBS observed in our study likely reflect part of the capacity of the DMN to respond with plastic adaptations. The fact that such changes were observed in a cortical region critically affected by Aβ aggregation in early AD(54, 61) opens the possibility that MRS-GABA responsiveness could be investigated as a putative physiological index of aberrant plasticity mechanisms in this condition. Studies looking for specific associations between such MRS responses and the status of primary pathophysiological markers of AD should help to clarify this issue. Further, dynamic modulation of neurotransmitters assessed with MRS has proved to be sensitive both to abnormal brain responses in neurological conditions(31) and to detect pharmacological interventions in the prodromal stage of AD(62). Hence, the possibility of studying plasticity dysfunction mechanisms by exerting experimental control over primarily and incipiently affected cortical regions holds promise as a putative physiological marker to monitor the effects of pharmacological and cognitive therapies.

The interpretation of molecular mechanisms accounting for the neurochemical modulation cannot be fully elucidated, as MRS quantifies total neurochemical concentrations and cannot distinguish between different pools(63). However, GABA-MRS seems to mainly capture tonic extracellular GABA(64). MEGA-PRESS-acquired GABA peaks contain contributions from both mobile brain molecules and homocarnosine, and partial contamination of the signal cannot be discarded. Nevertheless, these factors are unlikely to interact with the results. GABA modulations in our study may be related to upregulation of the glutamic acid decarboxylase enzyme that synthetizes GABA from glutamate(23, 31). Glutamate is the main excitatory neurotransmitter of the brain and glutamatergic neurons, though it is not uniquely involved in synaptic transmission, particularly in metabolic roles. We assessed Glx, a composite measure comprised of glutamate and glutamine, although glutamine seemed to be unmodified after stimulation(33). Dynamic changes in glutamate concentration may be explained within the context of altered enzymatic activity in response to changes in neuronal firing(33, 65). Interpretation results is also challenging as GABA and

glutamate-MRS changes may not necessarily reflect what baseline MRS concentrations reflect, and stimulation may not modulate just one aspect of the neurochemicals (i.e. theta-frequency stimulation may promote upregulation of synaptic strength(66)). Some limitations of our study need to be highlighted. First, some limitations are inherent to the technique and current knowledge on what it assesses(63, 67). Second, the small sample used in our study limits the study of intragroup variability. Third, we did not counterbalance the order of left IPL and PCU spectra acquisition.

In conclusion, this study provides the first evidence that stimulation in the DMN induces dynamic changes in neurotransmitters in both local and distal areas. MRS may provide unique information about plasticity mechanisms outside motor areas. The feasibility of modulating neurotransmitters in the DMN allows the possibility of studying aberrant plasticity in core areas of several neuropsychiatric disorders, such as schizophrenia, clinical AD and/or in relation to pathogenic measures such as Aβ deposition. Finally, we present the first evidence that neurotransmitter modulation is predicted by connectivity patterns, which highlights the relation between intrinsic network dynamics and neurotransmitter concentration.

Methods

Participants**:** Thirty-six healthy right-handed young subjects (age=23.50[2.00]; 7 males) were randomly assigned to one of three experimental groups (Sham, iTBS or cTBS). Four subjects (1 male) were discarded from the MRS analysis, due to poor quality data. These subjects were not included in any analysis; data from 2 left IPL spectras were removed due to lipid contamination, and one PCU spectra was removed due to MRS acquisition problems. Final analyses included 30 and 31 subjects for the left IPL and the PCU spectroscopy respectively. None of the participants had any neurological or psychiatric disorder or TMS contraindications(68). All subjects gave informed consent and the protocol was in accordance with the Declaration of Helsinki and approved by the local ethics committee.

Experimental design: The main experimental protocol consisted of a TMS session in-between MRI acquisitions. In both MRI sessions, two MRS were acquired in the PCU and the left IPL, along with structural and rs-fMRI acquisitions (see Figure 1). Prior to the main day of the experiment, subjects

underwent an MRI acquisition with hr-3D structural and rs-fMRI datasets, acquired for segmentation, co-registration and targeting purposes.

TMS: TBS was applied between two MRI sessions, according to previously described protocols(21). For the sham group, a sham device was used to mimic either cTBS or iTBS protocols. The mean intensity of stimulation was 36(7.1)% of maximum machine output, and no differences between groups were detected (F=1.245, p=.372). TBS was performed in a room adjacent to the MRI scanner and was neuronavigated. The stimulation target was defined individually as the area in the left IPL that showed highest functional correlation within the DMN. Detailed TMS protocols, motor thresholds acquisitions included, are described in SI.

MRI acquisition: All subjects were examined on a 3T MRI scanner (Magnetom Trio Tim, Siemens, Germany). Three 3D structural datasets (2 mr-3D and 1 hr-3D) and two 5' rs-fMRI datasets were acquired during the experimental design. Four 9 cm^3 ^{1}H-MRS MEGA-PRESS sequences (256 spectral averages) were acquired, two in the pre-stimulation MRI acquisition and two immediately after TBS stimulation. MRS VOIs were located in the left IPL (Figure 2c) and the PCU. MR acquisition parameters are fully described in SI.

Analysis

Neuroimaging analyses were performed with a combination of SPM (http://www.fil.ion.ucl.ac.uk/spm/), FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) and AFNI (http://afni.nimh.nih.gov/afni/) software, as well as tailored in-house scripts. Spectra data were analyzed with LCModel V.6.3-1a(69) (Figure 2a,b).

MRS analyses: For both "edit OFF" and difference spectra, we calculated the best fit of the experimental spectrum as a linear combination of model spectra. Fitting for "edit OFF" was performed over the spectral range from 0.2 to 4.2 ppm. MEGA-PRESS edited spectra were analyzed using a simulated basis set and fitting was performed between the 1.9 to 4.0 ppm spectral range. tCR was extracted from "edit OFF" spectra, and tNAA, GABA and Glx measures were extracted from the difference spectra. Only Cramér-Lao lower bounds (CRLB)<15% were considered. If not specified, metabolites of interest were expressed as tNAA ratios, further corrected with a CSF partial volume factor.

Structural analyses: Mr-3D images were co-registered with linear transformations to the previously acquired hr-3D images, which were segmented into tissue type to allow comparisons of the overlapping percentage between pre- and post-voxels and tissue type composition between experimental groups.

Connectivity analyses: To evaluate the relation between distal neurotransmitter modulation and connectivity, a seed-to-seed connectivity analysis with 6-mm radius spherical ROIs was performed. PCU-ROI was created in the center of gravity generated by overlapping pre- and post-TBS spectroscopic VOIs, while Stim-ROI was placed in the coordinates used to guide stimulation (see SI). After GM masking, rs-fMRI preprocessed ROIs timeseries were extracted and correlated for each subject. Preprocessing of the rs-fMRI pipeline is detailed in SI.

Data analysis: The main hypothesis, that the neurotransmitter change was due to stimulation, was tested using 3x2 repeated measures ANOVA with group (sham*cTBS*iTBS) and time (pre*post) as independent variables using PASW 18.0 software (IL, USA). To study GABA and Glx covariation and the relationship between connectivity and neurotransmitter modulations, bivariate Pearson's correlations were performed. The appropriate tests were used for secondary analyses. When not specified, data is presented as mean (SD) and significance is considered at $p<0.05$.

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Figures and Tables

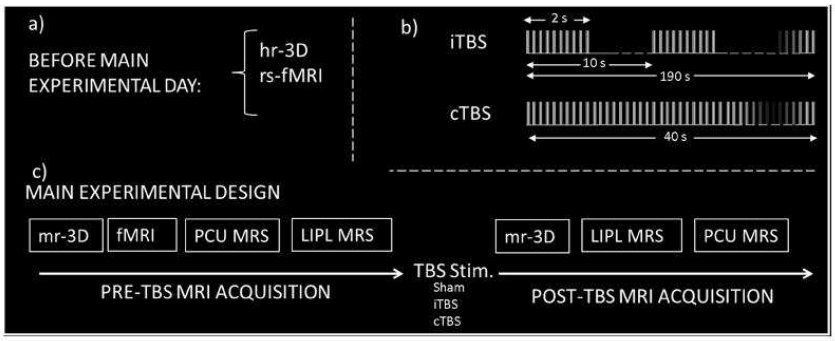

Figure 1. Protocol schema. a) Datasets acquired before the day of the experiment, b) schema of patterned repetitive transcranial magnetic stimulation used in the study and c) timeline schema of the main experimental design.

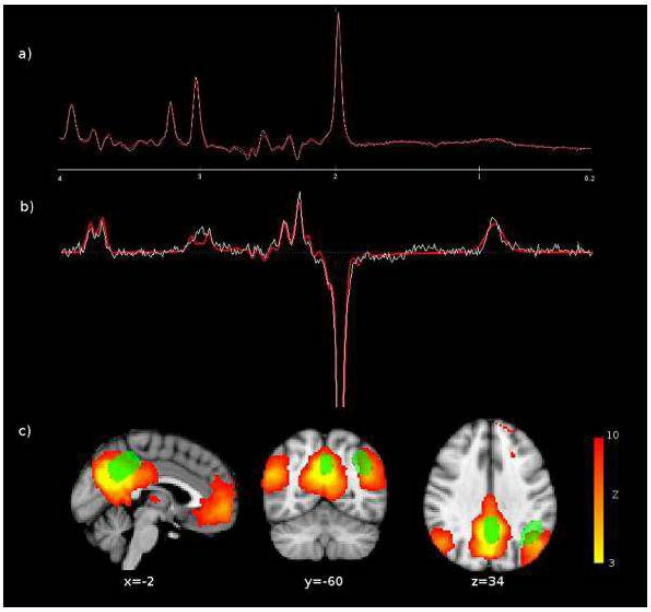

Figure2. Magnetic Resonance Spectroscopy sequences a) non-edited *"Edit-OFF"* and b) GABA-edited spectra acquired in the left IPL from a random subject. Grey dotted line represent baseline, white line the data and the red line represents the fitting. c) Mean positioning of PCU and Left IPL spectras (n>20) in Green, overlapping a DMN networkpublished elsewhere(2), arbitrarily thresholded at z=3 in Red-Yellow scale.

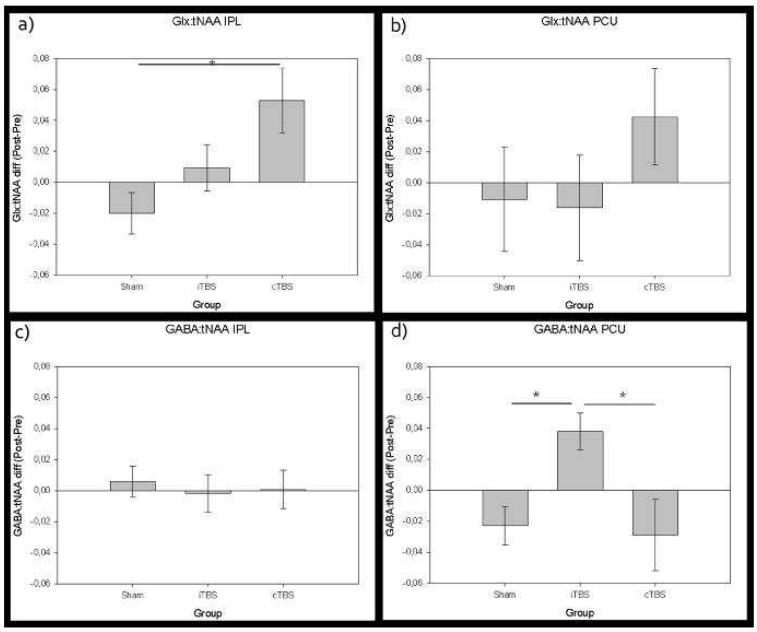

Figure 3. rTMS effect over Glx in the a) left IPL and in the b) PCU. rTMS over GABA in the c) left IPL and the d) PCU. Bars±SEM represent post-pre changes on metabolite concentrations.

