FUNCTION AND BRAIN STRUCTURE IN AGING WITH AND WITHOUT COGNITIVE IMPAIRMENT

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Present work contains four studies that have been carried out in the Neuropsychology Research Group of the Psychiatry and Clinical Psychobiology Department at the Faculty of Medicine, University of Barcelona. This group, lead by Prof. Carme Junqué, belongs to the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS). The following studies have been partially funded by a Spanish Ministerio de Educación y Culutra research project award (SEJ2004-06710/PSIC) to D. Bartrés Faz, as well as with grants from the University of Barcelona to C. Solé Padullés.

Envellir és com escalar una gran muntanya;
mentre es puja les forces minven, però la mirada és més lliure,
la vista més àmplia i serena.
Ingmar Bergman

A la joventut aprenem, a la vellesa entenem.
Marie von Ebner Eschenbach

Els arbres més vells donen els fruits més dolços.
Proverbi alemany

Agraïments

Ben entrada la primavera de l'any 2001, dos anys després d'haver acabat la carrera, tot fullejant apunts vells de neuropsicologia, vaig recordar de sobte que era allò el que m'agradava, així que se'm va posar al cap que volia formar-me en neuropsicologia i d'aquesta petita i creixent obsessió va sorgir la necessitat de posar-me en acció. Em vaig posar en contacte amb la Dra. Junqué per mail, i a base d'insistir em vaig "colar" al departament. Així és com em sentia al principi. Una afortunada que havia aconseguit entrar. Vull agrair a la Dra. Carme Junqué que m'obrís la porta del seu equip, donant-me la oportunitat de formar-me al voltant de persones de les quals he après i m'han fet possible presentar avui aquesta tesi. La primera d'elles va ser la Dra. Imma Clemente. Amb ella vaig tenir el primer contacte cara a cara amb aquest món de la recerca i em va preparar pel que m'havia d'esperar. Gràcies Imma pels teus consells, per estar sempre allà, per fer-me de directora i d'amiga, per acompanyar-me amb cotxe a buscar pacients, pel bon humor que desprens passi el que passi, per la gran ajuda que em vas donar durant el DEA, no ho oblidaré, i per aquestes estones que hem passat al laboratori de genètica ampliant gens sense parar juntament amb la Mar Matarín, ara doctora, a la qual li agraeixo que em deixés aprofitar-me de les seves mostres per aprendre genètica; li dono les gràcies també per l'amistat durant els meus primers dos anys de tesi i ara per la seva companyia des de l'altra banda de l'Atlàntic.

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Al David

Page

Foreword

The present thesis to obtain the degree of Doctor through the Neuroscience Program offered by the University of Barcelona is the result of different studies that have been carried out at the Department of Psychiatry and Clinical Psychobiology, Faculty of Medicine. During the first 2 years of this work I have obtained the DEA (Diploma d'Estudis Avançats).

Four studies will be presented hereunder, two of which have been accepted for their publication into international journals.

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Glossary of Abbreviations

AACD	Age-Associated Cognitive Decline
AD	Alzheimer's disease
APOE	Apolipoprotein E
BA	Broadmann Area
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CR	Cognitive Reserve
fMRI	functional Magnetic Resonance Imaging
GM	Gray Matter
HRF	Hemodynamic Response Function
MCI	Mild Cognitive Impairment
MTL	Medial Temporal Lobe
PCR	Polimerase Chain Reaction
PFC	Prefrontal Cortex
PET	Positron Emission Tomography
TMS	Transcranial Magnetic Stimulation
VBM	Voxel-Based Morphometry
WM	White Matter

1. INTRODUCTION

1.1 NEUROSCIENCE OF COGNITIVE AGING

The term cognitive aging describes a pattern of mild age-related impairments in cognitive functions. Understanding age-associated changes in cognition is challenging for several reasons. First, it is often difficult to separate the effects of normal aging from those of pathological processes that compromise cognition. Most older adults experience some form of age-related neural pathology, because aging is strongly associated with risk for Alzheimer's disease, Parkinson's disease, diabetes, hypertension and arteriosclerosis. Further, although longitudinal studies of aging are not non-existent, most of research on cognitive aging is of a cross-sectional nature in order to avoid highly expensive and long studies. Cohort differences are group differences that result from historical influences, such as educational opportunity, cultural factors and socioeconomic status. Such issues are being taken into account in studies of a cognitive reserve approach, which will be discussed in a later section. Finally, many brain and mental changes occur during aging and correlational approaches have been trying to link which particular changes in the brain affect which cognitive functions. The research for causal relationships between age and cognition has led us to the highly accepted assumption that age is related to a change in some, but not all, neurocognitve functions (Hedden & Gabrieli, 2004).

1.1.1 Age-related cognitive and cerebral changes

A large body of behavioural research on the effects of aging shows that its influence in distinct aspects of cognition is not unitary. Some authors have recently considered at least three descriptive patterns of age-related change in cognitive behaviour: life-long declines, declines that occur late in life and relative stability across life (Hedden & Gabrieli, 2004). The contrasts among these patterns indicate that, although aging might have global effects, it influences certain cognitive functions disproportionately. Functions that are thought to be basic mechanisms of the cognitive information processing architecture, such as processing speed, working memory and encoding of information into episodic memory, tend to decline across the adult lifespan. Longitudinal comparisons performed from age 20 to 60 showed that those changes were small or non-existent, being the speed of processing the most affected variable before the age of 60. Thus, changes would be detected after 60

in all domains with a similar slope as that evidenced in cross-sectional studies. Longitudinal data from that study also reported a more rapid decline at older ages or around 3 years before death (see Hedden & Gabrieli for a review, 2004). This acceleration indicates that pathology influences estimates of age-related cognitive changes in advanced age, whereas normal aging processes might be manifest in linear slopes.

On the other hand, well-practiced tasks or tasks that involve knowledge show little or no decline until very late in life. Short-term memory would be an example of late-life decline. Often measured by the digit span task, it involves phonological storage and it is characterized by slight declines during adult lifespan, with sharper declines observed after the age of 70 (Gregoire & Van der Linden, 1997). Measures of vocabulary and semantic knowledge are also stable until late in life, in both cross-sectional and longitudinal studies (Schaie, 1996; Park et al, 2002), although the latter tend to show greater declines in vocabulary after the age of 60. As stated above, these accelerated declines might be due to the influence of disease processes. The relative stability of semantic memory and knowledge until late in life indicates that life experience might lead to the knowledge and wisdom often exhibited by older adults (Baltes et al., 1995). Thus, they might use knowledge and experience to form more efficient or effective strategies when performing tasks in which younger adults rely on processing ability (Shimamura et al., 1995).

Apart from early or later in life declines in certain cognitive abilities, stability has also been proved in other cognitive processes. Autobiographical and automatic memory processes as well as emotional processing appear to be unchanged throughout life. Autobiographical memory for one's life events seem to be preserved along adulthood and a similar pattern is seen in centenarians (Fromholt et al., 2003). Similarly, tasks involving attribution of mental states to other individuals ('theory of mind' tasks) remain intact (Happe et al., 1998). Implicit memory, characterized by a lack of conscious awareness in the act of recollecting, is often stable or shows little age-related changes (La Voie & Light, 1994). On the whole, data available so far point to a relatively stability of overlearned or highly practiced skills, automatic processes and familiar information across life and to a relatively impairment of those memory functions requiring the formation of new connections such as recall of recent autobiographical experiences or new facts (for a review on autobiographical memory see Piefke & Fink, 2005).

A great amount of effort has gone toward the study of age-related changes in episodic memory. Episodic memory is the conscious recollection of events that have occurred in a person's experience and it is thought to be the result of several stages of processing, including an encoding stage in which the features of the incoming stimulus are analyzed and related to previously encountered information, and a retrieval stage in which stored information is searched for and brought to consciousness to be acted upon. Age-related difficulties in episodic memory could be related to deficits in encoding (Craik & Byrd, 1982) as well as to reductions in the adequacy of retrieval (Burke & Light, 1981). One proposed reason for encoding failure in older people is that they are less able to spontaneously initiate adequate encoding strategies, or to organize material in their attempt to learn it. In some cases, providing older adults with support for memory at the encoding stage, e.g. by giving them an efficient strategy can result in smaller age-related differences in their performance compared to young adults. As far as the retrieval stage of memory is concerned, older individuals have consistently been found to have more severe decrements on memory tasks requiring the free recall of learned information, compared to their ability to recognize previously encountered stimuli. In recognition, a familiarity process is at work, which means that a particular item has been previously encountered, whereas recall involves retrieving a memory of the exact circumstances in which the item was encountered. This difference between recognition and recall may be due to the more effortful nature of recall (for a review on episodic memory and aging see Grady 2000).

Attempts to understand age-related cognitive changes go along with the study of neurobiological aspects of aging, with the hope to distinguish normal from pathological processes and therefore develop useful behavioural, pharmacological and technological therapies. Post-mortem studies demonstrate decreased grey matter volumes in the brains of older adults as compared to younger ones (Haug & Eggers, 1991; Resnick et al., 2003). Rather than being a result of cell death, such declines seem to be related to lower synaptic densities in older adults (Terry, 2000). Neocortical synapse density declines progressively up to the age of 20. With such a progression, a non-demented brain would reach the reduced synaptic density that is seen in Alzheimer's disease (AD) at the age of 130 (Terry & Katzman, 2001). Nonetheless, synaptic changes are not uniform but they appear to be of a regional specific nature. Thus, while the prefrontal cortex (PFC) and medial temporal lobe (MTL) structures are specially affected, other regions such as the occipitial cortex remain relatively unaffected (West, 1996; Raz et al., 2003; Raz et al., 2004a). Another brain region

widely investigated in anatomical studies of aging is the white matter, which together with the PFC and the MTL shows important age-related changes associated with cognitive performance (Bartrés-Faz et al., 2001a). The white matter changes more relevant observed among elder subjects can be found in the anterior parts of the brain connecting the fronto-striatal system (Head et al., 2004). In this regard, literature has agreed to differentiate two different kinds of cognitive aging: one involving the frontal lobes and their connections with the basal ganglia nuclei and the other giving a principal role to medial temporal lobe structures (Buckner, 2004).

Patients with frontal lesions show behavioural deficits similar to older adults, namely a failure to suppress interfering information, perseverative errors and decreases in working memory capacity. Such observations made neuropsychologists speculate that prefrontal deficits were the underlying cause of cognitive aging (Moscovitch & Winocur, 1995; West, 1996). Posterior research evidenced that PFC undergoes the largest age-related volumetric changes in adulthood (Raz et al., 1998; Resnick et al., 2003), being estimated a decline of about 5% per decade after the age of 20 (Raz et al., 2003). Lateral regions of the PFC are mostly affected in healthy elders (Tisserand et al., 2002), whilst AD patients show greatest degeneration in the inferior PFC (Salat et al., 2001), though not in early stages of the disease (Thompson et al., 2003). These volume declines might be partially explained by decreased synaptic density in the PFC with aging, a fact that has also been observed in both monkeys (Bourgeois et al., 1994) and humans (Liu et al., 1996). Likewise, the human striatum, consisting of the caudate and putamen, has been shown to bear volume declines with age, though smaller as compared to the PFC. Specifically, it is believed to decrease about 3% per decade (Gunning-Dixon et al., 1998). The striatum has extensive connections with the PFC and is responsible for a large proportion of dopamine production. Besides lower dopamine concentration, dopamine D2 receptor density also decreases with age (Volkow et al., 1996 and 1998). This has been related to lower glucose metabolism in the frontal cortex as well as in the anterior, temporal cortex and caudate nucleus. There is, however, little information about age-related changes that occur between the ages of 30 and 60. Either volumetric or neurotransmitter changes in the PFC and the striatum have been associated with age-related declines in cognitive performance. In this respect, PFC volume was negatively correlated with perseverative errors on the Wisconsin Card Sorting Task (WCST) (Raz et al., 1998) and positively correlated with processing speed and executive ability, both considered measures of fluid intelligence (Schretlen et al., 2000).

Along with the attempts to relate age-related declines in the PFC with particular behavioural deficits, researchers are highlighting the study of prefrontal activity patterns in aging. Results from neuroimaging studies are controversial and will be discussed in a proper section, but in general it seems that older adults experience greater difficulty than younger adults in performing executive processes, and that this difficulty is sometimes manifested as a failure to activate PFC regions and other times as increased recruitment of PFC regions under relatively easy conditions.

Apart from the effects on grey matter density, above all of the PFC, aging is also accompanied by white matter lesions (Guttmann et al., 1998; Chen et al., 2001). Actually, greatest age-related alterations in white matter are in the PFC and the anterior corpus callosum (Bartzokis et al., 2003; Head et al., 2004). White matter abnormalities are associated with poor performance on tasks of processing speed, executive function and immediate and delayed memory, but not with declines in general intelligence measures (Gunning-Dixon & Raz, 2000). Specific age-related reduction in frontal white matter correlates with decreases in processing speed and reasoning ability (for a review see Hedden & Gabrieli, 2004). Both age-related grey and white matter changes in the frontal cortex are interpreted as mediating behavioural patterns of cognition within non-demented older people.

Besides the frontal lobes and connected regions, the importance of the hippocampus and related MTL structures to declarative memory makes them of particular interest for understanding age-related memory changes (Erickson & Barnes, 2003). Brain pathology can be detected in normal elderly subjects and used to predict future transition to MCI. This prediction is enabled by examinations revealing reduced glucose metabolism in the hippocampal formation (hippocampus and entorhinal cortex) as well as by the rate of medial temporal lobe atrophy as determined by MRI. Loss of volume in the entorhinal cortex would happen in a subtle and graded fashion in normal aging and could endure a decade or longer, but in dementia of the Alzheimer type changes would begin before its clinical diagnosis (Killiany et al., 2000; Dickerson et al., 2001). However, in contrast to the relatively large age-related changes that occur in the PFC and frontal white matter tracts, studies of the human MTL structures have observed relatively slight age-related changes in the absence of AD (Raz et al., 2004a). There is in fact no evidence of loss of neurons in

hippocampal CA subregions and the parahippocampus with age (Rapp et al., 2002), and dendritic growth seems to continue until after the age of 90. The volumetric declines of these structures on structural magnetic resonance imaging (MRI) studies are around 2-3% per decade in the volume of the hippocampus and the parahippocampal gyrus (Raz et al., 2004b), which only seem to be related to explicit memory impairment after the age of 60 (Raz et al., 1998; Rosen et al., 2003). Volumes of the subiculum and dentate gyrus have also found to decline with age in non-demented individuals (Small et al., 2002). Overall, available data indicate that normal aging has minimal structural effects on the hippocampus and adjacent MTL, although age-related functional changes might affect circuits that involve interactions between the PFC and the hippocampus, thereby causing age-related decrements in memory function that is mediated by hippocampal neurons (see figure 1).

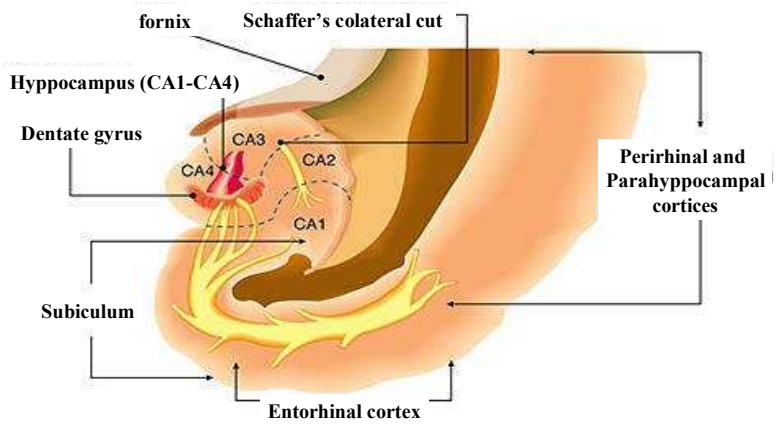

Figure 1. Medial temporal lobe (MTL) structures.

Nonetheless, measures of hippocampus and entorhinal cortex volumes provide secondary evidence for AD, since they are not specific for AD. Recent efforts to improve the diagnostic specificity of AD are being made by combining imaging with other biomarkers such as cerebrospinal fluid (CSF) and most recently by evaluating amyloid imaging using PET. This combination contributes to an early and specific diagnosis of AD (reviewed in de Leon et al., 2007).

In summary, there is a recurring distinction between cognitive decline associated with executive and attention difficulties and that associated with long-term, declarative memory; which parallels functional disruption in frontostriatal systems as well as medial temporal and associated cortical networks that are important to memory. Candidate causes are

vascular change linked to hypertension, neurotransmitter depletion and pathology arising from AD. These causes, while all strongly associated with age, may progress at different rates across individuals and combine their influence to affect memory (Buckner, 2004).

An important issue in cognitive aging is how an individual responds to change and therefore, researchers point to an increased variability with aging. Although it was first argued that variability might be an indication of pathological processes, more recent studies relate it with normal aging (Hedden & Gabrieli, 2004). For instance, varied life experience, physical or mental activity levels are examples of how variability across individuals could affect age-related cognitive changes. Addressing the issue of variability is of great importance since it is the backdrop for the 'Cognitive Reserve hypothesis', which will be thoroughly explored in the present thesis.

1.1.2 Mild cognitive impairment

The concept of cognitive impairment intervening between normal aging and very early dementia has been in the literature for many years (reviewed in Bartrés-Faz et al., 2001b). The construct of mild cognitive impairment (MCI) was proposed by Petersen and colleagues to designate an early, but abnormal, state of cognitive impairment (Petersen et al., 1999). In a subset of persons, in particular those who are destined to develop AD, there is a decline in cognitive function, which can be very subtle at first. Currently, the criteria for clinically probable AD identify people after a substantial degree of cognitive decline has taken place. The construct of MCI proposes to identify these individuals at an earlier point in the cognitive decline such that if therapeutic interventions become available, clinicians can intervene at this juncture. Within a continuum of normal aging and dementia, MCI is interposed between the cognitive changes of normal aging and what might constitute very early dementia. Of note is to mention that there is an overlap in the boundaries between normal aging and MCI and also between this condition and very early dementia, indicating that differences between both normal and pathological aging can be quite subtle (Petersen, 2004).

There have been a variety of terms used to discuss transitional stages between normal aging and early dementia in the literature. The term of 'age-associated memory impairment' (AAMI) was meant to characterize memory changes in aging which were felt to be a

manifestation of normal cognition (Crook et al., 1986), 'age-associated cognitive decline' (AACD) was proposed to refer to multiple cognitive domains presumed to decline in normal aging (Levy, 1994) and finally, the Canadian Study of Health and Aging used the term, 'cognitive impairment no dementia' (CIND), to characterize intermediate cognitive function of insufficient severity to constitute dementia (Graham et al., 1997). MCI has come to be recognized as a pathological condition, i.e. not a manifestation of normal aging, and has received a great deal of attention as a clinically useful entity.

As research on MCI has advanced, it has become apparent that several clinical subtypes of MCI exist (Petersen et al., 2001; Petersen 2003). Most research has focused on the amnesic-MCI (a-MCI) but other types have been recognized as well. Criteria for a-MCI include non-demented individuals with memory complaints, objective memory impairment for age, corroborated by neuropsychological tests (1.5 SD below age norms), general intellectual function and preservation of Activities of Daily Living (ADL) (Petersen, 2004). In a sample of normal older adults, approximately 3–5% of individuals will develop to MCI each year (Rusinek et al., 2003). There is increasing evidence that individuals with MCI have a greatly increased likelihood of progression to AD, with an annual rate of progression of 10–15% (Petersen, 1999). However, some proportion of MCI subjects does not seem to develop AD at all. Prospective studies of people with a-MCI have shown that episodic memory such as delayed recall of word lists and paired-associates learning (DeJaeger et al., 2003; Nestor et al., 2004), semantic memory, (Nestor et al., 2004; DeCarli et al., 2004) attention processing (Amieva et al., 2004) and mental speed can consistently predict which patients will develop dementia. Similarly, in a retrospective study of people with MCI who had developed AD, verbal and visual memory, associative learning, vocabulary, executive function and other verbal tests of general intelligence were impaired at baseline (Guarch et al., 2004).

In contrast to the normal age-related volumetric declines in subiculum and dentate gyrus, the entorhinal cortex shows declines in patients with AD and in individuals with MCI relative to healthy older adults (Small et al., 2000a and 2002). Although data are available evidencing that entorhinal-cortex atrophy might be a better predictor of AD progression than hippocampal volume loss (Dickerson et al., 2001) other predictors of conversion to dementia are also whole-brain and medial temporal lobe atrophy assessed with a standardised visual rating scale (Jack et al., 2003; Korf et al., 2004).

Individuals with MCI exhibit an increase in neurofibrillary tangles in the temporal lobes relative to normal elderly subjects that is correlated with their poorer memory performance (Guillozet et al., 2003), on the whole indicating that MCI is characteristic of the prodromal stage of Alzheimer's disease. By following participants after initial imaging and observing their progression from normal status to MCI and from MCI to AD, researchers have found that entorhinal cortex volume and metabolism are reduced in those participants who subsequently develop MCI or AD (Killiany et al., 2000; Dickerson et al., 2001; Rusinek et al., 2003).

Nonetheless, since results derived from MCI studies do not seem to be conclusive, several areas of controversy have arisen around its characterization. Several components accounting for such variability include three main issues. First, many research groups use rating scales as equivalent to the clinical diagnosis of MCI. For instance, the Clinical Dementia Rating (CDR) is a rating scale commonly used in research of aging dementia and some studies have equated a CDR of 0.5 (indicating questionable dementia) to MCI. CDR is not a diagnostic instrument and hence, subjects with a CDR of 0.5 may meet the criteria stated above for MCI or they may represent very mild AD. Second, MCI has been often diagnosed as compared to normal aging and this has been sometimes viewed as optimal aging. This approach may be classifying a large proportion of elders as being MCI since it is well acknowledged that even in the absence of significant disease, some cognitive decline is inevitable. Finally, a great deal of literature on MCI has been generated from clinical settings such as dementia or memory disorders, predisposing to a certain type of patient population. As a result of this, the MCI construct has been forced to be re-classified. Thus, apart from a-MCI, other clinical subtypes of MCI have been recognized as well, one involving various degrees of impairment in multiple cognitive domains (mcd-MCI), such as language, executive function and visuospatial skills, regardless of memory function, and another one focused on impairment in a single nonmemory domain (Zanetti et al., 2006).

Additionally, different aetiologies offer a more complex view of this field, attesting to the heterogeneous nature of MCI with several causes leading to the same symptoms (Chong & Sahadevan, 2005). While a-MCI might be the preclinical stage of AD (Petersen, 2000), little is known about the features and evolution of mcd-MCI, an entity initially defined as vascular MCI (v-MCI) by Frisoni and colleagues (Frisoni et al., 2002). Briefly,

the diagnosis of v-MCI was made according to a modified version of Erkinjuntti and colleagues' criteria for subcortical vascular dementia and included the following criteria: cognitive syndrome (dysexecutive syndrome and memory deficit with a progression of both), cerebrovascular disease (evidenced by brain imaging and neurological signs). For some researchers it may be the preclinical stage of vascular dementia (Wentzel et al, 2001). A recent 3-year-follow-up investigation studying the progression of a-MCI and mcd-MCI subjects has demonstrated that all patients who evolved to AD had been classified with a-MCI at base-line, whereas all patients who evolved to subcortical vascular dementia has been classified with mcd-MCI at base-line, corroborating previous findings by Wentzel and colleagues (Zanetti et al., 2006).

As things stand, researchers on aging are currently asking for guidelines to standardise the early assessment of cognitive impairment in order to avoid the non-uniform use of MCI criteria and to help researchers distinguish this condition from both normal aging and early dementia (Chong & Sahadevan, 2005). Despite all, MCI is a well established clinical entity and as such, the final diagnosis should rely on the clinician rather than on cognitive tests (see Petersen, 2004, for a review).

1.1.3 Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia in the elderly and one of the most serious health problems in the industrialized world. At present, AD afflicts 10% of individuals over 65 years of age, and more than 50% of people over 80 years (Mosconi et al., 2007). The risk of developing AD doubles approximately every 5 years between the ages of 65 and 85 (Brookmeyer et al., 1998; Petersen et al., 1999). The demographics of aging are in a great need to accurately diagnose AD and to specifically distinguish it from the many other possible causes of cognitive impairment.

The diagnosis of definite AD can only be made by neuropathological confirmation of people who have been studied in life and met criteria for dementia and therefore, the identification of biological and cognitive markers would be useful to improve early detection of AD and eventually develop effective prevention treatment. Specific pathological lesions whereby diagnosis of AD is based on refer to intracellular neurofibrillary tangles (NFT), extracellular β-amyloid deposition in the form of

extracellular senile plaques and blood vessel deposits as well as synapse loss and dysfunction (Ball et al., 1985; Braak & Braak, 1991; Price and Morris, 1999). Synaptic abnormalities are seen as the proximate cause of dementia in AD, and provide a better correlate of the pattern and severity of cognitive impairment than amyloid plaques or NFTs (Terry et al., 1991). One prominent hypothesis is that amyloid deposits (plaques) and soluble forms of amyloid lead to neuronal dysfunction (synaptic loss) and cell death. Due to this, major circuits are structurally disrupted in AD and there is selective vulnerability with respect to which neurons die and which are resistant to neurodegeneration (Morrison and Hof, 1997). Despite an initial predilection for the neocortex, amyloid-β depositions are also found in the MTL at later stages of disease (Arriagada et al., 1992; Giannakopoulos et al., 1994). On the other hand, NFT deposition originates in the MTL (hippocampus, transentorhinal and entorhinal cortices, and parahippocampal gyrus), which play a critical role in the neural control of memory functions, then begin to cluster in the adjacent inferior temporal and posterior cingulate cortex in mild AD, and finally spread to the parieto-temporal and prefrontal association cortices, which are involved in the neural control of perception, attention, and language, in mild and then severe dementia (Braak and Braak, 1996; Delacourte et al., 1999). Thus, both NFTs and amyloid plaques are associated with extensive synaptic loss in AD brains, with reductions ranging from 30% to 90% depending on the brain regions examined (Gomez-Isla et al., 1997; Price and Morris, 1999).

The abovementioned AD pathology is translated into functional and structural brain damage, mainly involving the brain regions where the concentration of cell loss is the greatest (see Morrison & Hof, 1997, for a review). Several studies have shown that the progression of AD pathology in the brain can be staged and that those changes develop many years before the clinical manifestations of the disease become apparent (Braak and Braak, 1991, 1996; Morris et al., 1996; Delacourte et al., 1999; Thompson et al., 2003). While severe entorhinal cortex and hippocampal atrophy is consistently found in mild AD patients, volume reductions in the cortical regions, particularly parieto-temporal, posterior cingulate/precuneus, and frontal cortices, are evident in moderate to severe AD (see Mosconi et al., 2007, for a review).

Regarding cognitive deficits, early stages of AD are hallmarked by deficits in declarative memory, specially episodic memory, such as difficulty in remembering short lists of words or objects (Huppert, 1994), although effects on executive function can also be detected

(Balota and Faust, 2001). When clinical neuropsychological tests are used to evaluate memory in AD patients, it is long ago clear that recall and recognition performance are impaired in both verbal and nonverbal domains (Wilson et al., 1983; Storandt and Hill, 1989). The loss of recent memories appears during Braak stages III and IV, when conspicuous neurofibrillary changes are restricted to the hippocampal and parahippocampal regions (Braak et al., 1996). Only during later stages (Braak stages V and VI), as the association areas of the neocortex receive the impact of NFT, remote memories also become affected. In stage I patients are never demented. Brains with widespread "tangle bearing" neurons in the higher neocortex and occipital cortex regions are Stage VI, where patients are always demented. Stages II-V in the Braak system are intermediary points in the journey from intact brain function to total incapacitation. This model is also in accordance with two functional neuroimaging reports, which found that hippocampal hypometabolism was associated with reduced episodic memory, in particular recent autobiographical recollection (Greicius et al., 2004; Eustache et al., 2004). In addition, these studies revealed pathology of the retrosplenial and posterior cingulated cortices, two brain regions related to autobiographical memory (see Piefke & Fink, 2005, for a review).