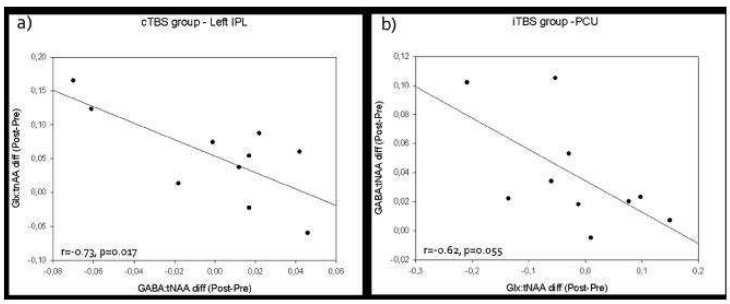

Figure 4. Negative correlation between Glx and GABA modulations after rTMS in the a) left IPL for the CTBS groups and b) in the PCU for the iTBS group.

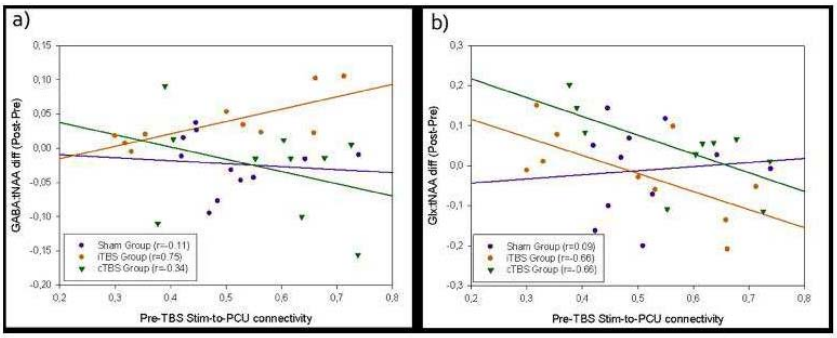

Figure 5. Relation between Stim-to-PCU connectivity and modulation after rTMS for each group for a) GABA and b) Glx MRS-concentrations. See also Table 2.

MRS descriptives

	n	pre-TBS GABA	post-TBS GABA	pre-TBS Glx	post-TBS Glx
Sham	10	.238(.04)	.244(.03)	.728(.06)	.708(.05)
iTBS	10	.265(.02)	.263(.04)	.761(.06)	.770(.05)
cTBS	10	.261(.03)	.261(.04)	.699(.06)	.752(.05)
All	30	.256(.03)	.256(.04)	.729(.06)	.743(.05)
Sham	11	.331(.05)	.308(.05)	.970(.10)	.960(.08)
iTBS	10	.302(.03)	.340(.05)	.993(.05)	.976(.11)
cTBS	10	.326(.04)	.297(.04)	.894(.09)	.936(.13)
All	31	.320(.04)	.315(.05)	.953(.09)	.958(.10)

Table 1. Descriptive MRS-metabolite concentrations for each group. Represented as tNAA ratios.

Correlation between PCU-StimROI connectivity and PCU neurotransmitter modulation		
	GABA:tNAA diff	Glx:tNAA
Sham	-0.11(0.760)	0.09(0.78)
iTBS	0.75(0.011*)	-0.66(0.038*)
cTBS	-0.34(0.338)	-0.66(0.039*)

Table 2. Table 2. Pearsons(r) correlation between PCU-StimROI connectivity and PCU neurotransmitter changes. See also Figure 5.

Supplementary material

SI METHODS

Targeting protocol

The stimulation target was individually defined as the coordinates within the left inferior parietal lobe (IPL) that showed the highest correlation with other default mode network (DMN) nodes. We operationalized this concept in a similar way as described in the literature(1, 2). We created 7 mm spherical ROIS in the medial prefrontal cortex (x,y,z=0,51,-7), the precuneus/posterior cingulate cortex (x,y,z=1,-55,17) and the right IPL (x,y,z=50,-64,27). Coordinates, obtained from previous studies(1, 3), were further transformed to functional space and a seed-based connectivity analysis of this ROIS within the left IPL was performed (thresholded at r=0.1). The targeted point was defined as the voxel with maximum conjoint correlation of the abovementioned connectivity maps. Coordinates (mean coordinates to MNI=-45,-67,32) were finally transformed to the stereotactic neuronavigated space.

Sequences acquired

The sequences were acquired with the following parameters.

Structural magnetic resonance Images (MRI): Three 3D structural datasets were acquired during the experimental design for each subject. One high-resolution 3D sequence (T1-weighted magnetization prepared rapid gradient echo [T1-weighted MPRAGE], sagittal plane acquisition, TR=2300 ms, TE=2.98 ms, 240 slices, slice thickness=1 mm, FOV=256 mm, matrix size=256×256) was acquired before the main day of the experiment, while two medium resolution 3D datasets were acquired in the pre- and post-stimulation MRI sessions (T1-weighted MPRAGE, sagittal plane acquisition, TR=1390 ms, TE=2.86 ms, 144 slices, slice thickness=0.625 mm, FOV=240 mm, matrix size=144x384).

Functional MRI (fMRI): Two 5' resting state fMRI datasets (T2*-weighted GE-EPI sequence, TR=2000, TE=16 ms, 36 slices per volume, slice thickness=3 mm, interslice gap=25%, FOV=240 mm, matrix size=128x128, 150 volumes) were acquired during the whole experiment, one prior to the main day of the experiment and the other during the pre-stimulation MRI acquisition.

MRS: 4 ^{1}H-MRS spectra were acquired with a MEGA-PRESS sequence during the study; two in the pre-stimulation MRI acquisition and two immediately after TBS stimulation. The spectra were located either in the PCU or the left IPL. MRS was acquired with the following parameters: MEGA-PRESS sequence; 256 spectral averages TR=1500 ms; TE=68 ms; voxel size=3.0*2*1.50 cm^3; approximate duration: 10 minutes. Voxel of interest (VOI) location was guided by functional and structural information. Double-banded pulses were applied at 4.7 ppm to suppress water and at 1.9 or 7.5 in alternate lines to edit the γ-CH_2 resonance of GABA at 3.0 ppm. The first post-stimulation left IPL and PCU spectral lines were acquired 11 and 20 minutes after stimulation. No differences in temporal lag between end of stimulation and sequence acquisition were found ($p>0.4$) for any test.

Resting-state data preprocessing

Rs-fMRI data preprocessing included removal of the first 5 volumes, motion correction, skull stripping, spatial smoothing (full width at half maximum [FWHM] = 7 mm), grand mean scaling and filtering with both high-pass and low-pass filters (0.1 and 0.01 Hz thresholds). Next, the data were regressed with six rigid body realignment motion parameters, mean white matter and the CSF signal. No global signal regression was used. Registration to an MNI standard space (3 mm voxel) through a two-step linear transformation was performed for group comparisons and transformation purposes.

Repetitive transcranial magnetic stimulation procedures

Theta-burst stimulation (TBS): TBS was applied in-between two MRI sessions with a MagPro x100 Stimulator (MagVenture A|S, Denmark) and an eight-figure coil. TBS was applied according to previously described protocols(4). Intermittent TBS (iTBS) bursts consisted of 3 pulses of 50 Hz at an intensity of 80% of the active motor threshold, repeated every 200 ms for 2". These trains were separated by an 8" interstimulus interval for a total of 20 repetitions (600 pulses-190"). Continuous TBS (cTBS) had the same 3 pulses at a 50 Hz pattern repeated every 200 ms, which were applied continuously without an interstimulus interval (600 pulses - 40"). For the sham group, a sham device was used to mimic either cTBS or iTBS protocols. TBS was neuronavigated with a stereotactic system (eXimia Navigated Brain Stimulation, Nexstim, Finland). TBS was applied in the the left IPL region that showed highest functional correlation within the DMN (see above).

Motor thresholds: The resting motor threshold and active motor threshold were determined for the right first dorsal interosseous muscle over the left primary motor cortex hand knob. Resting motor threshold was defined as the lowest intensity of the TMS required to elicit motor-evoked-potentials (MEPs) of at least 50 µv peak-to-peak amplitude in at least 6 of the 10 consecutive trials(5). Active motor threshold was defined as the minimum stimulus intensity that elicited at least 6 of 10 consecutive MEPs of 200 µv during isometric contraction of first dorsal interosseous.

Connectivity Analysis

DMN resemblance

A seed-based correlation analysis was performed using, as seed regions, ROIs created in the center of gravity of each spectroscopic VOI, to rule-out the possibility of mismatches in VOI location with respect to DMN. Six mm-radius spherical ROIs were thresholded with a grey matter mask and their timeseries from preprocessed rs-fMRI datasets were extracted and correlated voxelwise. Connectivity maps were later transformed to MNI standard space and voxelwise nonparametric permutation testing(6) (5000 permutations) was performed to determinate which areas were functionally connected to left IPL and PCU ROIs (one-sample t-test against 0). Threshold-free cluster enhancement correction(7) (TFCE) was used and maps were thresholded at $p<0.01$ family-wise error (FWE), corrected for multiple comparisons.

Additionally, subject-specific connectivity maps (left IPL-to-brain and PCU-to-brain connectivity) at standard space were compared to previously reported resting-state networks(8), which were available online (http://www.fmrib.ox.ac.uk/analysis/brainmap+rsns/). Cross-correlation analysis was performed by correlating each subject's connectivity maps to 10 validated resting-state networks (three visual networks, a cerebellar network, DMN, auditory, motor, executive and left and right frontoparietal networks). One-sample t-test (against 0) was performed and significance was set at $p<0.01$ after the Bonferroni correction (10 comparisons), to check which resting-state networks were similar to the seed-based connectivity maps extracted from our sample.

Specificity of left IPL-to-PCU connectivity as a predictor of neurotransmitter changes in PCU

Since Stim-to-PCU connectivity predicted the neurotransmitter response to TBS, we aimed to check the specificity of this measure. Consequently, we correlated neurochemical changes due to TBS and seed-to-brain connectivity maps, from PCU and Stimulation ROI timeseries. PCU-ROI and Stim-ROI are defined in the main text. For each experimental group, four analyses were performed, as Stim-to-brain and PCU-to-brain connectivity maps were independently regressed for GABA and Glx change. Nonparametric permutation testing (n=5000) was performed and results were considered at $p<0.05$ after TFCE, without multiple comparison correction, and a minimum voxel cluster extent of 500 voxels. These parameters were chosen to mimic as closely as possible the seed-to-seed analysis (see Methods).

SI RESULTS

MRS analysis

Main time*group ANOVA analyses (see Methods) were repeated without CSF partial volume correction and using tCR as an internal reference metabolite instead of tNAA. Both analyses replicated the main results (see Results). Glx ANOVA in the left IPL revealed a significant interaction both when expressed as tCR ratios ($F=4.967$, $p=0.015$) and without CSF partial volume correction ($F=6.752$, $p=0.004$). This suggests that Glx increased after cTBS. The GABA ANOVA in the PCU revealed a significant interaction both when expressed as tCR ratios ($F=5.545$, $p=0.009$) and without CSF partial volume correction ($F=4.958$, $p=0.014$). This suggests that GABA was higher than in the sham after iTBS stimulation. The other main interaction test did not reach significance ($p>0.05$). The influence of CSF and internal reference on neurotransmitter change measures is almost irrelevant in the present study.

Magnetic resonance spectroscopy analysis

The mean concentrations of neurotransmitter for each voxel and VOI tissue percentage are displayed in Table S1. Increased PCU metabolite concentrations are probably explained by increased grey matter (GM) relative to white matter (WM) concentration ratios in this area, in comparison with left IPL VOI. GABA and Glx have previously been found to be in higher concentrations in GM tissue than in WM. Plots representing the GABA and Glx change for every condition are shown in Figure S1. Increased GABA in the PCU is evident for the iTBS group, and increased Glx was observed in the left IPL for the cTBS group. High variability in the Glx measure of change was found in the PCU, which may overshadow Glx changes after cTBS stimulation in this area.