As mentioned earlier, deficits in executive function may also occur in mild AD. Most of the tasks that reveal a significant deficit are those that require concurrent manipulation of information, i.e., tasks that require set-shifting, self-monitoring, or sequencing (Trail Making Test (TMT), part B). By contrast, performance on tasks that assess cue-directed attention and verbal concept formation do not appear to be significantly impaired in very mild patients (i.e., patients with mini-mental state exam scores ≥ 22). It therefore seems most likely that the underlying disability of the mild AD patients on executive function tasks is the result of a primary difficulty with the concurrent manipulation of information. For example, AD patients perform well on part A of the TMT, a test that requires sustained attention and simple sequencing, but their performance is impaired on part B, which requires tracking two overlearned sequences simultaneously (i.e., numbers and letters) and switching rapidly from one sequence to the other. The development of cognitive symptoms in addition to memory is likely due to the progression of neuropathological change in cortical regions known to underlie these functions. In the case of executive dysfunction two explanations can be offered. The first pertains to pathological changes in subcortical structures, such as the basal forebrain, that modulate cortical function. The basal forebrain receives afferent projections from numerous subcortical

structures and projects to numerous cortical and subcortical regions; it can therefore serve as a source of integrated information to the cortex. The second likely source for the executive function deficits seen in early AD pertains to the loss of neocortical synapses and long cortico–cortical projection systems seen in AD (Albert, 1996).

1.1.4 Functional neuroimaging in aging

The discipline of *cognitive neuroscience of aging* focuses on the links between cognitive and cerebral aging (for a review see Cabeza, 2001). These studies employ a variety of methods, but the most powerful are functional neuroimaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). PET and fMRI are called hemodynamic techniques because they investigate indirectly neural function by measuring changes in blood flow. The resolution of these hemodynamic measures is limited both temporally and spatially. While spatial resolution is good when compared to electromagnetic techniques such as event-related potentials (ERPs) and magneto-encephalography (MEG), they have coarse temporal resolution (in the scale of seconds). The slowness of the hemodynamic response is shown by blood flow change that can last 10 seconds elicited by a neural event that lasts a fraction of a second. This represents a clear disadvantage when compared to the temporal resolution of electromagnetic techniques, which measure neural activity directly and can identify changes in the order of milliseconds. However, PET and fMRI techniques are particularly useful for revealing large-scale distributed networks of brain regions. PET and fMRI measure blood flow changes differently. PET measures hemodynamic changes relatively directly by marking blood with a radioactive tracer (for example, $O_{15}H_2O$). In contrast, fMRI measures blood flow changes through changes in blood oxygenation. When a brain region is activated, oxy-hemoglobin in the region increases beyond the actual oxygen demands, and the associated decrease in deoxyhemoglobin concentration yields the signal detected with MRI (Blood Oxygenation Level Dependent contrast mechanism, or BOLD). Both techniques reflect neural activity. In particular, cerebral blood flow and regional cerebral glucose metabolism are reliable indices of neuronal/synaptic function whereas increases and decreases of BOLD signal from fMRI reflect increases and decreases in neural activity respectively (Shmuel et al., 2006) mainly in the form of local field potentials (Logothetis et al., 2001). Compared to PET, fMRI is more sensitive to motion artefacts, but it is less invasive and expensive than PET. The design of PET and fMRI studies is most crucial, as the

understanding of any observed changes in brain activity is critically dependent on how well the design helps reduce the number of possible interpretations of the effect. In most studies, the design involves comparing blood flow in a target task (for example, learning face-name pairs) and a reference task (for example, recognition of a previously learned face-name pair). Regions showing relative increased activity in the target task compared to the reference task (so-called "activations") can then be assumed to reflect cognitive processes more engaged by the target task than by the reference task (for example, associative memory). PET and fMRI experiments can be designed in many different ways and several different statistical approaches can be adopted to identify changes in brain activity relating to the experimental question. Regardless of the type of design and statistical technique, the specific location of observed changes is often expressed in the form of three-dimensional coordinates according to a common standardized 3D space such as the Montreal Neurological Institute (MNI) or the atlas of Talairach and Tournoux (1988): x (left/right), y (anterior/posterior), and z (superior/inferior).

Much of the recent research on aging has focused on investigating the relationship between age-related changes in brain structure/function and concomitant changes in cognitive/behavioural abilities (Rajah and D'Esposito, 2005). Since signals measured by BOLD fMRI and PET are coupled to 'real' changes in neural activity, studies using these techniques can reveal how neural correlates of different cognitive functions change as a function of aging. Nonetheless, when using fMRI and PET to examine age-related differences in brain activity, one must keep in mind that normal aging affects the cerebrovascular system, which in turn affects the neurovascular coupling that is the basis of the signals measured by these techniques. The cerebrovascular changes observed in normal aging have been shown to decrease the signal-to-noise ratio, the amplitude and the spatial extent of the BOLD response (D'Esposito et al., 2003).

While age-related changes in temporal, parietal, occipital and cerebellar activity when performing different perceptual, memory and executive tasks do not appear to be very regular, changes in PFC activity are remarkably consistent. PET and fMRI studies have observed functional differences in frontalstriatal circuits during healthy aging across a range of tasks. In particular, there seems to be a reduced activity in the left PFC, which possibly reflects age-related deficits in episodic encoding and/or semantic retrieval operations. In contrast, right PFC activity in older adults is as strong as or even stronger than younger

adults (Cabeza et al., 1997; Anderson et al, 2000; Grady et al 2002, Stebbins 2002). On the other hand, during episodic memory retrieval, age-related decreases in activation were typically found in right PFC and right parietal regions, whereas age-related increases in activation were evidenced in left PFC, as well as in bilateral anterior cingulate and cuneus/precuneus regions (see Cabeza, 2001, for a review). Thus, age-related changes on PFC activity have been described in terms of their effects on the overall lateralization of PFC activity. Since episodic encoding and semantic retrieval activity is typically left-lateralized in younger adults, age-related reductions in the left PFC activity often led to a more bilateral pattern of PFC activity in older adults. Likewise, since PFC activity during episodic retrieval is usually right-lateralized in younger adults, age-related increases in left PFC also tended to make PFC activity more bilateral in older adults. This pattern is known as Hemispheric Encoding/Retrieval Asymmetry (HERA) model (Tulving et al, 1994; Nyberg et al., 1996 and 1998). Additionally, during working memory tasks, PFC activity usually occurs in the hemisphere less activated in young adults and therefore these changes also tended to produce a less asymmetric pattern of PFC activity in older adults. The effects of aging on PFC function can be hence summarized in the following statement: PFC activity is less asymmetric in older adults than in younger adults. This pattern is the most consistent finding of all functional neuroimaging studies of cognitive aging and has been postulated as a general aging phenomenon called Hemispheric Asymmetry Reduction in Old Adults or HAROLD, which is inconsistent with the HERA model (Cabeza, 2001). Age-related asymmetry reductions during episodic memory retrieval have been demonstrated for different kinds of tests (recall and recognition) and different kinds of stimuli (verbal and pictorial), and therefore they appear to be a robust and general phenomenon. In 1997 such asymmetry reductions were interpreted as reflecting compensation (Cabeza et al., 1997). Thus, to counteract neurocognitive deficits, older adults engage both hemispheres for tasks that require basically one hemisphere in younger adults. According to this view, age-related decreases or absences in activation reflect deficits in brain function (Cabeza et al., 2004) and the concomitant increases in activation show either successful compensation for these deficits, when there are no age-differences in performance, and 'attempted' compensation for these deficits, when there is an age-related decrement in performance (Grady et al., 1999; Madden et al., 1999; Reuter-Lorenz et al., 2000; Cabeza et al., 2000; Cabeza, 2002; Grady, 2002;). Apart from some studies showing increased or equivalent performance in older adults engaging more regions, other evidence supports the compensation hypothesis. Recovery of function after unilateral brain

damage is facilitated by the recruitment of homologous regions in the unaffected hemisphere and this has been evidenced in both PET and fMRI studies (see Cabeza, 2001, for a review).

Another point of view, the so-called dedifferentiation view, posits that age-related changes in functional activations reflect deficits in neurotransmission, which in turn cause decreases in signal-to-noise ratio and less distinct neural representations (Li et al., 2001). It follows that decreases in activation reflect a deficit due to reductions in regional process-specificity, while increases in activity show generalized spreading of brain function due to reduced specialization of function, which may or may not be compensatory. Therefore, this view suggests that overall there is no change in regional process-specificity in cortical function across lifespan, but that this specificity becomes more generalised with normal aging. Deficits in function would be caused by deficits in neurotransmission resulting in noisier internal cortical representations (deficits in function). Both functional compensation and dedifferentiation perspectives assume that there are primary deficits in function with age that precipitate functional compensation. However, the former does not state precisely what neural changes precipitate deficits in function, whereas the dedifferentiation perspective does. According to the dedifferentiation view, age-related increases in activity may not always be compensatory, since age-related reductions in regional process-specificity may result in aberrant neural activity. In some cases this may benefit task performance (compensation) and in other cases it may be detrimental to task performance. Therefore, the dedifferentiation perspective does not neglect the possibility that age-related increases in PFC activity may be compensatory (Rajah and D'Esposito, 2005).

Also linked with the HAROLD model, it has been observed that MCI and AD patients rarely display bilateral activations, and most often exhibit declines in PFC activation (Remy et al., 2004; Dannhauser et al, 2005; Trollor et al., 2005). A few studies, however, have observed increased PFC activations in these patients relative to controls, a finding that may be important for the determination of whether such additional activation should be considered compensatory (Remy et al., 2005; Rosano et al., 2005).

Apart from functional changes in the PFC cortex, aging also substantially affects connectivity among different regions and of particular interest are the connections between the MTL and PFC, which have been proved to be of high importance for memory

performance. In a study of successful encoding of picture stimuli, older adults displayed less parahippocampal activation than their younger counterparts, but displayed similar levels of activation in inferior PFC regions. Furthermore, the correlations between parahippocampal and inferior PFC activation were positive in younger adults, but negative in older adults, suggesting that successful encoding could be accomplished by some older adults through preserved activation in PFC, even as parahippocampal activation declined (Gutchess et al., 2005). In another study (Cabeza et al., 2004) involving three different task domains (working memory, visual attention, and episodic recognition), older adults were found to have less activation in the hippocampus than younger adults. This decrease was accompanied, however, by a corresponding increase compared with younger adults in dorsolateral PFC activation. Such results suggest that increased activation in the PFC reflects plasticity in healthy older adults that may partially compensate for functional declines in MTL memory systems (Reuter-Lorenz et al., 2005).

Few studies have integrated volumetric and functional information about age-related brain changes, but one such study (Rosen et al., 2005) found a positive correlation among older adults between left entorhinal volume and right frontal activation during verbal memory encoding. This finding is consistent with the view that more successful aging (as indexed by larger entorhinal volume) is associated with compensatory recruitment of contralateral PFC. These changes in MTL–PFC connectivity appear to be restricted to healthy aging; because MCI and AD minimally affect the connections between MTL and PFC, and it is therefore likely that the memory deficits observed in these pathologies derive largely from direct atrophy of MTL structures.

Focusing on pathological aging conditions, PET examinations have often used 2-[18F] fluoro-2-deoxy-D-glucose (FDG) as the tracer, which has the unique ability to provide quantitative estimates of the local cerebral metabolic rate of glucose, expressed as μmol/g min. Studies using FDG-PET report that as compared to age-matched healthy normal controls, AD patients show regional metabolic reductions involving the parieto-temporal and posterior cingulate cortices, and the frontal areas in advanced disease (see Mosconi et al., 2007). Along with this, there are also reports of hippocampal metabolic abnormalities in AD (de Leon et al., 2001; De Santi et al., 2001; Nestor et al., 2003; Mosconi et al., 2005, 2006). This pattern of hypometabolism is currently accepted as a reliable in vivo hallmark of AD, because of its high sensitivity in distinguishing AD from normal aging and it is

directly influenced by pathology processes. Thus, Klunk and colleagues observed that amyloid deposition in parietal cortex correlated negatively with glucose metabolism (Klunk et al, 2004). fMRI studies of AD patients observed marked differences in posterior parietal activation, near posterior cingulate and retrosplenial cortex (Lustig et al. 2003).

As regards MCI patients, FDG-PET studies showed a wide spectrum of cortical abnormalities in MCI, ranging from absent or very mild metabolic deficits (Reed et al., 1989; Powers et al., 1992; Small et al., 1994; McKelvey et al., 1999), particularly in the case of very mild subjects (De Santi et al., 2001; Mosconi et al., 2005), to severe parieto-temporal and posterior cingulate cortex hypometabolism in MCI patients of the amnestic type and in those close to converting to AD (Minoshima et al., 1997; Berent et al., 1999; Herholz et al., 1999; Arnaiz et al., 2001; Chetelat et al., 2003; Nestor et al., 2003). Longitudinal FDG-PET studies in MCI are consistent in demonstrating that excessive hypometabolism is associated with incipient AD (Arnaiz et al., 2001; Chetelat et al., 2003; Drzezga et al., 2003; Anchisi et al., 2005; Mosconi et al., 2005). On the other hand, a recent study with fMRI showed that people with MCI who would go on to develop AD recruited a larger extent of the right parahippocampal gyrus upon the encoding phase of memory testing, which reflects a compensatory response to accumulating AD pathology (Dickerson et al 2004).

fMRI and PET techniques are considered to be of a univariate approach, since they estimate regional activity. In contrast, multivariate analyses evaluate interactions between regions, reflecting a dynamic aspect of brain circuits. In this sense, functional connectivity, defined as statistical dependencies or correlations among neurophysiologic events (Friston, 2005), is one way to characterize such interactions. Exploratory functional connectivity analyses using patterns of covariance have been used in previous PET studies to demonstrate that relative to younger subjects, elderly individuals use different brain resources while maintaining a similar memory performance (Della-Maggiore et al., 2000 and Grady et al., 2003). Additionally, it has been shown that the interactions between the hippocampus and cortical regions are altered in disorders affecting medial temporal lobe structures such as Alzheimer's disease (Grady et al., 2001).