Resemblance to DMN

The connectivity of the left IPL and PCU ROIs resembled the patterns of DMN (Figure S2a,b, Table S2). Both regions were connected to DMN nodes such as the PCU, bilateral IPL, lateral temporal cortex, and medial prefrontal cortex. Additional structures such as the inferior and superior frontal gyrus or cerebellum were found to be functionally connected to both ROIs. Both patterns were topographically very similar, although the left IPL connectivity map was left-lateralized. Sparse negative correlations in the right supramarginal gyrus were only found for PCU ROIs. The lack of negative connectivity was probably due to a lack of global signal regression during preprocessing. Individual connectivity maps were correlated to validated RSN published elsewhere(8). Both left IPL and PCU connectivity maps were significantly correlated to DMN (r=.171, p=8.9e-9; r=.38, p=1.27e-24) after the Bonferroni correction. Additionally, left IPL was found to be positively correlated with the right and left frontoparietal network (r=0.096, p=2.7e-5; r=0.221, p=8.17e-16). Negative correlations to the motor network were found for both connectivity maps (r=-0.060, p=0.001; r=-0.087, p=1.82e-4), while the PCU connectivity map also negatively correlated with the auditory network (r=-0.074, p=0.001). These results confirmed the resemblance of both connectivity maps to the DMN network, while the slight dissimilitude is in accordance with the centrality of PCU within this network (Figure S2c). The other correlations were not statistically significant (p>0.01) after the Bonferroni correction.

Specificity of Stim-to-PCU connectivity measures as a predictor of neurotransmitter change in the PCU.

Voxelwise connectivity maps were generated from both Stim and PCU ROIs and voxelwise correlations of these maps with Glx and GABA changes were obtained for each experimental condition (Figure S3, Table S3). Although a total of 24 analyses were carried out, only those groups in which connectivity was able to predict neurotransmitter changes are discussed (for unpublished analyses please contact the corresponding author). Results showed good specificity of Stim-to-PCU connectivity measures as predictors of neurotransmitter change in the PCU after iTBS. These neurotransmitter changes were only predicted by the connectivity of Stim-ROI to posterior DMN areas. Similarly, the neurotransmitter changes were only predicted by the connectivity of PCU-ROI to posterior left-lateralized areas positively connected with PCU. Less specificity was found for the Stim-to-PCU connectivity measure when Glx change was predicted after cTBS. Neurotransmitter changes after sham stimulation were unrelated to DMN connectivity. Although inconclusive because of its exploratory character and its permissive threshold, this analysis suggests that neurotransmitter changes might be due to "real" intrinsic connectivity parameters instead of methodological aspects of this study. The analysis also suggests the relatively high specificity of Stim-to-PCU connectivity as a predictor of neurotransmitter change.

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Figures and Tables

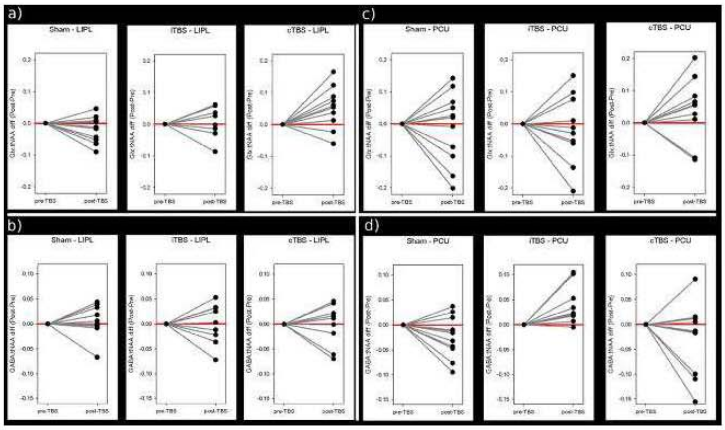

Figure SI1. Individual changes in neurotransmitters for each group, metabolite and region. Plots represent deviation in post-stimulation MRS-assessment from baseline (pre).

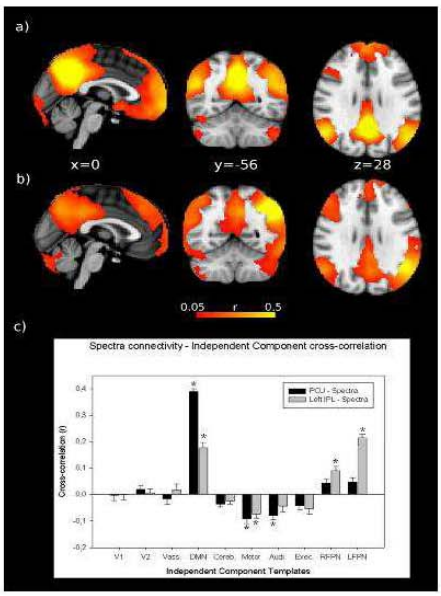

Figure SI2. Areas significantly correlated with a) PCU and b) left IPL spectral centers of gravity rs-fMRI timeseries (FWE<0.01). For illustrative purposes, these areas are displayed as Red-Yellow maps representing the Pearson(r) correlation. C) Relationship between left IPL and PCU connectivity maps for each subject and large scale resting-state networks published elsewhere(8). Correlations were considered significant at p<0.01 Bonferroni corrected.

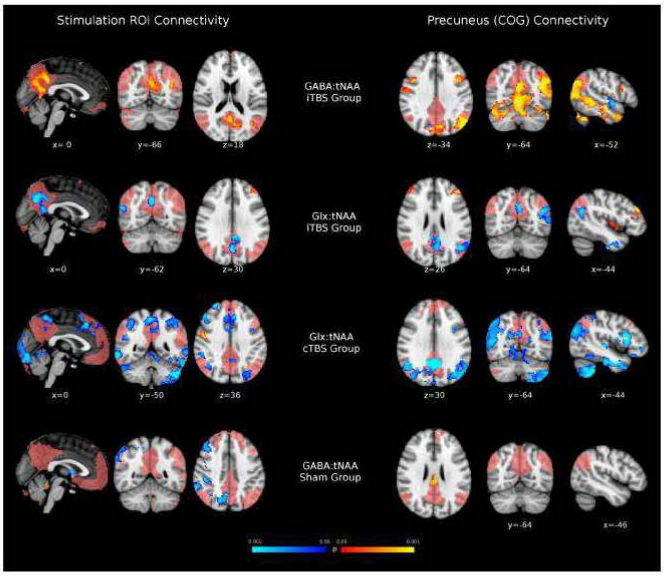

Figure SI3. All-brain exploratory correlation between Stim-ROI timeseries (left side) and precuneus-ROI timeseries (right side) with neurochemical changes, to illustrate the specificity of Stim-to-PCU connectivity to predict neurochemical modulation. Red-Yellow colors represents positive correlations between neurochemical change and connectivity, while the Blue-LightBlue scale represents negative correlation with those two measures (p<0.05 uncorrected; cluster size>500). Due to space limitations, not all groups are displayed. See also Table SI3.

MRS descriptives

Mean						Change(Post-Pre)			
	N	Min	Max	Mean	SD	Min	Max	Mean	SD

IPL GABA:tNAA	30	.19	.34	.26	.03	-.07	.05	.00	.04
IPL Glx:tNAA	30	.61	.86	.74	.05	-.09	.17	.01	.06
IPL GM	30	38.42	58.31	47.81	5.34	-7.46	8.23	.53	3.65
IPL WM	30	30.91	58.82	46.02	6.59	-14.72	9.08	-.39	5.08
IPL CSF	30	2.29	15.84	6.18	3.20	-4.38	7.16	-.14	2.02
IPL GM/(WM+GM)	30	.40	.63	.51	.06	-.09	.12	>.01	.05
IPL overlapping	30	66.71	94.02	84.20	5.70	----	----	----	----
PCU GABA:tNAA	31	.29	.41	.32	.04	-.15	.10	-.00	0.06
PCU Glx:tNAA	31	.75	1.19	.95	.08	-.20	.20	.00	.10
PCU GM	31	65.33	81.00	73.67	3.40	-1.89	1.38	-.23	.82
PCU WM	31	7.99	17.15	12.93	2.55	-3.81	1.80	-.18	1.20
PCU CSF	31	5.87	22.92	13.37	4.26	-.79	3.37	.42	1.06
PCU GM/(WM+GM)	31	.81	.91	.85	.03	-.02	.04	>.01	.01
PCU overlapping	31	33.53	91.77	73.32	13.81	---	----	----	----

Table SI1. Descriptive information displaying tissue composition, overlapping and neurochemical concentration indices of the MRS assessment.

All brain connectivity of specta rs-fMRI timeseries

	Cluster Index	Voxels ($2mm^2$)	MAX p (max r)	X	Y	Z	Area
<u>COG-connectivity</u>							
<u>PCU</u>							
Positive conn	1	28301	1 (0.83)	0	-52	38	Precuneus
	2	14645	1 (0.42)	0	62	-8	Frontal Pole

	3	6686	1 (0.21)	26	-86	-28	Right Lateral Occipital Lobe
Negative conn	1	166	1 (0.20)	56	-28	38	Right Supramarginal Gyrus
COG-connectivity Left IPL							
Positive conn	1	39027	1 (0.70)	-40	-58	38	Left Angular Gyrus
	2	12639	1 (0.33)	-36	12	60	Left Middle Frontal Gyrus

Table SI2. Correlation between PCU-StimROI connectivity and PCU neurotransmitter changes. See also Figure 5 in the main text.

Correlation between brain connectivity and neurochemicals change

Stim Connectivity

	Cluster Index	Voxels	MAX	X	Y	Z	Area
iTBS							
ΔGABA:tNAA+	1	1444	0.998	-6	-70	28	Precuneus
ΔGABA:tNAA-							
ΔGlx:tNAA+	1	940	0.992	30	6	0	Right Putamen
ΔGlx:tNAA-	1	1105	0.998	0	-66	30	Precuneus
cTBS							
ΔGlx:tNAA+							
ΔGlx:tNAA-	9	8381	1	-26	-84	-48	Cerebellum
	8	3126	1	34	22	48	Right Middle Frontal Gyrus
	7	1454	1	64	-50	-10	Right Middle Temporal Gyrus
	6	1384	0.992	44	-52	26	Right Angular Gyrus
	5	1374	0.992	-38	-54	54	Left Superior Parietal Lobule

	4	978	0.999	-28	-28	-8	Left Hippocampus
	3	945	1	28	48	14	Right Frontal Pole
	2	915	0.997	2	-50	58	Precuneus
	1	890	1	-22	14	54	Left Superior Frontal Gyrus
Sham							
ΔGABA:tNAA+	1	581	0.999	-34	-38	-10	Left Parahippocampal Gyrus
ΔGABA:tNAA-	3	1735	0.998	46	-30	46	Right Postcentral Gyrus
	2	965	0.993	40	24	44	Right Middle Frontal Gyrus
	1	626	0.998	18	-72	42	Right Lateral Occipital Cortex
Precuneus - Centre of Gravity							
iTBS							
ΔGABA:tNAA+	2	15248	1	-54	-58	-34	Cerebellum
	1	2354	0.999	54	-16	-18	Right Middle Temporal Gyrus
ΔGABA:tNAA-	1	2961	1	-28	10	-34	Left Temporal Pole
ΔGlx:tNAA+	2	1130	1	-32	34	34	Left Middle Temporal Gyrus
	1	1064	1	20	38	-18	Right Frontal Pole
ΔGlx:tNAA-	2	2524	0.999	-68	-18	-12	Left Temporal Temporal Gyrus
	1	908	0.998	-2	-66	36	Precuneus
cTBS							
ΔGlx:tNAA+	1	583	0.999	28	66	-10	Right Frontal Pole
ΔGlx:tNAA-	3	10480	1	-8	6	-20	Subcallosal Cortex
	2	10058	1	62	-50	-12	Right Inferior Temporal Gyrus
Sham							

ΔGABA:tNAA+2	1158	0.998	10	-6	58	Right Juxtapositional Lobule
1	589	1	-26	18	60	Left Superior Frontal Gyrus
ΔGABA:tNAA-						

Table SI3. All-brain exploratory correlations between Stim-ROI and Precuneus-ROI timeseries with neurochemical changes. Due to space limitations, not all the groups are described. See also Figure SI3.