1.1.5 Transcranial magnetic stimulation

1.1.5.1 Transcranial magnetic stimulation: basic principles

Transcranial magnetic stimulation (TMS) is based on the principles of electromagnetic induction where a brief and high-amplitude pulse of current, lasting for approximately 100 to 200 ms, is discharged into an electromagnetic coil held over the cranium. This current produces a magnetic field perpendicular to the current, which in turn causes an electric field perpendicular to itself. Since the tissue has electrical conductivity itself, the electric field leads to an electric current in a circular direction. Thus, the electric field affects the transmembrane potential, causing depolarization and firing of the neuron. Local depolarization of neurons may lead to macroscopic responses at a behavioural level. The TMS apparatus has two main devices: a power pulse generation unit that charges a bank of capacitors able to produce high discharge currents (from 1 to up to 4 Tesla) and an electromagnetic stimulating coil which delivers magnetic pulses of up to several Tesla. By means of the coil, which is connected to the stimulator by a copper cable carrying the currents, the abovementioned capacitors are discharged and very short magnetic fields are created (about 200 μs) (reviewed in: Tormos et al., 1999; Ruohonen and Ilmoniemi, 2003).

The most widely used types of coils are the circular coil and the figure-of-eight coil. In the circular coil, the copper conductor is placed in one or several turns into a ring-shaped configuration. This type of coil does not have any single magnetic field focus, but a maximum current in the whole external winding, a fact that produces a ring-shaped magnetic field around the coil. On the other hand, the figure-of-eight or 'butterfly coil' consists of two circular ring-shaped coils next to each other covered by a butterfly shaped coil mantle (see figure 2). The copper conductors are placed inside the two inner circular coils in a way that the currents circulate in opposite directions. Hence, the magnetic fields converge at the intersection of the coils, resulting in a more focused magnetic field distribution as compared to the circular coil and representing therefore an advantage over circular coils (Cohen et al., 1990). Specifically, the magnetic field amplitude underneath the centre of a figure-of-eight coil is about twice as high as the secondary peaks occurring at the wings. As regards the magnetic field strength, the cortical area directly affected by TMS is maximally 2-3 cm deep (Epstein et al., 1990; Rudiak et al., 1994). Moreover, there is

another type of coil, the double-cone, which is able to stimulate 3 to 4 cm deep (Maccabee et al., 1990; Tearo et al., 1994; Terao et al., 2000). In any case and as it will be mentioned below, several studies have stablished that TMS affects not only directly targeted cortical regions but also remote cortical and subcortical areas by transsynaptic connections (Dressler et al., 1990; Paus et al., 1997).

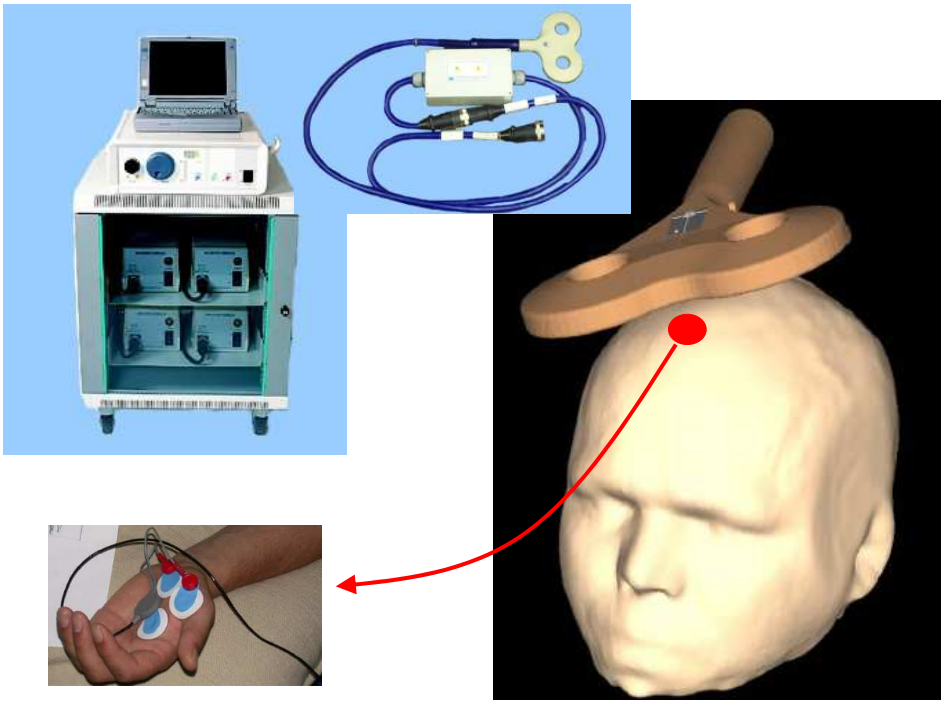

Figure 2. TMS with a figure-of-eight coil to motor cortex causes muscle activation measured by electromyography (EMG).

When designing a TMS experiment, the two most determinant parameters to be set are the frequency and the intensity of the magnetic pulses. Concerning the frequency, magnetic pulses can be administered once at a time, so that after each stimulation a response is recorded (single-TMS) or in trains of magnetic pulses (repetitive TMS, rTMS). In this latter version, a series of magnetic pulses can be administered at a given frequency (usually in the range of 1 to 25hz). Due to its excellent temporal resolution, single pulse TMS has been widely used in neuropysiological studies of the motor system, and also in cognitive studies mainly of the visual system to investigate chronometrics of brain connectivity (Pascual-Leone & Walsh, 2001). In contrast, rTMS is able to modulate CNS activity for times of periods beyond the stimulation itself and influence transynaptic connections (Paus et al., 1997; Maeda et al., 2000; Pascual-Leone et al., 1999). As it will be metioned below, these propierties have favoured the increased utilization of rTMS in cognitive studies and

extended its applications in psychopathological conditions (for reviews see Bartrés-Faz et al., 2000a and Bartrés-Faz et al., 2000b).

Stimulation intensity is also critical, since there is great inter-individual variability of susceptibility to cortical excitation as well as to seizure thresholds. In general, TMS devices can deliver magnetic pulses up to 2 or more Tesla. However, due to the abovementioned individual response of each central nervous system, the intensity of TMS pulses should be defined according to individual cortical excitability. The most common method to determine the response of a given intensity of magnetic stimulation in an individual is to stablish its motor threshold (MT). The MT is usually based on the motor evoked potentials (MEP) recorded with surface electromyographic electrodes in a particular muscle (i.e. the abtuctor pollicis brevis or the 1st dorsal interosseus) in response of single TMS pulses in its corresponding cortical representation of the contralateral hemisphere. In this regard, the motor threshold for a given individual can be for instance defined as the lowest stimulation intensity capable of producing a MEP motor evoked potential of at least 50 μV or a visible twitch, in more than 5 trials out of 10. Thus, for a given experiment where the intensity of stimulation could be set for example at 90% of MT, the intensity of the magnetic pulses delivered by the TMS device would be different across individuals depending on their MT, but its effect on the CNS should be comparable.

As a general rule, it is established that rTMS using either high (<1hz) frequencies and/or high intensities produce increased excitability in the underlying site of stimulation, whereas the administration of trains of TMS pulses at low frequency (<1hz) and/or intensities have the opposite effects on brain tissue (i.e. decrease cortical excitability). Although this seems to be an oversimplistic interpretation, this general rule is normally supported by the findings from the literature and can be considered as useful for practical purposes when designing an experiment aimed to increase or decrease regional cortical excitability.

Finally and regarding safety of TMS, several considerations should be noted. TMS can be considered a tool for non-invasive brain stimulation, and the most common side-effects are mild headaches or transient variations of the auitory threshold that recover spontaneously. A more serious consequence of repetitive TMS (rTMS) is its potential to induce epileptic seizures when applied at high frequencies and intensities. Since 1996,

several seizures after rTMS administration have been described and in order to avoid additional accidents, safety guidelines have been published, which restrict the use of rTMS to particular stimulation frequencies and parameter combinations (Chen et al., 1997, Wassermann, 1998). Following the use of these guidelines, no more seizures have been reported and this technique is safely used in many laboratories around the world.

1.1.5.2 Cognitive studies with TMS

Shortly after its introduction in the late 1980's as a neuropysiological tool to investigate the integrity of the motor system (which remains a fundamental application of the technique), cognitive studies of TMS and later rTMS began to emerge in the early 1990's (reviewed in Bartrés-Faz et al., 2000a). Since then, an increased number of TMS studies have targeted cognitive functions both in normal subjects and pathological conditions (see figure 3).

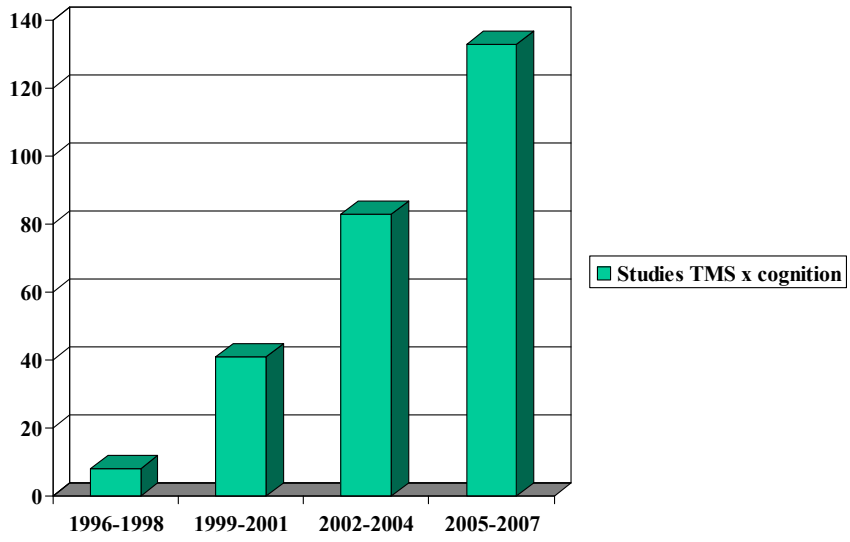

Figure 3. Graphic depicting the increasing research within the area of TMS and cognition. A total amount of 265 studies were found in a pubmed search (key words: transcranial magnetic stimulation and cognitive).

Virtually, all cognitive areas including the different types of learning and memory, attention, language, perception and executive functions have been investigated in single or repetitive TMS experiments. Most of these reports have indicated that TMS has a principal disruptory effect on brain function. For example, notable experiments were conducted in the earlies and middles 1990's demonstrating that rTMS had the potientallity to induce

'speech arrest' in normal individuals when premotor areas of the left frontal lobe were stimulated at high frequency while subjects were performing a naming task (e.g. Pascual-Leone et al., 1991). Similarly, when stimulating particular brain regions, memory and visual perception were also disrupted (Grafman et al., 1994; Pascual-Leone et al., 1994). Other investigations have been able to reveal the importance of forward and backprojections within the visual system pathways by specifically disrupting primary and secondary visual areas using single pulse TMS (Pascual-Leone & Walsh, 2001).

Many other studies have been published to date showing that TMS can transiently influence cognitive function in humans (see reviews by Walsh & Rushworth, 1999; Grafman & Wassermann, 1999; Jahansahi & Rothwell, 2000; Robertson et al., 2003; Chambers & Mattingley, 2005). Clearly, the relevance of these studies lies in the potentiality to establish brain-behavior relationships where a given targeted cortical area results in a behavioral consequence. For insance, rTMS of the dorsolateral prefrontal cortex (DPFC) transiently impairs encoding and retrieval mechanisms in human memory either for visuospatial (Rossi et al., 2001) or verbal stimuli (Sandrini et al., 2003; Rami et al., 2003), demonstrating that DPFC function is determinant for long-term memory process. The capacity of establishing causal relationships between brain regions and cognitive processes by inducing transient 'virtual lesions' (Pascual-Leone et al., 1999), is among the most important contributions of TMS. In this respect, this approximation surpasses the pure correlational interpretations allowed in functional neuroimaging studies where the activation (or deactivation) of a given area during cognitive processing tells about a relationship between both aspects but can not speak about a putative relation in terms of causality. The potentiallity of TMS to set causal relationships between brain and behavior becomes enhanced by the increased spatial resolution conferred when concomitantly functional and structural brain imaging is used in TMS experiments.

The combination of TMS with methods of functional brain imaging is of great utility for the understanding of the human brain, since it provides the opportunity to stimulate brain circuits and simultaneously monitor changes in brain activity and behaviour, hence enabling to identify brain-behaviour relationships. Brain can be imaged either 1) before TMS to identify and target the site of stimulation, 2) during TMS to assess cortical connectivity and excitability and 3) after TMS to evaluate its possible long term effects (Paus, 1999; Sack 2003).

Despite many rTMS investigations have revealed its capacity to interfere with cognitive functions, an emerging body of evidence has also found the opposite efect, that is, transient enchancements of intellectual performance. Thus, rTMS applied on the left prefrontal cortex on healthy subjects cognitive performance can also be enhanced, rather than disrupted (Rossi & Rossini, 2004). rTMS resulting into a positive effect include several cognitive domains, such as short-term memory (Pascual-Leone et al., 1993; Wassermann et al., 1996), analogic reasoning (Boroojerdi et al., 2001), picture and action naming (Topper et al., 1998; Mottaghy et al., 1999; Cappa et al., 2002), choice reaction task (Evers et al., 2001) as well as language identification tasks (Andoh et al., 2006). The exact mechanisms by which TMS can positively modulate cognitive function are unknown but it has been speculated that in these cases, magnetic stimulation could exhert an excitatory effect on functionally relevant task-supporting activation (Sack & Linden, 2003) or the inhibition of brain circuits that are competing with brain areas directly related to the cognitive process being assessed (Robertson et al., 2003) (see table 1).