Chapter 5: Summarized Results

1. Main Results

Globally, the results showed the feasibility of experimentally using TMS plasticity inducing protocols to modulate memory networks assessed either as BOLD signal activation or as neurotransmitter concentrations. The results also showed the key role of intrinsic functional connectivity to understand distal responses to TMS. The results highlight the relevance of the frontal and DMN networks in order to understand decline or preservation of memory function in aging.

The principal results of the three studies are summarized in the following sections:

2. Study I

Main results after applying real or sham iTBS over the left IFG in healthy elders in order to study behavioural and brain functional changes during an episodic task included the following:

1) Intermittent TBS did not produce changes in performance in an episodic memory task.

2) TMS significantly changed BOLD responses during the episodic memory task. Functional brain TMS-induced changes were found specifically when subjects were performing a deep encoding task in local (left inferior frontal gyrus) and distal areas (occipital cortex).

3) Areas where iTBS effects were found belong to memory encoding networks.

4) TMS effects correlated with task-related connectivity shifting measures at pre-iTBS encoding task.

5) When subjects were at “resting-state” no stimulation changes in connectivity or in spontaneous brain activity measures were detected.

3. Study II

Main results obtained after studying the anatomical, perfusion and cognitive correlates of antero-posterior DMN connectivity in healthy elders are summarized as follows:

1) Antero-posterior mPFC-PCU DMN connectivity is significantly reduced in ageing.

2) In ageing, mPFC-PCU coupling is related to cognition, specifically to memory performance.

3) mPFC-PCU DMN connectivity is associated with measures of GM and WM (VBM, MD, GWC) integrity in several brain areas, not restricted to DMN.

4) Perfusion measures, assessed with ASL technique, are unrelated to antero-posterior DMN connectivity.

5) mPFC-PCU DMN structural correlates appear to be located in areas of high age vulnerability.

4. Study III

In this study, TBS stimulation was applied in-between two MRI acquisitions that included MRS and fMRI sequences. The main findings to be highlighted include:

1) TBS applied to the left IPL induced brain dynamic neurotransmitter changes both locally and distally in a modality-dependent fashion. Specifically iTBS increased GABA, distally, in the PCU while cTBS increased Glx in the left IPL, the area underneath stimulation.

2) The magnitude of the induced neurotransmitter modulation in the PCU was strongly associated with the strength of baseline functional connectivity between the TMS-targeted area and this distal region.

Chapter 6: General Discussion

This thesis aimed to characterize and modulate large-scale brain networks involved in long-term episodic memory through multimodal MRI assessment and patterned TMS stimulation focused in the implications for cognitive aging. This chapter will be divided in three sections where the following topics will be discussed: characterization and alterations of memory networks in aging, the relevance of plasticity and NIBS techniques to understand brain aging, and memory functions and the relevance of multimodal assessment to better understand functional networks in aging.

1. Memory networks characterization and age-related alterations

It is increasingly accepted that behavioral and cognitive functions arise from interactions within and between distributed brain systems (Bressler and Menon, 2010) being fMRI an optimal technique to study brain from this perspective. Regarding memory, several brain circuits have been highlighted such as MTL-PFC dynamic interactions or the DMN (Andrews-Hanna et al., 2007; Grady et al., 2003). Normal aging is accompanied by disruptive alterations in the engagement and coordination of large-scale brain systems that support memory functions both at rest and during task contexts (see Ferreira and Busatto, 2013; Grady, 2012 for reviews). In this sense, prefrontal regions connected to temporal and posterior areas and the DMN network are highly altered in aging and crucial for memory processes. The present thesis extended these evidences by studying in elders the specific role of PFC in an episodic encoding task with different levels of processing and by characterizing DMN alterations in aging.

PFC cortex has attracted considerable interest in cognitive neuroscience of aging as it have been pinpointed as a higher-order area that engages multiple plasticity

mechanisms trying to compensate the negative impact of age over cognitive function (Cabeza et al., 2002). In **Study I**, we investigate the involvement of PFC in different episodic encoding processes and how PFC influenced, in a task-dependent fashion activity in mnesic networks. Concretely, we studied left IFG involvement under deep (semantic) and shallow (perceptual) encoding by means of patterned TMS. Left IFG region has been highlighted as fundamental for semantic encoding processes in fMRI literature along with DLPFC (Kapur et al., 1994; Otten et al., 2001; Wagner et al., 1998). Using a TMS interference paradigm Innocenti and colleagues (2010) causally demonstrated specific involvement of the DLPFC in deep encoding processes as stimulation applied over this region reduced memory performance only when it was semantically encoded. While our results did not show behavioural effects of patterned TMS as previously hypothesized according to previous studies from our group (Solé-Padullés et al., 2006), we found task-dependent effects of TMS over fMRI activity in local and distal posterior areas. That is, left IFG TMS only affected brain networks when subjects encoded stimuli in a semantic processing fashion. These results are in good agreement with a specific PFC role in deep LoP processing (Innocenti et al., 2010) as well as with the role of left IFG in semantic control (Jefferies, 2013). Left IFG may selectively exert top-down modulation over posterior, relatively unspecific, areas hierarchically located before visual areas involved in semantic recognition. These results are in agreement with several neuroimaging studies that reach similar conclusions by using disparate cognitive paradigms (Çukur et al., 2013; Kherif et al., 2011; Urooj et al., 2013). This top-down modulation might reflect either an early attentional shift towards facilitating deep encoding or instead a continuous feedforward network that leads to enhanced recognition during semantic encoding. Memory decline in aging seems greater in shallower LoPs, such as in perceptual encoding, that during semantic encoding (see Park and Gutchess, 2000 for a review). However, older people seem to have problems to spontaneously use semantic strategies which are susceptible to be amenable to cognitive training (Kirchhoff et al., 2012; Logan et al., 2002). Top-down modulation of semantic processing might be a compensatory mechanism by which elders can relatively maintain deep encoding performance in aging when semantic encoding strategies are provided. Top-down mechanisms, though affected by age

(i.e. inhibition processes, Gazzaley and D'Esposito, 2007), might change and adapt during lifespan in an attempt to maintain function. In a verbal memory task, older adults showed increased parahippocampal-frontal connectivity and reduced hippocampal-frontal connectivity which was interpreted as evidence that older adults compensate for hippocampal deficits for relying more on other structures and processes (Daselaar et al., 2006). Similar results have been reported in other memory paradigms (Addis et al., 2010; Dennis et al., 2008). Thus when deep encoding strategies are provided to older people, left IFG might engage an attentional shift towards early visuals areas with the objective to compensate deficits when spontaneously codifying new verbal information. Knowledge of the biological processes that led to successful encoding of material in aging is necessary to elaborate effective strategies and obtain functional modifications of memory networks and behavioural gains.

Study II and **Study III** were focused on the DMN, which investigated throught rs-fMRI, has found to be highly susceptible to the effects of ageing (see Ferreira and Busatto, 2013; Hafkemeijer et al., 2012 for reviews), DMN integrity, especially inter-nodal connectivity, has been consistently related to memory functions, particularly in elders populations (Andrews-Hanna et al., 2007; He et al., 2012; Sala-Llonch et al., 2014b; Wang et al., 2010a). Our results, in **Study II** expand this previous knowledge with a large, homogeneous sample of older adults by finding that antero-posterior DMN connectivity is altered in ageing and that, in elders, this connectivity predicts memory function. We found that coupling between posterior midline and mPFC nodes, key nodes of the DMN, were especially affected by age in agreement with several other studies (Andrews-Hanna et al., 2007; Biswal et al., 2010; Bluhm et al., 2008; Campbell et al., 2013; Mevel et al., 2013). In addition the link between memory and DMN network integrity during resting-state is increasingly been accepted (Andrews-Hanna et al., 2007; Wang et al., 2010a). As a good example, a recent study by our group conducted in an independent sample also found a relationship between functional DMN connectivity measures and memory using an independent sample and graph-based analysis (Sala-Llonch et al., 2014b). We also found that DMN connectivity correlates belong to areas of high age-related

susceptibility suggesting that connectivity might be present at early stages of aging before other macrostructural changes are evident. That is in agreement with the observation that middle-age healthy subjects (Biswal et al., 2010; Bluhm et al., 2008; Evers et al., 2012) already have reduced DMN connectivity.

DMN disconnection in ageing does not seem to reflect (successful) compensatory mechanisms; rather, this disconnection is likely to be a reflection of brain ageing and inefficient processing related to the centrality of these areas regarding the connectome (de Pasquale et al., 2012). Deficient coupling at rest might reflect inefficient allocation of neural resources necessary for correct performance during episodic memory tasks (Stevens et al., 2008). Age-related DMN disconnection at rest in ageing then, interpreted as brain deficits to dynamically shift and adapt to the external demands can be easily linked to alterations of and/or by plasticity mechanisms (Fjell et al., 2014; Freitas et al., 2013; Oberman and Pascual-Leone, 2013). The suggestion that regions characterized by a high degree of life-long plasticity are vulnerable to the detrimental effects of normal ageing (this age-vulnerability also renders them more susceptible to additional, pathological AD-related changes, Fjell et al., 2014; Mesulam, 1999) is a provocative hypothesis that is gaining attention and is coherent with our results. The relevance of plasticity has been both considered as an adaptive mechanism and as a factor of risk that may lead to pathology (Oberman and Pascual-Leone, 2013). DMN areas are among the ones with the highest neuroplasticity capacity in the cerebral cortex, as they play a central role in brain functioning and specifically in learning and memory processes. This increased demand of plasticity may thus make this system especially vulnerable during the lifespan lifespan (Fjell et al., 2014) and functional disconnection might well represent an early marker of brain ageing, which could partially emerge from maladaptive plasticity mechanisms during the lifespan.

2. Controlled induction of plasticity in memory networks: Assessment of circuitry and local plasticity

Several animal studies revealed that neurobiological substrates of age-associated cognitive decline are associated with alterations in synaptic plasticity such as the balance between LTP and LTD thresholds and a more rapid decay of hippocampal LTP which are further associated with memory function (Barnes, 2003, 1979; Bliss, 2003; Burke and Barnes, 2006; Rosenzweig and Barnes, 2003).