Either causing transient disruptions or improvements, most TMS experiments have been conducted in young or middle aged individual (both normal or pathological). Consequently, very little is known about the putative cognitive effects of rTMS in the elder population. A study by Moser (Moser et al., 2002) found a beneficial effect of high-frequency rTMS in executive function among depressive adults with a mean age of 60 years. Rossi et al. (2004) compared the effects of rTMS applied to the left or right dorsolateral PFC on visuospatial recognition memory in 66 healthy subjects divided in two classes of age (<45 and >50 years). In younger subjects, rTMS of the right DLPFC interfered with retrieval more than left DLPFC stimulation, while in elders there was interference when applying rTMS in either right or left PFC. Thus, the presence of bihemispheric effects of rTMS on memory performance may represent a direct evidence of a compensatory role of the left PFC to support effective retrieval and supports the HAROLD model (Rossi et al., 2004).

Study	Participants	Design	Results
Töpper et al. *Exp Brain Res* 1998	65 participants	Simple pulses (110%), different latencies before and after stimulus in: left motor cortex, Wernicke ipsi and contralateral.	**NAMING AFTER VISUAL CONFRONTATION** TMS 500 and 1000ms before the stimulus over Wernicke reduces naming latency.
Mottaghy et al. *Neurology* 1999	15 participants	2 sec. trains, 20Hz before stimulus over: Wernicke ipsi and contralateral, Broca.	**NAMING AFTER VISUAL CONFRONTATION** TMS over Wernicke reduces naming reaction time.
Olivieri et al. *Brain* 1999	14 patients with right lesion. 14 patients with left lesion.	Simple pulses over intact frontal and parietal areas following 40ms electric stimulation uni or bimanual.	**CONTRALATERAL NEGLECT** Left frontal stimulation in patients with right hemispheric damage reduces contralateral extinction.
Boroojerdi et al. *Neurology* 2001	16 participants	3 trains of 5Hz (90%) over left and right DLPFC, left motor cortex and sham during the task.	**ANALOGIG REASONING** Reduction of response time.
Hilgetag et al. *Nat Neurosci* 2001	7 participants	1Hz 'off-line' paradigm, 10 min after stimuli presentation over left and right parietal lobe	**EXTINTION** Right parietal stimulation results into improvement in detection of stimuli presented ipsi and bilaterally.

Study	Participants	Design	Results
Grosbras et al. *J Cogn Neurosci* 2002	17 participants	Simple pulses over left and right frontal eye field (FEF) 53 msec before and 70 msec after stimuli.	**VISUOSPATIAL ATTENTION** Stimulation over right FEF reduces time response in a visuospatial attentional task.
Grosbras et al. *Eur J Neurosci* 2003	22 participants	Simple pulses over FEF following masked stimuli presentation.	**VISUAL PERCEPTION** Stimulation over FEF, specially in the right hemisphere increases probability to detect a masked stimulus presented 40ms later.
Naeser et al. *Brain Lang* 2005	4 cronic non-fluent aphasic patients	1Hz during 10 days over contralateral Broca area.	**LANGUAGE** Improvement in three patients 2 months and 8 months later in naming, sentence length or verbal fluency with semantic instruction.
Schutter et al. *J Psychiatry Neurosci* 2006	12 participants	3 sessions of 20' in one day (80% MT) 1Hz left orbitofrontal, left DLPFC and sham stimulation.	**EMOTIONAL MEMORY** Improvement in recall of faces showing positive emotions.
Andoh et al. *Neuroimage* 2006	12 participants	1 Hz, 110% 'Wernicke' and 'Broca' 'on-line' and fMRI guided (Wernicke)	**LINGUISTIC DECODIFICATION** Improves speed of comprehension during stimulation over Wernicke.
Andoh et al. *Cereb Cortex* 2007	14 participants	1 Hz Rtms, 50 Hz burts of rTMS (theta burst stimulation [TBS]) and sham over Wernicke. Frameless stereotaxy was used.	**WORD DETECTION TASK/AUDITORY SYSTEM** Response times were facilitated with 1 HZ rTMSin detection of native words. TBS facilitated detection of foreign words.

Table 1. Main TMS studies that resulted into cognitive amelioration.

To date, no investigation using TMS in combination with functional imaging techniques has been carried out in the elder population and the putative positive cognitive effects of magnetic stimulation remain thus unexplored. Further, the potential modulation of biological variables such as genetic variations on the effects of TMS on brain and cognitive function has never been adressed before.

1.1.6 Genetic correlates of brain function

Several genetic variants have been proved to modulate cognitive function as well as brain activity patterns in aging (reviewed in Solé-Padullés et al, 2004). Hitherto, the genetic variant which has been more clearly shown to affect brain function in humans is the ε4 allele of the Apolipoprotein E (APOE) gene. In comparison with the other isoforms, the ε4 variant has been associated with higher cholesterol levels, increased aggregation of amyloid, less protection against amyloid-induced oxidative neurotoxicity, less efficient repair of neurons and synapses, less protection against the hyperphosphorylation of the microtubuleassociated protein tau, the formation of neurofibrillary tangles, and a reduction in the outgrowth of neurons (Strittmatter et al., 1993; Miyata & Smith, 1996; Mahley, 1998). Although any of these processes could have a role in the development of AD, some could have an additional role in neurological development.

As compared to the non-carriers, healthy subjects bearing the ε4 genotype already show reduced metabolism and increased atrophy in the same regions as clinically affected AD patients (Reiman et al., 1996, 1998, 2001, 2004; Small et al., 1995, 2000b). Although mild, these are considered brain abnormalities prior to the onset of cognitive symptoms. Furthermore, the metabolic reductions were independent of brain atrophy (Reiman et al., 2004; Small et al., 1995). In middle-age ε4 carriers it has been evidenced that the metabolic reductions are progressive and correlate with reductions in cognitive performance (Reiman et al., 2001; Small et al., 1995).

fMRI studies in ε4 carriers parallel PET investigations finding abnormalities in these individuals as compared to non-bearers. A common finding in many studies has been the observation of increased activations in asymptomatic ε4 carriers several brain regions during cognitive processing. These areas have included the left prefrontal region, bilateral orbitofrontal, superior temporal, and inferior and superior parietal regions, during the

performance of a paired-associates learning and recall task (Bookheimer et al., 2000) as well as in the left parietal cortex during a letter fluency task (Smith et al., 2002). More recently, Bondi et al. (2005) found that among elders with normal cognitive function, carriers of the ε4 allele showed greater magnitude and extend of fMRI BOLD signal during picture learning relative to non-carriers in bilateral fusiform and medial frontal gyri, left inferior and middle frontal, right superior parietal, and right hippocampal and parahippocampal cortices. These findings were essentially replicated mainly for the left parahippocampal region using a verbal paired-associate learning task in a sample of younger subjects with both the APOE ε4 allele and positive history of dementia (Fleisher et al., 2005). The same group has reported right hemisphere overactivations in multiple regions among APOE ε4 positive elders using the same paired associate task (Han et al., 2007). Finally, Wishart et al. (2006) found increased activation among cognitively intact APOE ε3/ε4 relative to ε3/ε3 bearers in the medial frontal and parietal regions bilaterally as well as in the right prefrontal cortex during a two-back working memory task. Altogether, these results have been interpreted as supporting evidence that APOE ε4 subjects require increased cognitive work reflected by enhanced activity of supplementary brain regions as compared to non-carriers (Bondi et al., 2005, Bookheimer et al., 2000, Han et al., 2007 and Smith et al., 2002). Conversely, decreased activation during a covert object naming experiment has also been reported in ε4 carriers at high risk for AD (Smith et al., 1999) with additional regional decreases at follow-up examinations (Smith et al., 2005). This decreased activity was found in occipital, inferotemporal and frontal regions. Other studies have further observed reduced activations in left inferior parietal lobe and bilaterally in the anterior cingulate region during a semantic encoding task (Lind et al., 2006) as well as in the right hippocampus and entorhinal cortex during the encoding of novel stimuli (Trivedi et al., 2006).

Despite these reported studies evidencing distinct patterns of brain activity for ε4 carriers during cognitive tasks, little is known regarding the usage of dynamic brain networks by means of functional connectivity. This approximation could offer new knowlegde of how this relevant genetic variation influences brain circuits during cognitive processing in the elder.

1.1.7 Cognitive Reserve and brain function in normal and pathological aging conditions

1.1.7.1 Cognitive Reserve

Cognitive reserve (CR) applied to the field of aging research, refers to the hypothesized capacity of the mature adult brain to sustain the effects of pathology that would cause clinical dementia. This hypothesis predicts that for a given burden of neuropathology, those individuals with higher reserve will be able to remain clinically assymptomatic or in the whole will have a lower risk of dementia than individuals with less reserve. In a converse and complementary way, and in the context of similar clinical manifestations, the model predicts that individuals with higher CR will posses more neuropathology in their brains as those with lower CR. The concept of CR was initially proposed to account for the repeated observation that there was no direct relationship between the severity of brain damage and its clinical expression (Katzman, 1993; Satz, 1993; Stern, 2002). Katzman et al. (1988) were the first to observe a relationship between the clinical expression of dementia and brain volume in a sample of 137 nursing home residents. They found that 10 participants with AD-related neuropathology confirmed in postmortem examination demonstrated an equal cognitive performance to that of residents without any brain pathologies. The absence of clinical manifestations by these participants was attributed to their greater brain reserve. According to this 'hardware' or 'passive' view, although brain reserve would be reflected by measures such as brain volume, its ultimate correlate most probably corresponds to neuronal or synaptic density (Mortimer et al., 2003).

A complementary explanation was offered more recently by an active model, such as Stern's (2003a). This approach considered what might comprise cognitive reserve and proposed active and passive components. Among active components, level of education, complex occupations requiring continuing education and premorbid intelligence estimated using the Wechsler Adult Intelligence Scale (WAIS) Vocabulary subtest or the National Adult Reading Test are included. Other several factors such as higher quality in educational and occupational attainment as well as the involvement in cognitively and socially stimulating leisure activities can also be considered as CR proxies (Scarmeas in Stern, 2007, chapter 11). As stated, passive components would comprise brain characteristics, such as brain volume and ultimately synaptic density (Stern, 2003a) that add capacity to efficient processing of information, enhanced retrieval of memories and problem solving. In the active model, CR is understood as the ability to optimize performance by 'recruiting'

alternative brain networks, reflecting the use of different cognitive strategies. Due to the functional perspective of this model, the term of compensation was introduced to refer to the use of structures or brain networks not normally used in a nondamaged brain, to compensate for the deficit. Therefore, whereas passive models consider the reserve in terms of anatomical variables, active models define it in terms of individual task processing differences. Evidence supporting active models has been provided by PET studies on AD and healthy individuals (Scarmeas et al., 2003a, 2004a). These studies show brain networks in which the amount of increased activation from low to high demands of a visual recognition task is correlated with cognitive reserve.

Epidemiological studies have established low educational attainment and low occupational status as important risk factors for AD (Zhang et al., 1990; Launer et al., 1999; Cullum et al., 2000). In a longitudinal study of memory decline in AD, more rapid decline was detected in those patients with higher educational attainments and occupational status. This association suggested that a greater burden of Alzheimer neuropathology was required if highly educated individuals were to develop dementia. In a study by Bennet and colleagues, the association between AD pathology (particularly senile plaques) and cognition was modified by educational level (Bennet et al., 2003).

As regards premorbid IQ as a proxy for CR, lower childhood intelligence is a risk factor for late onset but not early onset dementia (Whalley et al., 2000). This association is even stronger at later ages of onset, suggesting that if childhood intelligence is a reliable indicator of CR, this reserve becomes more important with later age of onset. In the British 1946 Birth Cohort Study, Richards et al. (2004) showed that cognitive decline between age 43 and 53 partly accounted for childhood intelligence irrespective of educational and occupational attainment and certain health parameters.

Lifestyle differences between individuals might also explain differences in dementia risk, especially when lifestyle factors include exposures that might increase the risk of vascular disease. A more active lifestyle was found to be protective of late life cognitive function in several studies (Elwood et al., 1999; Dik et al., 2003) and that is consistent with a report that cognitive function in mid life is associated with greater physical activity in childhood (Richards et al., 2003). In a religious order study (Wilson et al., 2002), longitudinal data were collected from 801 older Catholic nuns, priests, and brothers

without dementia. Cognitive activities were registered and later shown to be associated with retention of cognitive function and reduced risk of dementia after controlling for age, sex and education. Authors concluded that continuing effortful cognitive activity in later life might reduce decline in global cognition. Leisure activities rated in a non-demented general population sample and whether they involved higher or lower cognitive effort, were also found to have a cumulative effect on the risk of incident dementia (Scarmeas et al., 2001). Generally, it is assumed that cognitive aging is not easily detected before age 60. In the British 1946 cohort study (Richards et al., 2003), leisure activities were associated with better cognitive performance at age 43, and physical exercise at age 36 was linked to a significantly slower rate of memory decline from age 43 to 53 years. Although physical activity is associated both as a cause and consequence of better general health, the study does not identify biological pathways mediating the protective effects of leisure on mid life cognition. In the Swedish Twin Studies, Crowe et al. (2003) compared leisure activities between same sex twin pairs discordant for dementia. Factor analyses of activity reports obtained 20 years earlier identified three activity factors: intellectual/cultural, self-improvement and domestic activity. The authors concluded that greater participation in intellectual-cultural leisure activities was associated with a lower risk of AD in women, but not men. Studies of physical activity (Gomez-Pinilla et al., 1998) in juvenile rodent models of corticogenesis suggest that greater physical activity is related to greater induction of neurotrophic growth factors and this may explain better cognitive function in old people who exercise (Kramer et al., 1999). Likewise, nutritional factors in early life may also extend their influence from cognitive function in childhood to mid life and thus contribute to cognitive reserve (Richards et al., 2002).

1.1.7.2 Cognitive Reserve influence on brain function

The relationship between an estimate of premorbid intelligence and cerebral glucose metabolism was investigated by Alexander et al. (1997) in AD patients of similar severity disease. Higher IQ ability was inversely correlated with cerebral glucose metabolism in specific brain regions. This finding suggests that more cerebral pathology is required in those of higher original intelligence to produce the clinical features of dementia. Likewise, patients with higher occupational attainment (Stern et al., 1995), higher education (Stern et al., 1992) or more engagement in intellectual, social, and physical activities (Scarmeas et al., 2003b) manifested more prominent cerebral blood flow or metabolism deficits (and hence

more pathology) when controlling for clinical severity. In other words, as compared with low CR individuals, subjects with higher CR can manifest milder clinical deficits despite comparable burden of pathologic involvement. These observations support the prediction that individuals with more CR can tolerate more pathology.

In order to further investigate the neural basis of CR, some investigators have started using brain imaging not only during rest but also during cognitive activation. Most of these studies were performed for healthy participants (Gray et al., 2003; Stern et al., 2003b; Scarmeas et al., 2003a). However, one PET activation study that noted associations between a general index of CR (obtained from a combination of the National Adult Reading Test, Vocabulary subtest of the Wechsler Adult Intelligence Scale and years of education) and brain activation during a recognition memory task was performed in AD patients (Scarmeas et al., 2004a). The directionality of the association between CR and cerebral activation differed between normal aging and AD.

The biological nature of how CR confers protection against dementia can be conceived in two ways. The first refers to the *neural reserve* or anatomic factors such as bigger brains or higher number of synapses or neurons, which would tolerate more loss before exhibiting impaired function (Scarmeas & Stern, 2004). The second one emphasizes the efficiency of brain networks, irrespective of anatomic characteristics. Even though the number of neurons and synapses is the same, enhanced synaptic activity, more efficient connectivity or more ability to use alternate brain networks may be more likely in individuals with higher CR. As mentioned earlier, the term of neural *compensation* should be used in a situation where the physiological effects of aging or brain pathology cause the alteration of a brain network, resulting in a network that would not normally be used by unaffected individuals (Stern et al., 2005). With increased task difficulty, recruitment of additional brain areas has been observed, even in healthy young adults (Grady et al., 1996; Rypma and D'Esposito, 1999; Jansma et al., 2000; Jha and McCarthy, 2000; Glahn et al., 2002). Very few studies have looked at the relation between measures that would be proxies for CR and task-related activation in healthy elders (Gray et al., 2003; Habeck et al., 2003; Scarmeas et al., 2003a; Stern et al., 2003b) and fewer in pathological aging (Scarmeas et al., 2004a).

In summary, current functional neuroimaging studies evaluating the relationship between brain activity and performance in the face of a particular CR background provide

information about the neural implementation of the two aspects of CR above all in normal aging: neural reserve and compensation. Further studies are required to investigate such relationships among cognitively impaired elders without dementia as well as dementia conditions, particularly of the AD type.

1.2 APPROACH AND OBJECTIVES

From the above reviewed literature, it should be concluded that there are several numbers of variables that can influence brain function and anatomy during healthy and pathological aging and thus contribute to explain the cerebral and behavioral characteristics of our seniors. Among the genetic factors, the apolipoprotein E (APOE) ε4 allele has been proved to affect how brain activates at-rest or while performing a particular cognitive task among healthy elders and cognitive impaired patients (Smith et al., 1999; Bookheimer et al., 2000; Burggren et al., 2002; Scarmeas et al., 2004b; Bondi et al., 2005; Reiman et al., 2005; Dickerson et al., 2005; Scarmeas & Stern, 2006; Han et al., 2007). On the other hand, studies investigating the role of environmental variables such as education, ocupation or other factors considered within the construct of cognitive reserve have only recently been studied in terms of how they modulate cognitive function and particularly brain structure and activity in samples of normal elders and early stages of dementia. Finally, the experimental manipulation of brain activity using a non-invasive method such as TMS, offers a unique approach to investigate how cerebral responses to magnetic stimulation are modulated by the abovementioned genetic and environmental variables influencing brain activity thorough life in the elders.

In the present thesis we aim to study the influence of the above reported genetic and environmental variables and their interactions on brain function among healthy and pathological aging: Aging-Associated Cognitive Decline (AACD), Mild Cognitive Impairment (MCI) and mild Alzheimer's disease (AD).

The objectives of the present thesis are the following:

1) To study the effects of Transcranial Magnetic Stimulation (TMS) on brain activity and memory performance in a sample of aging-associated cognitive decline (AACD).
2) To study the effects of the interaction between TMS and APOE genotype in AACD.
3) To study how a particular genetic background according to the APOE gene affects brain connectivity in a sample of AACD while performing a visual learning task.
4) To study the influence of cognitive reserve proxies in brain structure and atrophy in order to test the brain reserve hypothesis on the one hand, as well as investigate how the level of cognitive reserve may influence brain activity patterns in order to explore active models of CR (i.e. compensation) among healthy elders, MCI and mild AD.

2. METHODOLOGY

The present thesis comprises four main studies which required several methods and techniques widely used in neuroscience. First of all, neuropsychological tests have been essential for a proper diagnosis and recruitment of the sample to be enrolled in each of the studies. Moreover, neuroimaging techniques, and particularly functional magnetic resonance imaging (fMRI) have been needed to explore how brain works by measuring the hemodynamic response related to its neural activity. Transcranial magnetic stimulation (TMS) was used in-between fMRI sessions in two studies in order to test rTMS effects on both brain function and cognitive performance in elders with memory complaints. Finally, cognitive reserve variables were also investigated to determine how they affected brain structure and function in normal aging and in pathological conditions (Alzheimer's disease).

2.1 NEUROPSYCHOLOGICAL ASSESSMENT

A global evaluation of cognitive function was measured with the Mini-Mental State Examination by Folstein et al. (1975). Besides, specific assessments of language (Token Test and Boston Naming Test), praxis (imitation and perfomance to command of ideomotrive praxis) and gnosis (Poppelreuter's embedded figures and Luria's watches) or together with abstract reasoning (Wechsler Adult Intelligence Scale III (WAIS-III) Similarities subtest) and memory function (Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and Rey Auditory Verbal Learning Test) were undertaken in order to classify patients into a particular clinical category from cognitive preservation to moderate cognitive impairment: healthy aging, AACD, amnestic MCI or mild AD.

2.2 FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI)

Two fMRI designs were used. For the first two studies, a face-name declarative memory learning task with posterior retrieval and recognition was employed, modified from Sperling et al. (2001) and also previusly used in our laboratory in a sample of adolescents with antecedents of prematurity (Giménez et al., 2005). In the third study, a picture memory task with subsequent forced-two choice recognition test was employed, modified from Dickerson et al. (2004).

fMRI images obtained from abovementioned designs where analysed using the general lineal model to show patterns of brain activity that differed across distinct groups and in

one study we used functional connectivity by means of the coherence analysis approach, which reveals cerebral regions showing sincronic brain activity.

2.2.1 Functional connectivity

To study functional connectivity of the hippocampus, we used coherence analysis, a method that measures the linear time-invariant relationship between two signals (Sun et al., 2004). To identify networks of functional connectivity for the hippocampus, we generated coherence maps using the task-specific coherence between the reference voxels, or seed and all other regions in the brain. The method is outlined in four steps: (1) selection of a seed; (2) generation of condition-specific time series; (3) estimation of condition-specific coherence maps; (4) group analysis of condition-specific coherence maps. This analysis measures task-related interactions between regions and across groups.

2.3 TRANSCRANIAL MAGNETIC STIMULATION (TMS)

A MAGSTIM SUPER stimulator and a double-cone coil were employed. The intensity of TMS pulses was set at 80% of motor threshold. The intersection of the coil was placed over the left primary motor cortex. Ten rapid TMS trains, with a duration of 10s each, were applied during a period of 5 minutes and at a frequency of 5 Hz. Every 30s, subjects were then delivered 10s of stimulation followed by a rest period of 20s. For the sham or placebo condition, the coil was positioned tangentially to the head, with its edge resting on the scalp, so that no magnetic pulses were actually administered.

2.4 APOLIPOPROTEIN GENOTYPE

Genomic DNA was isolated from peripheral blood leukocytes. At the APOE locus, the polymorphism of the three common genetic variants, ε2, ε3 and ε4, due to Cys-Arg substitutions at amino acid positions 112 and 158 was analyzed. The polymerase chain reaction was used to amplify the alleles of the APOE gene as described by Wenham et al (1991).

2.5 COGNITIVE RESERVE (CR) PROXIES

Three main CR proxies were defined: 1) educational and occupational attainment, 2) the Wechsler Adult Intelligence Scale Vocabulary subtest was administered as a measure reflecting premorbid IQ and finally 3) a customized questionnaire including items such as leisure activities, cognitively stimulating activities, physical and social life (see annex).

2.6 STATISTICAL ANALYSIS

The Statistical Package for Social Sciences (SPSS v.11.5.1, 12.0 and 14.0) was used to investigate group differences in demographic, clinical, neuropsychological perfomance and CR measures by means of ANOVA and chi-square tests when appropriate. Further analyses included a two way ANOVA for pre and post TMS data and partial correlations for the CR study. For the analyses of structural MRI and fMRI data the Statistical Parametric Mapping (SPM2) software, running in Matlab 6.5 (MathWorks) was used. Individual and group analyses were performed. Two sample t-tests, Analysis of Variance (ANOVA) and regression analyses were undertaken depending on the specific study.

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3. RESULTS

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Repetitive Transcranial Magnetic Stimulation Effects on Brain Function and Cognition among Elders with Memory Dysfunction. A Randomized Sham-Controlled Study

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In the present study, we aimed to investigate the effects of repetitive transcranial magnetic stimulation (rTMS) on memory performance and brain activity in elders presenting with subjective memory complaints and a memory performance within the low normal range. Forty participants underwent 2 functional magnetic resonance imaging (fMRI) sessions, in which they were administered 2 equivalent face–name memory tasks. Following each fMRI, subjects were asked to pair faces with their corresponding proper name. In-between, high-frequency rTMS was applied randomly using real or sham stimulation in a double-blind design. Only subjects who received active rTMS improved in associative memory significantly. This was accompanied by additional recruitment of right prefrontal and bilaterial posterior cortical regions at the second fMRI session, relative to baseline scanning. Our findings reflect a potentiality of rTMS to recruit compensatory networks, which participate during the memory-encoding process. Present results represent the first evidence that rTMS is capable of transitorily and positively influencing brain function and cognition among elders with memory complaints.

Keywords: associative memory task, face–name memory encoding, functional magnetic resonance imaging, repetive transcranial magnetic stimulation

Introduction

Transcranial magnetic stimulation (TMS) is a noninvasive technique, which delivers magnetic pulses reaching the cerebral cortex through the scalp. It is generally accepted that high-frequency (>1 Hz) repetitive transcranial magnetic stimulation (rTMS) induces an increase in cortical excitability, whereas low-frequency (≤1 Hz) rTMS reduces it (Pascual-Leone and others 1994; Chen 2000; Gorsler and others 2003), although these assumptions have been challenged by recent neuroimaging studies in nonmotor areas investigating functional connectivity (Speer and others 2000). Previous studies have demonstrated that rTMS is able to modulate the activity of a particular cortical region, resulting in transsynaptic effects on other distant areas (Paus and others 1997). Furthermore, applying rTMS simultaneously with functional imaging techniques allows the study of brain-behavior relationship (Mottaghy and others 2000, 2003; Sack and Linden 2003). The potential effects of rTMS in modulating human cognitive functions, including memory, have been manifested in a number of studies (Grafman and Wassermann 1999; Pascual-Leone and others 1999, 2000; Jahanshahi and Rothwell 2000). In this regard, most published reports showed interferences on neuropsychological performance, but recent evidence is now available indicating a positive effect of rTMS on several cognitive domains, such as short-term memory (Pascual-Leone and others 1993; Wassermann and others 1996), analogic reasoning (Boroojerdi and others 2001), picture naming (Topper and others 1998; Mottaghy and others 1999), and language identification tasks (Andoh and others 2005). Whereas these findings have been mainly described in young and healthy subjects, little is known about the putative cognitive effects of rTMS in the elder population. Nonetheless, in a study by Moser and others (2002), a beneficial effect of high-frequency rTMS was observed in executive function among depressive adults with a mean age of 60 years.

In the present report, we sought to investigate whether the above-mentioned positive effects of rTMS can also be observed in a group of otherwise healthy elders presenting with subjective memory complaints and low memory performance. Should these effects be evidenced in a memory task, a second aim was to determine if such cognitive improvement was accompanied by concomitant changes in brain activity measured by functional magnetic resonance imaging (fMRI).

Materials and Methods

Subjects

Forty adults with memory complaints enduring at least 1 year and above 50 years of age were recruited from 2 health centers and 1 hospital in Barcelona. Participants did not meet criteria for dementia according to Diagnostic and Statistical Manual fourth edition criteria and a neuropsychological assessment, including measures of global cognitive function (mini-mental state examination ⩾ 24), language (Token test, Boston Naming Test), praxis (imitation and performance to command), gnosis (Poppelreuter's embedded figures and Luria's watches), and abstract reasoning (Wechsler Adults Intelligence Scale III Similarities subtest). None of the participants suffered from other psychiatric or neurological disease based on medical evaluation. Moreover, possible cases of clinically depressive mood were ruled out through a Hamilton Depression Scale cutoff score of 15. Besides memory complaints and normal performance in the remaining cognitive areas, our subjects exhibited a performance in the low normal range (–1 standard deviation [SD] below standardized age-matched norms) in at least one of the

following secondary memory tests: Rey Auditory Verbal Learning Test, Visual Reproduction (Wechsler Memory Scale Revised), or Benton Visual Retention Test.

Magnetic Resonance Imaging Acquisition

Scans were obtained on a GE Signa 1.5T (General Electric, Milwaukee, Wisconsin). High-resolution T_1-weighted images were acquired for anatomical identification with a Fast Spoiled Gradient-Recalled Echo three-dimensional sequence (Digital Imaging and Communications in Medicine) format: Repetition time [TR]/Echo time [TE] = 12/5.2, Inversion time = 300, Number of Exitations = 1, FOV = 24 × 24 cm, 256 × 256 matrix). Whole-brain volumes were acquired in an axial plane yielding contiguous slices with slice thickness of 1.5 mm. Functional images were acquired using a T_2*-weighted gradient echo planar imaging (TR = 2000 ms, TE = 40 ms, FOV = 24 × 24 cm, flip angle of 90°). Twenty axial slices were obtained for each brain volume with a slice thickness of 5 mm and a gap of 1.5 mm.