While fMRI has become a useful tool to investigate the consequence of plasticity in humans, repetitive TMS is additionally able to explore in vivo the state of such mechanisms. TMS is capable to non-invasively and controllably induce engagement of plasticity mechanisms and to make use of them to improve cognition and behaviour (Oberman and Pascual-Leone, 2013). In **Study I**, we failed in using patterned TMS as a tool to modulate memory performance (as expected; Solé-Padullés et al., 2006). However, lack of behavioural results allowed as to interpret network changes by TMS as a physiological probe and to discuss induced modulations as the existence of a specific role of left IFG in a semantic encoding network that consisted in a task-dependent semantic top-down modulation (see **Memory networks** characterization and age-related alterations for detailed discussion)

Instead in **Study III** we use patterned TMS to evaluate the state of local plasticity mechanisms. While plasticity mechanisms are continuously operational, the ability to change and adapt becomes increasingly less efficient with advancing age (Freitas et al., 2013). In humans and by means of TMS several studies replicated earlier animal findings mostly within sensoriomotor cortex (see Freitas et al., 2013; Zimerman and Hummel, 2010 for reviews). As example Freitas et al. (2011) found that the duration and the magnitude of corticospinal excitability modulation by patterned TMS was inversely correlated with age, suggesting that LTD-like plasticity becomes increasingly less efficient with age. We demonstrated, for the first time in

humans, the feasibility to evaluate local plasticity mechanisms in key cores of the DMN by modulating GABA and Glutamate neurotransmitters concentration after patterned TMS. Specifically GABA modulation in the posteriomedial cortex which reflects engagement of plasticity mechanisms, represents a unique opportunity to examine local plasticity mechanisms in a crucial area for both healthy and pathological aging. It has been proposed that diminished local cortical plasticity might be tied to the brains engagement of macrostructural plasticity modifications whose consequences are evidenciated by fMRI (Freitas et al., 2013). Even more crucial is the feedfoward relationship between synaptic plasticity mechanisms and Aβ amyloid protein, which follows a pattern of aggregation closely overlapping the topography of the DMN (Buckner et al., 2005). Aβ oligomers, highly involved in AD pathogenesis, disrupt glutamatergic transmission (Hsia et al., 1999) and inhibitory circuitry (Ulfig, 1988). Conversely, neuronal activity regulates Aβ concentrations (Kamenetz et al., 2003). Abnormal levels of synaptic activity may lead to toxic Aβ secretion (Bero et al., 2011), suggesting that plasticity mechanisms and Aβ are part of a synaptic activity regulatory mechanism that is disrupted in AD (Palop and Mucke, 2010). Our study constitutes a novel approach to study plasticity, in a cortical region critically affected both by healthy aging and by Aβ aggregation in early AD (Buckner et al., 2009, 2005). Pararelly MRS-GABA in combination to TMS seems a useful technique to study aberrant plasticity mechanisms in areas outside the motor cortex (Marjańska et al., 2013).

This work also contributes to understand how TMS impacts the brain. NIBS literature established both that TMS effects propagates transynaptically to distal areas (Bestmann et al., 2008) and that its induced-effects are state-dependent, that is, TMS impact over the brain depends on the state of the system when stimulated (Silvanto et al., 2008). Both studies demonstrated distal effects of patterned TBS either reflected by GABA concentration modulation or by an increase of BOLD signal during an encoding task. Importantly though, both studies demonstrated state-dependency effects of TMS as its magnitude depended on functional connectivity between the stimulated area and the area where the effects were assessed. In **Study I** we demonstrated a correlation with pre-stimulation task-dependent shift in

connectivity and distal change of activity during semantic processing. In **Study III**, we showed a correlation between distal neurotransmitter modulation and pre-stimulation DMN connectivity. Both findings provide novel information as the measure used in the first study, a shift in connectivity, and the relationship between neurotransmitters modulation and intrinsic connectivity was unpublished. Still, the results are in good agreement with several studies that highlighted the role of functional connectivity as a moderating variable of TMS effects (Cárdenas-Morales et al., 2013; Fox et al., 2012b). TMS impact over brain and behavior proved to be related both to direct connectivity measures (Andoh and Zatorre, 2013; Nettekoven et al., 2014) and indirect compensatory circuits (Cárdenas-Morales et al., 2013; Lee and D'Esposito, 2012). Consequently intrinsic functional connectivity have been proposed as a method to improve TMS targeting (Eldaief et al., 2011; Fox et al., 2012b; Halko et al., 2010) as well as previous modulation of intrinsic connectivity could represent a novel technique to improve TMS effects.

In conclusion, to understand memory function decline and preservation in aging it is absolutely necessary to invoke the concept of plasticity. Synaptic plasticity is not only inherently tied to learning and memory but diminished synaptic plasticity in aging and the engagement of macrostructural plasticity changes in an attempt to maintain normal functioning are also determining memory network and its engagement during memory tasks. Studying how plasticity mechanisms relate at different levels of expression in the next few years will be necessary to obtain a comprehensive knowledge of brain aging. NIBS, and more specifically patterned TMS, represents a unique technique able to interrogate for plasticity mechanisms and its consequence over memory networks in aging and a powerful scientific tool to use in combination with other neuroimaging techniques.

3. Multimodal analysis of memory network in aging

Brain aging is a multifactorial complex phenomenon characterized by changes at different levels from cellular and synaptic dysfunctions to macrostructural functional and structural connectome modifications. While BOLD signal provides a surrogate measure of brain activity that allows the study of the brain as a dynamic interaction of distributed brain areas, fMRI results alone are often of limited interpretability as no causality can be inferred (Logothetis, 2008). Consequently, in the last years, multimodal studies of aging started to appear such as those relating neurotransmitter with rs-fMRI properties (Duncan et al., 2014), or those relating structure and function (Greicius et al., 2009) which alloweda broader and more complex interpretations of the aging brain.

All the studies of the present thesis have been carried on employing a multimodal approach (see **Chapter 3**: Methods). In **Study II**, perfusion, white matter and grey matter indexes were used in an attempt to provide a more comprehensive description of altered connectivity in ageing as knowledge of the causes and consequences of decreased antero-posterior DMN connectivity remains limited. We found that perfusion was unrelated to antero-posterior DMN connectivity while both grey matter and white matter measures were related to the DMN coupling. Grey matter correlates of DMN were limited to posterior areas suggesting that the disconnection produces a different pattern of grey matter deterioration in frontal and posterior areas as suggested by other studies (Chen et al., 2011; Li et al., 2013) and highlighting the relevance of posteriomedial areas as the main DMN hub (Campbell et al., 2013; de Pasquale et al., 2012). The greater correlations were found between DMN connectivity and white matter integrity measures. Integrity of several long-range antero-posterior tracts, not limited to cingulum bundle, correlated with DMN connectivity suggesting that structural integrity is partially supporting functional networks within the DMN (Khalsa et al., 2013; van den Heuvel et al., 2008). However, results suggested that other tracts, besides those directly connecting mPFC and posteriomedial nodes (van den Heuvel et al., 2009) are related to maintenance of DMN in aging, an interpretation which concords with few other studies (Luo et al., 2012; Teipel et al., 2010). The most striking result was that GWC measures showed a high topological concordance with DMN. That is, DMN

connectivity was greatly predicted by GWC indices within DMN. GWC might, in great measure, reflect integrity in thinly myelinated white matter near grey matter boundaries (Cho et al., 1997; Magnaldi et al., 1993; Westlye et al., 2009). Consequently, white matter near cortical surface may be closely associated with the maintenance of the synchronous function among neural networks, upon which optimal cognition would depend. Such observations open new avenues to explore the relationships between structural andfunctional connectivity characteristics of the ageing brain, as thinly myelinated white matter tracts have been reported to have enhanced age-related susceptibility (Bartzokis et al., 2004; Tang et al., 1997).

In **Study I** and **Study III**, use of the TMS along with fMRI allow the extraction of causal inferences from fMRI signal changes providing greater explanatory power to the results. In **Study III**, we additionally relate rs-fMRI connectivity to neurochemical concentration. The possibility to quantify main excitatory and inhibitory neurotransmitters in the brain in vivo with MRS allows the possibility of relating these neurochemicals with rs-fMRI properties (Donahue et al., 2010; Hu et al., 2013; Kapogiannis et al., 2013; Northoff et al., 2007). As Glutamate and GABA are mediating neuronal activity (Logothetis, 2008) its concentrations have been repeatedly related to fMRI signal (see Duncan et al., 2014 for a review). Regarding plasticity mechanisms, tDCS modulation of BOLD signal in a motor learning task was related to local baseline GABA concentration (Stagg et al., 2011), while, complementarily, we showed that DMN connectivity at baseline predicted GABA and Glutamate modulation in distal (posteriomedial) DMN areas. That suggests a complex relationship between baseline neurochemical and network properties and the responsiveness of the system to controlled induction of plasticity through more studies are needed to extract conclusive inferences. Altogether, multimodal and causal analysis will be of utmost importance to outline a comprehensive explanation of brain aging and the subsequent memory function decline.

Chapter 7: Conclusions

The main conclusions that can be derived from the studies of this thesis are the following:

1) Long-range functional networks specifically frontal and DMN networks are fundamental to understand memory function amd dysfunction in elders.

2) Antero-posterior DMN connectivity is reduced in aging and predicts memory performancefunction.

3) In elders, antero-posterior DMN connectivity is related to grey matter and white matter integrity in areas of high age-related susceptibility, comprising, but not limited, to DMN.

4) Left IFG exerts top-down influences over primary visual areas during semantic encoding, which might be related to the relative preservation of deep encoded material in elders.

5) Application of patterned TMS over encoding networks is able to induce physiological changes that outlast the period of stimulation in both local and distal areas as reflected by BOLD signal changes and neurotransmitters modulation.

6) Patterned TMS over memory networks shows state-dependency phenomenon both at rest and during tasks as the induced effect over the brain is moderated by intrinsinc network functional connectivity.

7) Neurotransmitters modulation assessed with MRS in response to patterned TMS is a useful tool to interrogate local plasticity properties critical areas for healthy and pathological aging.

Resum de la tesis

Els països desenvolupats estan experimentant increments substancials en la proporció de població envellida (Cohen, 2003). La vellesa comporta un deteriorament tant cognitiu com motor, i, més important, representa el major factor de risc per a varies malalties neurodegeneratives com la malaltia de Alzhèimer (AD; Hebert et al., 2003). És d'una importància socioeconòmica primordial promoure la independència funcional i la qualitat de vida en la vellesa. Donat que en aquests moments el declivi neurocognitiu constitueix el major perill per a un envelliment sà en la nostre societat, és absolutament necessari conèixer aquells factors que es relacionen amb fragilitat o protecció neurocognitiva com n'és l'expressió de les xarxes cerebrals que sostenen la cognició.

La psicologia cognitiva ha caracteritzat durant dècades els canvis cognitius en l'envelliment (Glisky, 2007; Park and Schwarz, 1999) entre els quals destaca la memòria episòdica a llarg termini (Craik et al., 1987; Zacks et al., 2000) afectada des de principis de l'edat adulta (Park and Reuter-Lorenz, 2009). La memòria episòdica fa referència a aquella memòria relacionada amb successos autobiogràfics que han ocorregut en un lloc i un moment particular i que es poden evocar de forma explícita. El declivi mnèsic en l'envelliment està afectat en els diferents processos de formació de memòria com són la codificació, l'emmagatzematge i el record. No obstant diferents tipus de subprocessos, com pot ser la forma (nivell de profunditat; Craik and Lockhart, 1972; Logan et al., 2002) en que un estímul s'ha adquirit moderarà l'afectació deguda l'edat. Diferents estructures, entre les quals destaquen les del lòbul temporal medial (MTL; hipocamp, parahippocamp, còrtex entorrinal), del còrtex prefrontal (PFC) i les estructures de la Default-Mode Network (DMN) són fonamentals per a la memòria episòdica. Per altre banda, les tècniques de neuroimatge han permès caracteritzar el cervell envellit per un aprimament cortical, reduccions volumètriques de substancia gris, expansió ventricular, densitat reduïda de substancia blanca, caiguda de neurotransmissors i alteracions en l'expressió funcional del cervell. No obstant, part dels canvis produïts durant l'envelliment són

de caràcter compensatori a degut a la manifestació de mecanismes de plasticitat (Grady, 2012b). El cervell és un òrgan dinàmic i adaptatiu que intenta mantenir un control cognitiu *homeostàtic* i que canvia de forma dinàmica durant de la vida a través de la promoció de mecanismes de plasticitat. En l'envelliment aquests canvis tracten de mantenir la funció cerebral davant els insults biològics produïts per l'edat a través de reorganitzacions de xarxes o el reclutament de regions addicionals durant tasques cognitives (Freitas et al., 2013).

La neurociència cognitiva en l'envelliment a tractat d'unir els coneixements tant de la neurociència con de la psicologia cognitiva al estudiar aquells mecanismes cerebrals associats a canvis cognitius en la vellesa; és a dir, comprendre i explicar quins són els mecanismes biològics subjacents associats al declivi o a la preservació de la funcions cognitives en ancians. A nivell estructural la pèrdua de memòria episòdica en la vellesa s'ha associat a alteracions de substancia grisa tant en regions prefrontals com en regions temporals medials i a una pèrdua d'integritat entre aquestes dos àrees. A nivell funcional la ressonància magnètica funcional (fMRI) ha resultat una tècnica de gran ajuda per l'alteració de l'expressió de les xarxes en l'edat. Inicialment el PFC generà un interès especial, ja que en aquestes regions s'hi ha observat consistentment canvis de tipus compensatori, essencialment augments de reclutament neural en tasques mnèsiques, interpretats com a mecanismes de resposta a la pèrdua de capacitats cerebrals implicats en el manteniment de la funció cognitiva (Cabeza et al., 2002; Spreng et al., 2010). Tanmateix aquests mecanismes compensatoris no son necessariament exitosos sinó que poden resultar fallits o, fins i tot, aberrants. Recentment, s'han relacionat les pèrdues de memòria en l'envelliment i el deteriorament de la DMN (Andrews-Hanna et al., 2007; He et al., 2012; Sala-Llonch et al., 2014b). La DMN es defineix com una xarxa neuronals a gran escala (RSN), és a dir, com un conjunt de regions cerebrals la activitat de les quals fluctua sincrònicament al llarg del temps (Damoiseaux et al., 2006; Fox et al., 2005), que compren estructures posteriors medials, parietals inferior laterals, estructures temporals medials i mitges i el còrtex prefrontal medial (Laird et al., 2009). Significativament, aquestes regions cerebrals estan fortament relacionades amb la AD i la deposició de βeta-Amiloide (βA), així com els seus principals nodes són

regions claus en el *conectome* cerebral (Buckner et al., 2009; van den Heuvel and Sporns, 2013). Les alteracions de la DMN han estat generalment avaluades en contexts lliures de tasca, en *resting-state (*rs-fMRI); no obstant, les disfuncions són també evidents durant la realització de tasques cognitives, sent una menor desactivació d'aquestes àrees davant de tasques de codificació mnèsica una observació paradigmàtica (Miller et al., 2008). En els darrers anys, han aparegut estudis multimodals que permeten analitzar conjuntament informació estructural i funcional i representen una excitant aproximació alhora de proporcionar una explicació comprensiva de l'envelliment cerebral (Andrews-Hanna et al., 2007; Daselaar et al., 2013). La connectivitat avaluada a través de rs-fMRI, sembla esta fortament mediada per varis factors com poden ésser la integritat de la substancia blanca, la gris, els dèficits dopaminèrgics i la deposició de βA (Ferreira and Busatto, 2013).

La plasticitat pot ésser conceptualitzada com aquella propietat intrínseca del cervell humà que permet al sistema nerviós sobreposar-se a les limitacions del genoma i adaptar-se a les pressions ambientals, als canvis fisiològics i a les experiències (Oberman and Pascual-Leone, 2013; Pascual-Leone et al., 2005). No és possible, doncs, entendre el funcionament psicològic normal sense invocar el concepte de plasticitat cerebral, sent aquest darrer el mecanisme essencial per l'aprenentatge i el desenvolupament. En l'envelliment la plasticitat local es redueix (Freitas et al., 2011b), no obstant, les conseqüències d'aquesta a nivell macrostructural són evidents com és el cas dels canvis compensatoris prefrontals anteriorment esmentats (Grady, 2012b). Mentre els mecanismes de plasticitat són essencials per a adaptar-se a les demandes també poden conduir a patrons d'adaptació fallits o patològics (Oberman and Pascual-Leone, 2013). En aquest sentit un sistema capaç de reorganitzacions flexibles també pateix el risc d'arranjaments corticals indesitjats que poden comportar patologia cerebral. Contextualitzat en l'envelliment, s'ha proposat que la DMN, com a àrea central del *conectome* i clau per a la formació de noves memòries, és una de les àrees amb més necessitat d'utilitzar mecanismes de plasticitat i, com a conseqüència, més susceptible de ser afectada per processos aberrants d'origen plàstic (Fjell et al., 2014). L'estudi dels mecanismes de plasticitat

amb l'edat s'ha realitzat clàssicament amb animals on s'han descrit nombroses alteracions en els mecanismes de potenciació i depressió a llarg termini (LTP, LTD; Barnes, 2003). Tanmateix en humans s'ha beneficiat enormement de la introducció de les tècniques d'estimulació cerebral no-invasiva (NIBS) ja que permeten la caracterització de la plasticitat local i de xarxes de manera experimental causal i no invasiva (Oberman and Pascual-Leone, 2013).

Entre les NIBS, destaca la Estimulació Magnètica Transcranial (TMS) que es basa en el principi de la inducció electromagnètica de Faraday actuant a través de l'aplicació de breus camps magnètics en una bobina que traspassen el crani i provoquen la despolarització de les neurones subjacents (Wagner et al., 2007). Els mecanismes d'acció, pobrament dilucidats, suggereixen un impacte de la estimulació sobre els límits axonals (Huerta and Volpe, 2009; Ridding and Rothwell, 2007). La mesura més directa dels efectes de la TMS s'obté en el sistema motor a través del registre dels potencials evocats motors. No obstant, combinada amb altres tècniques com la fMRI o la RM per espectroscòpia (MRS) permeten mesurar canvis cerebrals associats a la TMS fora el còrtex motor. La TMS aplicada de forma repetitiva és capaç d'induir modificacions transitòries de l'activitat del còrtex subjacent que es mantenen durant varis minuts després de l'estimulació i que es reflecteixen per canvis de magnitud dels potencial evocats, per l'expressió de les xarxes cerebrals o per canvis en mecanismes cel·lulars, sinàptics i moleculars. Les bases neurofisiològiques dels mecanismes que governen les respostes cerebrals de la TMS repetitiva es poden entendre dins el marc de canvis que reflecteixen variacions de plasticitat cortical (Di Lazzaro, 2013; Thickbroom, 2007). Nous paradigmes d'estimulació, com la Theta-Burst Stimulation (TBS) s'han desenvolupat amb la finalitat d'emular els mecanismes similars als de LTP i LTD amb més precisió (Huang et al., 2005). Estudis fisiològics i farmacològics amb TBS també suggereixen una implicació glutamatèrgica i GABAèrgica similar als fenòmens de plasticitat sinàptica (Huang et al., 2007; Stagg et al., 2009a).

Els canvis transitoris degut a l'aplicació de NIBS en l'activitat regional cortical poden provocar canvis conductuals, cognitius i emocionals (Guse et al., 2010). Un dels dominis cognitius alterats a través de la TMS són els processos de codificació i

reconeixement mnèsic així com les xarxes que suporten aquests processos (Rossi et al., 2004). Varis estudis han aconseguit modular exitosament a través de l'estimulació el rendiment cognitiu de persones envellides (Manenti et al., 2013a; Solé-Padullés et al., 2006). Així mateix també permet estudiar de forma causal la implicació de certes regions en processos cognitius. En el cas de la memòria l'ús de les NIBS s'ha focalitzat en la implicació del PFC (Blumenfeld et al., 2014). Com exemples, n'és la observació d'una implicació bihemisfèrica de la memòria en l'envelliment (Rossi et al., 2004) o la selectiva implicació del PFC en processos de codificació semàntica (Innocenti et al, 2010). Així mateix, la TMS és una eina útil per a avaluar l'estat dels mecanismes de plasticitat cortical *local.* Varis estudis han demostrat, en correspondència amb la literatura animal, disminucions dels mecanismes de plasticitat durant l'envelliment en humans (Freitas et al, 2011). Així mateix els mecanismes de plasticitat en subjectes amb envelliment patològic estan clarament alterats. L'avaluació de l'estat plàstic del cervell pot servir com una marcador de deteriorament. Així mateix la modulació de la plasticitat cerebral pot minimitzar, retardar o prevenir parcialment simptomatologia associada a aquests estats.

A nivell macroestructural la combinació de la TMS amb tècniques de neuroimatge ha permès estudiar el impacte de l'estimulació en l'expressió de les xarxes ajudant a entendre els mecanismes subjacents als canvis cognitius (Reithler et al., 2011b; Stagg et al., 2010b). Entre les aportacions destaca el fet que el impacte de la TMS s'observa també en àrees interconnectades a la zona estimulada degut a mecanismes transinàptics. També cal destacar l'efecte modulatori de les característiques individuals del subjecte i l'estat específic del sistema estimulat sobre l'efecte de la TMS. El impacte de l'estat del sistema sobre l'efecte de l'estimulació s'engloba sota el concepte de l'*state-dependency* (Silvanto et al., 2008). La fMRI s'han mostrat útil alhora de revelar canvis tant d'activació com de connectivitat en xarxes després de la inducció controlada de plasticitat (Eldaief et al., 2011; Peña-Gómez et al., 2012; Polanía et al., 2011; Solé-Padullés et al., 2006) que poden ajudar a entendre i predir canvis cerebrals i cognitius de l'estimulació. Una altre eina a destacar és la MRS que permet mesurar la concentració de metabòlits, incloent els neurotransmissors GABA i glutamat, en una regió determinada del cervell a través

d'espectres de domini de freqüència. La possibilitat de mesurar concentració de neurotransmissors *in vivo* en resposta a la TMS permet una aproximació més propera a la fisiologia i altament relacionada amb els mecanismes de plasticitat cerebral. Paral·lelament, s'han trobat relacions entre els nivells de GABA i Glx (compost de Glutamat i Glutamina) i la senyal BOLD tant en repòs com davant de demandes cognitives (Hu et al., 2013; Kapogiannis et al., 2013; Muthukumaraswamy et al., 2009). Així mateix s'han trobat canvis de neurotransmissors en resposta a l'aplicació de NIBS (Clark et al., 2011; Stagg et al., 2009a, 2009b) indicant que la MRS representa una eina sensible a la inducció controlada de plasticitat.

OBJECTIUS

L'objectiu principal d'aquesta tesis és aprofundir en la caracterització i la modulació de les xarxes a gran escala implicades en la memòria episòdica a llarg termini focalitzat en les implicacions i aplicacions en l'envelliment cognitiu a través de l'ús multimodal de tècniques de neuroimatge i la inducció controlada, de forma experimental, de mecanismes de plasticitat cortical. Un segon objectiu és revelar la relació entre els mecanismes de plasticitat, estudiats a través de la TBS, i la connectivitat intrínseca del cervell avaluada a través de la fMRI.

Per a assolir aquest objectius s'han realitzat tres estudis, breument detallats a continuació:

Estudi I

L'objectiu d'aquest estudi s'ha centrat en la modulació, a través de l'aplicació de TMS, del rendiment en una tasca de memòria episòdica així com de les xarxes neurals que suporten aquesta funció a través de l'aplicació de TBS.

Estudi II

Aquest estudi s'ha focalitzat en la avaluació de la relació entre la connectivitat entre el còrtex prefrontal medial i l'escorça posteriomedial pertanyents ambdós a la DMN i mesures de perfusió, integritat de la substancia blanca i de la substancia gris en subjectes amb envelliment sà.

Estudi III

L'objectiu d'aquest estudi s'ha enfocat en la modulació de la concentració de neurotransmissors en nodes locals i distals de la DMN (inferior parietal esquerre i precuneus) a través de la inducció controlada de plasticitat i avaluat a través de la MRS.

MÈTODES/RESULTATS

Estudi I:

S'ha realitzat un disseny experimental on s'ha aplicat TBS intermitent (controlada per un grup amb estimulació fictícia) entremig de dos adquisicions de fMRI en repòs i durant una tasca de memòria episòdica amb dos nivells de processament (semàntic/perceptual) en una mostra de vells sans. Es buscà un efecte de la estimulació tant a nivell cognitiu, rendiment en la tasca mnèsica, com a nivell de xarxes implicades en les funcions de memòria.