Baseline fMRI Session and Memory Assessment

We used a 10-block design task with alternating "repeated" and "experimental" conditions (5 blocks each). The whole experiment had a duration of 300 s (30 s per block). The task started with a repeated block, which consisted of the presentation of 2 face–name pairs that were learned before the fMRI session. These 2 stimuli were presented five times each in an alternating way (presentation time: 2 s and interstimuli period: 1 s). Following this block, participants were presented 10 face–name pairs previously unfamiliar to the subjects (experimental block). The presentation time and interstimuli period for these 10 stimuli were equivalent to those presented during the repeated block. The same repeated and experimental blocks were thereafter presented in an alternating way until the total duration of the experiment (10 blocks, 300 s) was reached. During the repeated blocks, subjects were asked to keep their attention on the displayed face–name pairs even though they were already known. On the other hand, in the experimental blocks individuals were given explicit instructions to try to remember which name was associated with which face for later testing. Following the fMRI session, participants were assessed using an associative memory procedure, where they had to match the names and faces given separately. For this purpose, individuals were shown 10 printed photographs as well as 10 written names and were instructed to pair each name with the corresponding face as they remembered from the fMRI session. Only the stimuli used in the experimental blocks were presented during the associative memory task, and thus, only correct/incorrect face–name matches were recorded as responses. The maximum score for this task was 10 (all names correctly matched with the corresponding face).

Repetitive Transcranial Magnetic Stimulation

Individuals were randomly assigned to 2 groups: an active rTMS (n = 20) and a sham rTMS (n = 20). One subject initially belonging to the placebo condition had to be excluded from the whole study due to visual problems that precluded her to accomplish the fMRI task. Hence, the study finally enrolled 39 subjects in a double-blind design, in which neither the participants nor the researcher in charge of the memory assessment knew who received real or sham stimulation.

rTMS was applied using the so-called "off-line" paradigm (Robertson and others 2003) because magnetic pulses were administered during a rest period set between the first and second fMRI examinations. A MAGSTIM SUPER stimulator and a double-cone coil were employed. The intensity of TMS pulses was determined at 80% of motor threshold, which corresponded to the lowest intensity able to elicit a visible twitch of the first dorsal interosseus muscle of the right hand in at least 5 out of 10 trials. For this purpose, the intersection of the double-cone coil was positioned over the left primary motor cortex. For the active rTMS session, it was slightly diverted to the plane of the interhemispheric cissure and moved anteriorly approximately 5 cm to reach the prefrontal cortex.

Ten rTMS trains lasting 10 s each were delivered during a 5-min period at a frequency of 5 Hz. Specifically, every 30 s, subjects were given 10 s of TMS followed by a 20-s rest period. For the sham condition, the coil was positioned tangentially to the head, with its edge resting on the scalp.

Post-rTMS fMRI Session and Memory Assessment

Immediately after the rTMS, patients underwent a second fMRI examination. The time elapsed in-between was in minutes: 10.07 (mean), 2.30 (SD). The characteristics of this second fMRI session were exactly the same as those described earlier for the baseline session with the exception that during the 5 experimental blocks we used 10 new unfamiliar face–name pairs. Once the scanning procedure was finished, participants were retested for associative memory as explained earlier. The order of presentation of the fMRI sessions (baseline and post-rTMS) was counterbalanced across subjects. Figure 1 illustrates the experimental procedure in 3 stages.

Data Analysis

fMRI Data

Images were processed with SPM2 (Statistical Parametric Mapping) running in Matlab 6.5 (MathWorks, www.mathworks.com). Original magnetic resonance images registered in format GE-advanced (1 two-dimensional file per slice) were organized into three-dimensional files (150 volumes per subject) by means of MRICro software (University of

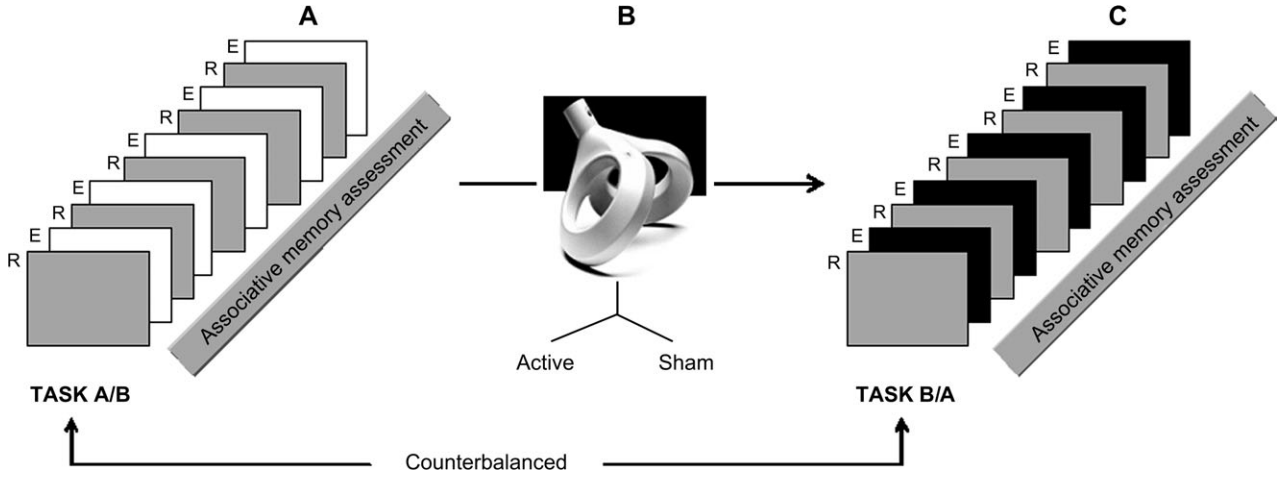

Figure 1. Experimental design. (*A*) Baseline fMRI session: alternating 5 repeated (R) and 5 experimental (E) blocks. The former consisted of the presentation of 2 previously learned face–name pairs, whereas the latter included 10 unfamiliar face–name pairs. This was followed by an associative memory assessment. (*B*) rTMS session applied either in an active (n = 20) or a sham (n = 19) condition. (*C*) Post-rTMS fMRI session: the E blocks comprised 10 new unfamiliar face–name pairs, whereas the R blocks remained the same learned items as in the first. fMRI session: the post-rTMS fMRI session was accompanied by a memory assessment analogous to the first one.

Nottingham, UK) and saved in ANALYZE 7.5 format compatible with SPM2. Following alignment along the anterior commissure–posterior commissure line and realignment of the scans to remove the effects of head movement, images were transferred into a standardized coordinate system. Normalized images were smoothed with an isotropic Gaussian kernel (full width half maximum) of 8 mm. Individual analyses were carried out for each subject to evaluate the increased activation observed during the experimental condition compared with that seen during the rest condition. Except when specifically stated, results derived from group analyses were examined at the voxelwise threshold of $P < 0.001$ (uncorrected for multiple comparisons) and at $P < 0.05$ threshold on the extent of clusters or with more than 20 contiguous voxels.

Neuropsychological Data

Scores for the associative memory task for different groups and conditions were analyzed using two-way analysis of variance (ANOVA) and Student's *t*-tests with the statistical program SPSS v.11.5.1.

The study was approved by the local ethics committee, and all the participants gave informed consent for their participation.

Results

Active and sham groups were comparable in terms of age, cognitive status, gender distribution, and educational attainment (Table 1).

Baseline Whole-Group fMRI Activations

Our analyses at baseline for the entire sample of subjects during the encoding memory condition relative to the resting condition revealed significant activations in several clusters including frontal and parietal regions, visual associative areas, cingulate gyrus, and cerebellum (Table 2). Further, second-level analyses attempting to correlate the behavioral data with patterns of fMRI activity revealed positive correlations (uncorrected $P < 0.005$) in the right middle frontal gyrus (Brodmann's Area [BA] 45/46, coordinates [*x*, *y*, *z*]: 42, 28, 12; cluster size: 0.24 cm^3; $t = 3.14$, $P = 0.002$) and bilaterally in parietooccipital regions (BA 7, coordinates: –60, –46, 40; cluster size: 1.24 cm^3; $t = 3.66$, $P = 0.001$, and BA, 19/40—coordinates: 22, –70, 46; cluster size: 0.37; $t = 3.48$, $P = 0.001$).

Table 1
Demographic and global cognitive characteristics of the participants

	Active rTMS ($n = 20$)	Sham rTMS ($n = 19$)	Statistical values	*P* values
Age	66.95 (9.43)	68.68 (7.78)	$t = 0.63$	0.54
MMSE[a]	26.50 (2.06)	26.16 (1.92)	$t = 0.51$	0.61
Educational attainment[b]	4/12/4/0	2/9/7/1	$\chi^2 = 2.89$	0.41
Gender[c] (M/F)	5/15	6/13	$\chi^2 = 0.21$	0.65

Note: Values are given in mean (SD).
[a]MMSE, mini-mental state examination.
[b]No studies/primary/secondary/superior.
[c]M, male; F, female.

Table 2
Brain pattern activation for the whole group ($n = 39$) during baseline fMRI

Talairach coordinates [*x*, *y*, *z*]	Region	BA	Volume (cm^3)	*t* Values	*P* values
[−36, 16, 24]	Left inferior frontal gyrus	44, 45	26.30	6.55	<0.001
[−38, −76, −12]	Left cerebellum	—	5.86	6.02	<0.001
[42, −50, −16]	Right fusiform gyrus	17	3.86	5.64	<0.001
[36, −14, 26]	Right supramarginal gyrus	40	4.06	5.51	<0.001
[−24, −66, 40]	Left superior occipital gyrus	19	4.74	5.29	<0.001
[−2, 8, 46]	Left cingulate gyrus	24	2.46	4.58	<0.001
[36, 16, 24]	Right middle/inferior frontal gyrus	6, 44	1.07	4.57	0.02

Effects of rTMS on Memory Performance

No major side effects of rTMS administration including seizures were reported by any patient. Associative memory scores did not differ between active and sham groups for both sessions (baseline associative memory: $t = 0.88$, df = 37, $P = 0.39$; post-rTMS examination: $t = 1.45$, df = 37, $P = 0.14$). Interaction between rTMS conditions (active vs. sham) and pre- versus postmemory performance was tested by a two-way repeated-measures ANOVA. A significant interaction between both factors was seen, evidencing that pre- and postmemory performance was different across rTMS groups ($F = 7.15$, df = 1, $P = 0.01$). To investigate a possible amelioration in the active group relative to the sham condition, a *t*-test for independent samples was conducted on a new variable (rate of change), which was created by subtracting the value of the associative memory task achieved in the first assessment from the score obtained in the second memory task. Significant differences in the *t*-test showed that the active group improved as compared with the sham condition, as reflected by positive values in the rate of change variable in the former (Table 3). Even though the mean value of the new created variable was slightly negative for the placebo condition (indicating better performance in the first memory evaluation), a related-samples *t*-test revealed no statistical differences in the 2 time evaluations ($t = –1.39$, df = 18, $P = 0.18$).

rTMS Effects on Brain Activity

Brain activation patterns were not different when comparing active and sham groups at baseline examination. However, the comparison between both groups at follow-up did show significant changes in terms of higher activation among the active group in the left anterior cingulate (BA 24—Talairach coordinates [*x*, *y*, *z*]: –6, 30, 34; cluster size: 3.07 cm^3; $t = 4.73$, $P = 0.001$) and right middle and superior frontal gyrus (BA 9—Talairach coordinates [*x*, *y*, *z*]: 42, 10, 38; cluster size: 0.84 cm^3; $t = 4.00$, $P = 0.03$). To address whether the brain activity in the active group was different from that of the placebo condition across the 2 fMRI sessions, a mixed ANOVA was conducted with rTMS condition as the intersubject factor and fMRI session as the intrasubject factor. This analysis showed a significant interaction affecting the right middle frontal gyrus (BA 8—Talairach coordinates [*x*, *y*, *z*]: 36, 36, 40; cluster size: 2.04 cm^3; $F = 15.18$, $P < 0.001$) and the right medial frontal lobe (BA 8—Talairach coordinates [*x*, *y*, *z*]: 6, 28, 44; cluster size: 1.48 cm^3; $F = 14.36$, $P < 0.001$). To further investigate the direction of the interaction, subsequent two-sample *t*-tests were undertaken. A first analysis showed that when contrasting the active group across the 2 fMRI sessions, the right inferior and middle frontal gyri together with middle and superior occipital gyri were additionally activated in the second fMRI acquisition (Fig. 2 and Table 4). These data indicate that such changes are rTMS related because no significant modifications could be evidenced within the group of subjects receiving sham stimulation.

Discussion

To our knowledge, the present study provides first evidence of rTMS ability to induce transient ameliorations in associative

Table 3
Associative memory task performance for the active and sham groups before and after rTMS as well as the rate of change between the 2 assessments

	Associative memory at baseline[a]	Associative memory at follow-up[a]	Rate of change (improvement)[a]	Confidence interval 95%	*t* Values	*P* values
Active rTMS ($n = 20$)	4.15 (2.94)	5.75 (2.99)	1.60 (3.08)	0.16–3.04		
Sham rTMS ($n = 19$)	5.00 (3.11)	4.37 (2.97)	−0.63 (1.98)	−1.58–0.32	−2.67[b]	0.01

[a]Mean (SD). Rate of change variable (improvement) reflects follow-up minus baseline associative memory. Positive values (active group) indicate increased memory performance at the second memory evaluation.
[b]*t*-Test for independent samples conducted for the improvement variable showing significant amelioration in the associative memory task following real rTMS group compared with the sham condition.