Els principals resultats després d'aplicar TBS intermitent en subjectes envellits són els següents: A) la TBS intermitent aplicada al gir inferior frontal esquerre no va produir canvis conductuals en una tasca de memòria. B) No obstant, canvis funcionals degut a la TMS es trobarem específicament quan els subjectes realitzaven tasques amb un nivell de processament profund/semàntic tant en zones locals com distals. C) Les àrees afectades per la TMS són regions que pertanyen a xarxes de memòria episòdica. D) Els efectes de la TMS estaven modulats per mesures de canvi de connectivitat dependent de la tasca avaluades amb la fMRI en estat basal.

Estudi II

S'ha analitzat una mostra relativament gran d'imatges de ressonància magnètica multimodals provinents de una població relativament homogènia de subjectes amb envelliment sà. Així mateix s'ha utilitzat una mostra secundaria, parcialment analitzada en altres estudis del laboratori (Sala-Llonch et al., 2014b) amb l'objectiu de fer comparacions d'edat. Es va adquirir en tots els subjectes seqüències de neuroimatge que incloïen informació estructural, de difusió, de perfusió i senyal BOLD en repòs. Es van realitzar correlacions de la connectivitat de la DMN amb mesures de cognició, mesures de perfusió, de integritat de substancia gris i de integritat de substancia blanca.

Els principals resultats de les correlacions entre la connectivitat de la DMN i mesures de cognició i integritat cerebral en una mostra de vells sans són: A) La connectivitat anteroposterior de la DMN està clarament reduïda en l'edat. B) En l'edat aquesta mesura de connectivitat es relaciona amb la cognició, especialment amb mesures de memòria. C) La connectivitat anteroposterior de la DMN es relaciona amb mesures d'integritat de substancia gris i blanca en varies regions cerebrals, no limitades a la DMN. D) Les mesures de perfusió no estan relaciones amb la connectivitat de la DMN. E) Els correlats estructurals de la connectivitat anteroposterior de la DMN es troben en àrees de alta vulnerabilitat a l'edat.

Estudi III

Es va aplicar TBS (intermitent, contínua i fictícia) al lòbul inferior parietal esquerre enmig de dos sessions de ressonància magnètica que incloïen rs-fMRI i MRS amb vòxels situats en nodes de la DMN (inferior parietal esquerre i precuneus). Es realitzaren comparacions per avaluar el impacte de l'estimulació sobre la concentració de neurotransmissors i la seva relació amb la connectivitat intrínseca.

El resultats d'aquest estudi mostren per primera vegada evidencies d'una modulació controlada dels neurotransmissors a la DMN a través de tècniques d'estimulació inductores de plasticitat. Cal ressaltar dos resultats principals: A) La TBS aplicada al inferior parietal esquerre induí canvis de neurotransmissors tant locals com distals. Concretament la TBS contínua provocà un augment local de glutamat mentre que la TBS intermitent provocà un augment de GABA al precuneus, el vòxel espectroscòpic distal. B) La magnitud de la modulació distal dels neurotransmissors s'associà a la força de la connectivitat basal entre l'àrea on s'aplicà l'estimulació i l'àrea espectroscòpica.

DISCUSSIÓ

Caracterització de les xarxes de memoria i de les alteracions degudes a l'edat

L'objectiu d'aquesta tesi va ser caracteritzar i modular les xarxes cerebrals a gran escala implicades en la memòria episòdica a llarg termini centrat en les implicacions

per a l'envelliment cognitiu a través de tècniques de neuroimatge i TMS repetitiva. L'assumpció que la conducta i la cognició sorgeixen de les interaccions entre els diferents sistemes cerebrals (Bressler and Menon, 2010) és cada vegada més acceptat sent la fMRI una tècnica òptima per a estudiar el cervell des d'aquesta perspectiva. Pel que fa a la memòria, diversos circuits cerebrals s'han destacat com, per exemple, les interaccions entre el MTL i el PFC o la DMN (Andrews-Hanna et al., 2007; Grady et al., 2003). L'envelliment sà s'acompanya de disfuncions en la connectivitat i l'activitat de les RSN que suporten la memòria, tant en estat de repòs com durant tasques (Ferreira and Busatto, 2013; Grady, 2012b). En aquest sentit, les regions prefrontals connectades a àrees temporals i posteriors i la DMN es mostren a la vegada alterades en l'envelliment i relacionades amb la memòria. Aquesta tesis ha expandit aquestes evidències a través de l'estudi del rol específic del PFC en una tasca de codificació episòdica amb diferents nivells de processament i de la caracterització de les alteracions DMN en l'envelliment.

El PFC ha estat àmpliament estudiat en el camp de la neurociència cognitiva en l'envelliment ja que s'ha identificat com una àrea altament involucrada amb mecanismes de plasticitat de tipus compensatori (Cabeza et al., 2002) . En l'Estudi I, s'investigà la participació del PFC en diferents processos de codificació episòdica i la seva influencia sobre les xarxes mnèsiques. Concretament, s'estudià la implicació del còrtex inferior frontal esquerre durant una codificació de material verbal visual amb dos nivells de profunditat: profund (semàntica) i superficial (perceptual) per mitjà de la TBS. A través de la fMRI s'ha destacat la rellevància del còrtex inferior frontal esquerra en processos de codificació semàntica juntament amb el còrtex prefrontal dorsolateral (Kapur et al., 1994; Otten et al., 2001; Wagner et al., 1998). L'aplicació de la TMS ha demostrat de forma causal la implicació específica del còrtex prefrontal dorsolateral en els processos de codificació profundes, ja que el material codificat semànticament s'interferia de forma específica degut a estimulació *on-line* (Innocenti et al., 2010). Tanmateix, els nostres resultats no van mostrar efectes conductuals en resposta a la TMS com s'havia hipotetitzat d'acord a estudis previs del nostre grup (Solé-Padullés et al., 2006). En canvi, es van trobar efectes dependents de tasca de la TMS reflectits com augments d'activitat fMRI en regions

locals i distals. És a dir, la TBS aplicada al còrtex inferior frontal esquerra afecta a les xarxes mnèsiques únicament quan els subjectes codifiquen estímuls semànticament. Aquests resultats concorden amb el rol específic del PFC en processos de codificació profunda (Innocenti et al., 2010), així com amb el paper de còrtex inferior frontal esquerra en el control semàntic (Jefferies, 2013). Aquesta regió sembla exercir de forma selectiva modulació tipus *top-down*, a àrees posteriors, relativament inespecífiques situades jeràrquicament abans que les àrees visuals implicades en el reconeixement semàntic d'acord amb varis estudis de neuroimatge (Çukur et al., 2013; Kherif et al., 2011; Urooj et al., 2013). Aquesta modulació tipus *top*-down podria reflectir tant un canvi atencional que facilitaria la codificació profunda com unes influencies tipus *feedforward* que conduirien a un major reconeixement durant codificació de tipus semàntic. El declivi de la memòria en l'envelliment és més gran en nivells de processaments menys profund que en codificacions semàntiques. Tanmateix, els vells semblen tenir problemes per reclutar espontàniament estratègies semàntiques que, en tot cas, són susceptibles de ser entrenades (Kirchhoff et al., 2012; Logan et al., 2002). La modulació *top*-down durant el processament semàntic podria ser un mecanisme de compensació pel qual els ancians poden mantenir el rendiment cognitiu durant taques de memòria. Els mecanismes *top-down* poden canviar i adaptar-se durant el transcurs vital en un intent de mantenir la funció. Per exemple, durant una tasca de memòria verbal, els subjectes envellits mostraren un augment de la connectivitat frontal-parahippocampal en contrast amb una reducció de la frontal-hipocampal interpretat com a evidència compensatòria (Daselaar et al., 2006). D'aquesta manera, dotar a ancians d'estratègies de codificació profunda pot ajudar a preservar la funció mnèsica degut a la influència *top-down* exercida pel còrtex inferior frontal esquerra específicament en codificacions semàntiques. El coneixement dels processos biològics que suporten la codificació exitosa de nou material en l'envelliment és necessària per a poder elaborar estratègies eficaces i obtenir modificacions funcionals de les xarxes de memòria i, conseqüentment, beneficis cognitius.

Els estudi II i III es van centrar en la DMN que, avaluada en rs-fMRI, es mostra molt susceptible als efectes de l'envelliment (Ferreira and Busatto, 2013; Hafkemeijer et

al., 2012). La integritat de la DMN, especialment la connectivitat inter-nodal, ha estat repetidament relacionada amb les funcions de memòria especialment en poblacions envellides (Andrews-Hanna et al., 2007; He et al., 2012; Sala-Llonch et al., 2014b; Wang et al., 2010a). Els nostres resultats en l'Estudi II ampliaren aquestes anteriors observacions amb una mostra gran i homogènia de vells demostrant que la connectivitat anteroposterior de la DMN es veu altament alterada en l'envelliment i que, en els ancians, aquesta connectivitat és predictiva de la funció de mnèsica. Els resultats mostren que la sincronia entre els nuclis prefrontals medials i posteriomedials de la DMN estan especialment afectats per l'edat, amb harmonia amb altres estudis (Andrews-Hanna et al., 2007; Campbell et al., 2013).

La desconnexió de la DMN en l'envelliment no sembla reflectir mecanismes compensatoris (reeixits); més aviat aquesta desconnexió sembla ser un reflex d'un deteriorament cerebral i un processament ineficient. La sincronia reduïda en repòs podria reflectir una distribució ineficient dels recursos neuronals necessaris pel correcte funcionament del cervell durant tasques de memòria episòdica (Stevens et al., 2008). La desconnexió de la DMN en l'envelliment es pot interpretar com un dèficit del sistema nerviós alhora de canviar la seva configuració de forma dinàmica i adaptar-se a les demandes externes i, en conseqüència, pot esser fàcilment vinculat a alteracions dels mecanismes de plasticitat (Fjell et al., 2014; Freitas et al., 2013; Oberman and Pascual-Leone, 2013). La hipòtesis que les regions caracteritzades per un alt grau de plasticitat al llarg de la vida són més vulnerables als efectes perjudicials de l'envelliment normal (i patològic; Fjell et al., 2014; Mesulam, 1999) és una idea provocadora molt coherent amb els nostres resultats. La plasticitat pot ésser adaptativa o maladaptativa i representar un factor de risc patològic. Àrees de la DMN es troben entre aquelles amb més capacitat neuroplàstica en l'escorça cerebral ja que tenen un paper central en el funcionament del cervell i específicament en processos d'aprenentatge i memòria. Aquesta alta capacitat plàstica pot convertir la DMN en un sistema especialment vulnerable al llarg de la vida (Fjell et al., 2014) mentre que la seva desconnexió podria representar un marcador primerenc d'envelliment del cervell, parcialment explicat per l'aparició de mecanismes de plasticitat maladaptatius.

Inducció controlada de plasticitat cortical en xarxes de memòria: Avaluació de la circuiteria i de la plasticitat *local*

La literatura animal ha revelat que part dels substrats neurobiològics subjacents al deteriorament cognitiu associat a l'edat estan associats a alteracions de la plasticitat sinàptica, com ara l'equilibri entre els llindars de LTP i LTD o la accelerada disminució de la durada del LTP (Barnes, 2003, 1979; Burke and Barnes, 2006).

Mentre que la fMRI s'ha convertit en una eina útil per a investigar les conseqüències de la plasticitat en els éssers humans, la TMS repetitiva és, a més, capaç d'explorar *in vivo* l'estat d'aquests mecanismes. La TMS és capaç de induir mecanismes de plasticitat de forma no invasiva i controlable i fer-ne ús per a obtenir millores conductuals i cognitives (Oberman and Pascual-Leone, 2013). Tanmateix, en l'Estudi I la manca de resultats conductuals (prèviament hipotetitzada, SolèSolé-Padullés et al., 2006) permeten interpretar els canvis en l'expressió de les xarxes degut a la TMS com un *marcador fisiològic* i discutir aquestes modulacions en les xarxes respecte l'existència d'un rol específic del còrtex inferior frontal esquerra en tasques de codificació semàntica (vegeu secció anterior).

En contrast, en l'Estudi III hem utilitzat la TBS per avaluar l'estat dels mecanismes *locals* de plasticitat. Mentre aquests mecanismes són intrínsecs al funcionament del cervell, la capacitat de canviar i adaptar-se resulta cada vegada menys eficient amb l'edat (Freitas et al., 2013). Varis estudis han replicat en humans aquests disfuncions estudiades prèviament en la literatura animal sobretot a través de l'aplicació de TMS sobre la escorça sensoriomotora). En aquesta tesis hem demostrat per primera vegada la viabilitat d'avaluar en éssers humans l'estat de mecanismes *locals* plasticitat en nuclis centrals de la DMN mitjançant la modulació de la concentració dels neurotransmissors GABA i Glutamat després de l'aplicació de TBS. Específicament, la modulació de la concentració de GABA en l'escorça posteriomedial representa una oportunitat única per examinar els mecanismes de plasticitat *locals* en una àrea crucial per a l'envelliment sà i patològic. S'ha hipotetitzat que la disminució de la plasticitat podria estar vinculada inversament en les modificacions macroestructurals observades en la fMRI, aparegudes per a compensar la falta de flexibilitat sinàptica ((Freitas et al., 2013). Encara més crucial

és la relació circular entre els mecanismes de plasticitat sinàptica i la proteïna ßA, que segueix un patró d'agregació estretament sobreposat a la topografia de la DMN (Buckner et al., 2005). Els oligòmers de Aß, altament involucrats en la patogènesi d'AD, interrompen la transmissió glutamatèrgica (Hsia et al., 1999) i la circuitaria inhibitoria (Ulfig, 1988); per contra, l'activitat neuronal regula les concentracions de Aß (Kamenetz et al., 2003). Els nivells anormals d'activitat sinàptica poden conduir a la secreció de Aß tòxics (Bero et al., 2011) suggerint que els mecanismes de plasticitat i la Aß formen part d'un mateix mecanisme de regulació de l'activitat sinàptica clarament alterada en la AD (Palop and Mucke, 2010). En aquesta tesis es defensa l'ús de la TMS com una aproximació novedosa per a estudiar la plasticitat en una regió cortical críticament afectada tant per l'envelliment sa com per l'agregació de Aß ((Buckner et al., 2009, 2005). Pararel·lament la tècnica de MRS-GABA en combinació amb la TMS representa una tècnica útil per a estudiar els mecanismes de plasticitat aberrants en àrees fora de l'escorça motora (Marjańska et al., 2013).

Aquest treball també contribueix a la comprensió de l'efecte de la TBS sobre el cervell. La literatura de NIBS ha establert tant que els efectes de la TMS es propaguen transinàpticament a zones distals (Bestmann et al., 2008) com que els seus efectes mostren el fenomen de *state-dependency*; és a dir: que el impacte de la TMS sobre el cervell depèn de l'estat específic del sistema quan és estimulat (Silvanto et al., 2008). Ambdós estudis van demostrar efectes distals de la TBS reflectides com un canvi en la senyal BOLD i com una modulació de neurotransmissors respectivament. Així mateix ambdós estudis van mostrar efectes *d'state-dependency* ja que els efectes de la TMS depenien de la connectivitat funcional entre l'àrea estimulada i l'àrea on es van avaluar els seus efectes. Mentre que aquests resultats representen una novetat, un per la mesura utilitzada, d'interacció psicofisiològica, i l'altre per la correlació amb la modulació neuroquímica, els resultats són altament congruents amb un seguit d'estudis que han posat de relleu el paper de la connectivitat funcional com a variable moderadora dels efectes de la TMS (Cárdenas-Morales et al., 2013; Fox et al., 2012b). El impacte de la TMS sobre el cervell i la conducta resulta mediatitzat tant per mesures de connectivitat directa (Andoh and Zatorre, 2013; Nettekoven et al., 2014) com per

circuits compensatoris indirectes (Cárdenas-Morales et al., 2013; Lee and D'Esposito, 2012). En conseqüència la connectivitat intrínseca s'ha proposat com un mètode per millorar la localització i eficàcia de la TMS (Eldaief et al., 2011; Fox et al., 2012b; Halko et al., 2010) així com utilitzar la modulació de la connectivitat intrínseca per a condicionar els efectes de la TMS.

Per entendre tant el declivi com la preservació de la memòria en l'envelliment és absolutament necessari invocar el concepte de plasticitat. La plasticitat sinàptica no només està intrínsecament lligada a l'aprenentatge i a la memòria, sinó que la disminució de la plasticitat i l'aparició de fenòmens compensatoris a gran escala són clarament presents en l'envelliment i determinen el funcionament de les xarxes mnèsiques i, darrerament, la funció cognitiva. Estudiar, en els pròxims anys, com els mecanismes de plasticitat es manifesten en diferents nivells d'expressió serà necessari per a obtenir un coneixement comprensiu del envelliment cerebral. Les NIBS i, específicament, la TBS, representa una tècnica única capaç d'interrogar, en xarxes implicades en la memòria, l'estat dels mecanismes de plasticitat i la seves conseqüències així com una eina científica potent utilitzada en combinació amb altres tècniques de neuroimatge.

Anàlisis multimodals de les xarxes de memòria en l'envelliment

L'envelliment cerebral és un fenomen complex i multifactorial caracteritzat per canvis a diferents nivells des de disfuncions cel·lulars i sinàptiques a modificacions macroestructurals dels *conectomes* funcional i estructural. Tot i que la senyal BOLD proporciona una mesura subrogada de l'activitat cerebral permetent l'estudi del cervell com una interacció dinàmica de diferent regions cerebrals, les observacions amb la fMRI per si soles sovint són d'una interpretabilitat limitada, a banda que no es pot inferir la causalitat (Logothetis, 2008). En conseqüència han començat a aparèixer estudis multimodals en l'envelliment en els quals l'expressió de les xarxes es relaciona amb altres mesures com ara de neurotransmissors (Duncan et al., 2014) o de integritat estructural (Greicius et al., 2009) i que permeten interpretacions més complexes del envelliment cerebral.

Tots els estudis d'aquesta tesis s'han realitzat amb un enfocament multimodal. En l'estudi II, es van utilitzar índexs de perfusió i de integritat de substància blanca i substància grisa en un intent de proporcionar una descripció més completa de les disfuncions de la connectivitat en l'envelliment ja que el coneixement de les causes i conseqüències d'aquesta alteració són limitades. La integritat de la substancia blanca i grisa estava fortament relacionada amb la integritat de la DMN mentre que la perfusió no es va relacionar amb les propietats funcionals d'aquesta xarxa. La integritat de la substancia grisa que correlacionà amb la DMN es van limitar a zones posteriors suggerint que la desconnexió produeix un patró diferent de deteriorament de la substància grisa en àrees frontals i posteriors (Chen et al., 2011; Li et al., 2013) i destacant la rellevància de les àrees posteriomedial com a node central de la DMN (Campbell et al., 2013; de Pasquale et al., 2012). Les correlacions més rellevants de la connectivitat anteroposterior de la DMN fou amb les mesures d'integritat de substancia blanca. La integritat de diversos tractes anteroposteriors, incloent part del tracte cingulat, es trobà correlacionat amb la connectivitat DMN suggerint que la integritat estructural està parcialment suportant la funcionalitat de la DMN (Khalsa et al., 2013; van den Heuvel et al., 2008). No obstant això, els resultats suggereixen que altres tractes, a banda del cingulat, estan relacionats amb el manteniment de la connectivitat de la DMN en concordança amb altres estudis (Luo et al., 2012; Teipel et al., 2010). Els resultat més sorprenents va ser la forta concordança topològica que van mostrar les mesures de contrast entre substancia blanca i gris (GWC) que correlacionaven amb la DMN, és a dir, la connectivitat de la DMN es relacionava fortament amb els índexs de GWC de la pròpia DMN. Les mesures de GWC emblen reflectir en gran mesura la integritat de la substància blanca, finament mielinitzada prop dels límits de la substància grisa (Cho et al., 1997; Magnaldi et al., 1993; Westlye et al., 2009). En conseqüència, aquesta tipus de substancia blanca podria estar estretament associada amb el manteniment de la funció sincrònica entre les xarxes neurals, de la qual en depèn la cognició. Aquests resultats permeten obrir noves vies per explorar la connectivitat estructural-funcional ja que la substància blanca finament mielinitzada pateix un gran deteriorament en l'envelliment (Bartzokis et al., 2004; Tang et al., 1997). Els resultats també suggereixen que la connectivitat de la DMN podria estar present en etapes

primerenques d'envelliment abans que altres canvis macroestructurals s'evidenciïn. Aquesta interpretació està d'acord amb l'observació que subjectes de mitjana edat (Biswal et al., 2010; Bluhm et al., 2008; Evers et al., 2012) ja mostren alteracions de la DMN.

En els estudis I i III, l'ús de la TMS juntament amb la fMRI ha permès inferir causalitat als canvis de senyal BOLD, proporcionant un major poder explicatiu dels resultats. En l'estudi III, a més s'ha relacionat la connectivitat de la DMN amb la modulació neuroquímica. La possibilitat de quantificar els principals neurotransmissors excitatoris i inhibitoris del cervell *in vivo,* a través de la MRS, permet relacionar aquests neuroquímics amb propietats d'expressió dels circuits (Duncan et al., 2014). Pel que fa als mecanismes de plasticitat, la modulació de la senyal BOLD degut a l'aplicació d'estimulació elèctrica transcranial durant una tasca d'aprenentatge motor s'ha relacionat amb la concentració de GABA en estat basal (Stagg et al., 2011), mentre que, de forma complementària, en un estudi de la present tesis s'ha demostrat el impacte de la connectivitat basal de la DMN sobre les modulacions de GABA i glutamat en nodes distals de la DMN. Tot plegat suggereix una complexa relació entre les propietats neuroquímiques i de xarxes a nivell basal i la capacitat de resposta del sistema davant de paradigmes inductors de plasticitat cerebral malgrat es requereixen més estudis per a inferir conclusions fiables. En resum, l'anàlisi multimodal i la possibilitat d'inferir causalitat són característiques de summa importància per a poder realitzar una explicació comprensiva i exhaustiva del envelliment del cervell i la conseqüent disminució de la funció mnèsica.

CONCLUSIONS

Les principals conclusions derivades dels estudis que conformen aquesta tesis són els següents:

1) Les xarxes cerebrals a gran escala, específicament les xarxes frontals i de la DMN són fonamentals per a poder comprendre el declivi i preservació de la memòria episòdica en l'envelliment.

2) La connectivitat anteroposterior de la DMN està disminuïda en l'edat i prediu la funció mnèsica.
3) En subjectes envellits, la connectivitat anteroposterior de la DMN es relaciona amb la integritat de la substancia gris i blanca en àrees de gran susceptibilitat als efectes de l'edat que compren, però no es limita, a la topologia de la DMN.
4) El còrtex inferior frontal esquerra exerceix influències de caràcter *top-down* sobre àrees visuals primàries específicament durant codificacions de tipus semàntic que sembla estar relacionat amb la preservació de material codificat de forma profunda en l'envelliment
5) L'aplicació de TBS sobre xarxes relacionades amb la codificació verbal es capaç d'induir canvis fisiològics que perduren al període d'estimulació tant en regions locals con distals reflectit a través de canvis en la senyal BOLD i de modulacions de la concentració de neurotransmissors.
6) L'efecte de la TBS sobre les xarxes de memòria mostra un efecte d'*state-dependency* degut a la connectivitat intrínseca del cervell tant en repòs com durant tasques mnèsiques.
7) La modulació de neurotransmissors en resposta a la TBS avaluada amb MRS és una tècnica útil per avaluar l'estat dels mecanismes *locals* de plasticitat en àrees crítiques per l'envelliment sà i patològic.

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