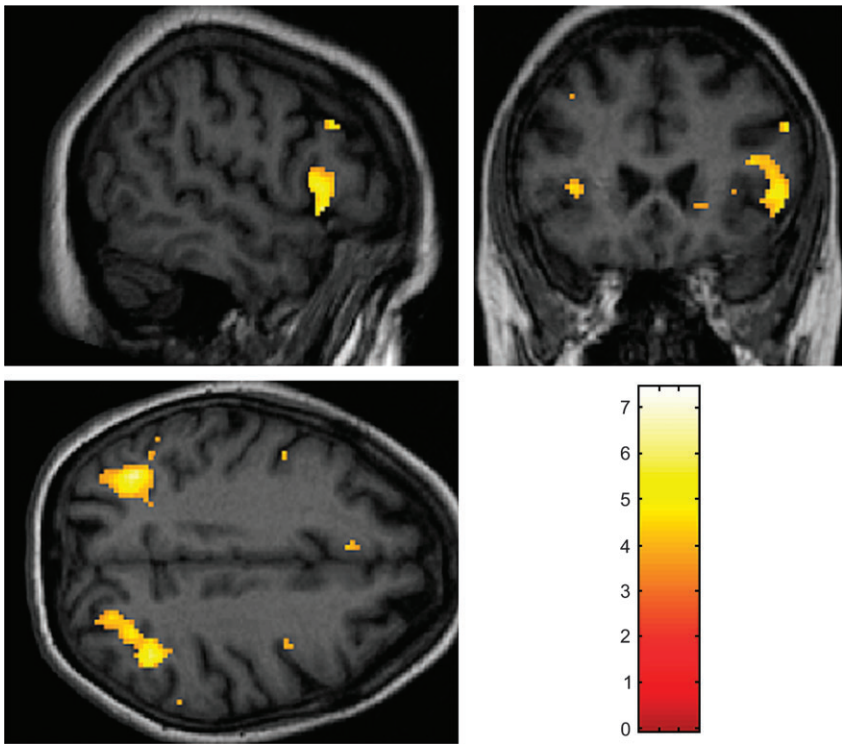

Figure 2. Images depict increased brain activation following rTMS relative to baseline within the active group (for precise anatomical localization of the significant regions, see Table 4).

Table 4
Brain regions significantly activated among the active group following rTMS session, relative to baseline

Talairach coordinates [*x*, *y*, *z*]	Region	BA	Volume (cm^3)	*t* Values	*P* values
[−36, −62, 34]	Left middle/superior occipital gyrus	39, 19	3.64	7.40	<0.001
[38, −52, 40]	Right superior occipital gyrus	19	3.07	6.01	<0.001
[52, 20, −4]	Right inferior frontal gyrus	45, 47	2.11	5.99	<0.001
[42, 40, 14]	Right middle frontal gyrus	46	1.05	5.12	0.001

memory assessed immediately after stimulation as well as measurable concurrent cerebral changes among elders with memory complaints and low performance in neuropsychological tests of secondary memory. Present results are in line with several studies now available demonstrating cognitive enhancement during (Mottaghy and others 1999; Boroojerdi and others 2001; Cappa and others 2002; Andoh and others 2005) and after rTMS administration (Pascual-Leone and others 1993; Hilgetag and others 2001; Moser and others 2002; Brighina and others 2003).

Using a face–name learning task, we obtained a whole-group activation pattern including the fusiform and cingulate gyri as well as the inferior parietal lobe, which is in accordance with previous findings using a similar task on healthy elder subjects, individuals with mild cognitive impairment, and mild Alzheimer's disease patients (Sperling and others 2003). Additional brain areas such as the inferior frontal lobe and secondary visual areas have also been related to learning of faces in healthy young subjects (Sperling and others 2001, 2003). Face perception involves recruitment of multiple and bilateral cerebral regions, including the extrastriate cortices (Haxby and others 2000). Likewise, secondary visual regions have been seen highly activated in a series of studies employing picture-encoding tasks (Grady and others 1999; Reber and others 2002).

Accordingly, our results evidencing increased blood oxygen level dependent (BOLD) signal bilaterally in the middle and superior occipital gyri among the active group following rTMS administration suggest that activation of such regions may play a role in successful face encoding and eventual memory performance.

Along with visual areas, other regions that showed contrasted brain response following rTMS between sham and active groups were the left anterior cingulate gyrus and bilateral regions of the dorsolateral prefrontal cortex. Interestingly, supplementary recruitment in the right inferior and middle frontal gyri as well as in the left middle/superior occipital gyrus was observed only in the active group during the second fMRI acquisition. The relevance of these left parietooccipital and right prefrontal regions relative to the memory task is highlighted by our results showing positive correlations between BOLD signal and behavioral measures. Despite these considerations, these latter findings should be interpreted with caution because the statistical threshold had to be lessened to observe significant results. The most probable explanation relates to the fact that BOLD measurements within the scanner reflected brain regions activated during a face–name learning task, whereas behavioral measures were related to an associative memory task. Hence, the weak associations found might be explained by the fact that encoding and retrieval semantic memory tasks might not share the exact neurofunctional correlates (Cabeza and Nyberg 2000; Fletcher and others 2001). Specifically, retrieval of information of face processing seems to rely on more anterior prefrontal areas than those observed in the present study during memory encoding (Ranganath and others 2003). To overcome this limitation, future studies possibly using event-related designs should be able to record direct behavioral responses during the scanning sessions.

Several lines of evidence suggest that the cognitive effect induced by rTMS in our study might be principally mediated by the additional recruitment of right prefrontal regions that were highlighted in our mixed ANOVA model as well as in the paired *t*-test comparison within the active group. First, encoding of faces and scenes has demonstrated a common area of activation within the right inferior frontal gyrus (Golby and others 2001). Second, studies with aged and young populations demonstrated that increased activity of prefrontal regions during semantic encoding correlated with the likelihood of eventual memory performance (Wagner and others 1998; Grady, McIntosh, and others 2001; Stebbins and others 2002; Gutchess and others 2005). Finally, former studies comparing young and older adults during encoding memory tasks have pointed out a more unilateral prefrontal cortex (PFC) activity in the former ones, but a bilateral pattern in the latter (Backman and others 1997; Grady, Furey, and others 2001; Gutchess and others 2005), which is in accordance with the brain activity pattern observed following rTMS in our active group. The different encoding network proposed for older subjects has been interpreted as compensatory. Consistent with this hypothesis, several studies have demonstrated that a bilateral recruitment of PFC regions is related to facilitation in memory tasks (Reuter-Lorenz and others 2000; Cabeza and others 2002; Rosen and others 2002). Notably, direct evidence of a compensatory role of the right dorsolateral frontal lobe among healthy elders has been recently reported in an rTMS study (Rossi and others 2004).

Despite being encouraging, further research is merited in order to corroborate these results. Of particular interest would be the study of brain-behavior relationships following rTMS in elders without any cognitive dysfunction. To the best of our knowledge, there are no studies of cognitive enhancement following TMS among normal aging, but there is some evidence that memory training within these individuals causes a reliable improvement even in a 2-year follow-up period (Ball and others 2002). Assuming that older adults may be benefited from cognitive rehabilitation and given the positive effects of rTMS found in previous and present report, an intriguing issue for ulterior studies might be to determine to what extent this technique is useful as an add-on instrument in cognitive training programs for this population. Despite these putative potential applications, in its current state, present findings must be interpreted solely as showing experimental evidence of rTMS ability to transiently influence brain function and not as indicating rTMS as a therapeutic tool for subjects with memory complaints and low performance in neuropsychological tests.

Several limitations or specific particularities of the present study influencing the interpretation of results should be considered. First, and regarding the characteristics of our patients, reported results should be set in the proper context. Due to the presence of a continuum of memory impairment from normal aging to dementia (Petersen and others 2001), the problem of a high heterogeneity within our sample might be an important issue to bear in mind. Thus, although setting our memory cutoff to –1 SD below age-matched norms allowed us to exclude normal memory performing elders, we cannot reject the possibility that our sample included a variety of cases ranging from elders showing only mild memory dysfunction to preclinical stages of Alzheimer's disease. Studies in more homogeneous groups are needed to precisely interpret the rTMS effects in distinct elder populations.

Second, previous studies have shown cognitive facilitation after rTMS ranging from minutes up to several days (Pascual-Leone and others 1993; Hilgetag and others 2001; Brighina and others 2003). Notwithstanding, in the current study, we have not assessed the duration of rTMS effects on cognition. There is evidence that with increased number of sessions of rTMS at high frequency, stronger effects on cortical excitability can be observed (Maeda and others 2000). Although speculative, it might occur that increasing cerebral activity from additional rTMS sessions is associated with more significant changes in cognitive performance. This possibility was, however, not addressed in the present report.

Finally, in the present report, we did not use any available neural navigation or frameless stereotaxic device systems as has been performed in recent cognitive studies combining rTMS with functional neuroimaging techniques (e.g., Andoh and others 2005). Thus, despite the coil being placed over the prefrontal region, we were unable to identify the specific anatomical localization of the site of rTMS. In the same line, the coil used in our study probably causes diffuse effects under the stimulated cortex and nearby functionally connected areas (Maccabee and others 1990). These 2 observations prevent from identifying a single cerebral area targeted by rTMS and directly related with the observed cognitive results. Another aspect to bear in mind regarding the double-cone coil relates to its magnetic proprieties. Stimulation elicited by smaller coils, like the figure-of-eight shaped, produces weaker magnetic fields (Weber and Eisen 2002). Conversely, the double-cone coil is considered the best tool for stimulation of deep brain structures up to 3–4 cm in depth (Maccabee and others 1990; Terao and

others 1994, 2000) and produces stronger and more distributed magnetic field (Roth and others 2002). These unique characteristics might affect cerebral excitability or transsynaptic connections, resulting in particular cognitive effects. Despite this, we cannot resolve with certainty that there is a relationship between coil characteristics and reported results because they have not been contrasted with alternative coils. In any case, our results encourage further investigations employing the double-cone coil in cognitive studies.

In summary, our study provides a first step evidencing the feasibility of rTMS to transiently improve memory performance with concurrent cerebral changes among elders with memory complaints and performance within the low normal range. Ongoing investigations in homogeneous samples designed to acquire direct correlations between behavioral data and brain activity as well as combining larger amount of rTMS sessions with a neural navigation device adapted to TMS are required to explore in depth the beneficial effects of magnetic stimulation on cognitive aging.

Notes

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Genetic modulation of rTMS effects on brain function among cognitively impaired elders

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Abstract

Behavioral effects of a focal brain lesion, which can be modeled by repetitive transcranial magnetic stimulation (rTMS), represent an interaction between the local disruption and the capacity of the rest of the brain to adapt to it. Genetic differences among individuals likely play a critical role in such an adaptation. The apolipoprotein E (APOE) ε4+ allele is a well-known genetic variant that influences cognitive processing in humans. Thus, differences in brain activity modulated by this genetic variation may condition the brain's responses to TMS during a cognitive task. Twenty participants (9 APOE ε4 allele carriers) underwent two fMRI sessions, in which they were administered two equivalent face-name memory tasks. In-between, high-frequency rTMS was applied to the prefrontal region to modulate its activity. Despite similar behavioral impact, in the second fMRI, ε4+ subjects recruited additional contralateral prefrontal cortical regions relative to baseline scanning, whereas APOE ε3/ε3 individuals showed additional activity only in the inferior parietal cortex bilaterally. Present results offer first evidence that genetic differences may play a fundamental role to understand the differential brain effects of focal brain insults in humans.

Keywords: repetive transcranial magnetic stimulation (rTMS), apolipoprotein E (APOE), aging-associated cognitive decline (AACD), functional magnetic resonance imaging (fMRI), face-name memory encoding, recognition memory, prefrontal cortex.

Introduction

A growing body of evidence from neuropsychological, neurophysiological, and neuroimaging studies in animals and humans suggests that interactions between brain regions engaged in functional networks underlie cognitive processing and determine behavior. Every cognitive function and goal-directed behavior may be best identified with a certain pattern of activation of specific, spatially-distributed, but interconnected, neuronal assemblies in a specific time window and temporal order. Defining network interactions is thus key to understanding normal cognition and the pathophysiology of its decline.

Following a focal brain insult (e.g. following a stroke), or as a consequence of the alteration of function in a specific brain region (for example due to a sustained change in afferent input or efferent demand), the affected neural network adapts fluidly. This dynamic, neural plasticity can confer no perceptible change in the behavioral output of the brain, lead to changes demonstrated only under special testing conditions, or cause behavioral changes that may constitute symptoms of disease or represent paradoxical functional facilitations.

Transcranial magnetic stimulation (TMS) provides a non-invasive technique to transiently disrupt the function of a given cortical target thus creating a temporary, “virtual brain lesion” (Pascual-Leone et al., 1999, Walsh et al., 2003). In combination with functional neuroimaging techniques, TMS provides an opportunity to study the mechanisms of dynamic network plasticity (Pascual-Leone et al., 2005; Pascual-Leone, 2006). TMS can be applied in trains of variable frequency and intensity to modulate the activity of a given cortical area, increase or decrease it transiently, while the subject performs a given behavior, and the brain activity associated with such behavioral activation is measured using fMRI. In this case, neuroimaging studies can evaluate the functional adaptation of brain activity to the controlled modulation of activity in an element of a neural network. For example, in a previous study we reported first evidence of a facilitatory effect elicited by a single session of rTMS on a face-name pairs learning task in a group of elders exhibiting memory

dysfunction. The behavioral effects in the group receiving real rTMS were accompanied by increased brain activity, as measured by fMRI, in frontal and parieto-occipital regions whereas no cognitive or cerebral effects could be observed in a group under sham rTMS administration (Solé-Padullés et al., 2006).

Genetic factors appear to critically influence network interactions and thus are likely critical contributors to the dynamic neural plasticity that allows the brain to adapt to focal disruptions. The apolipoprotein E (APOE) ε4 allele is the major genetic risk factor for sporadic Alzheimer's disease (Saunders et al., 1993) and previous studies have demonstrated that it exerts a robust influence on brain function among the elder (ej. Bookheimer et al., 2000; Bondi et al., 2005; Lind et al., 2006; Han et al., 2007, Bartrés-Faz et al., 2007). In the present study we investigated whether the genetic background regarding the APOE status influences the effects of rTMS on brain activity. For this purpose, 20 subjects with age-associated cognitive decline were administered two fMRI memory encoding tasks. In-between fMRI examinations we administered a single session of rTMS and compared the rTMS-induced cognitive and BOLD-fMRI changes between subjects carrying the APOE ε4 allele (n=9) and non-bearers (n=11). To our knowledge this is the first study combining rTMS and fMRI to demonstrate that individual differences in a common human genetic variant determine brain response to focal brain disruption.

Methods

Subjects

Twenty subjects (age 52 to 85 years) fulfilling the criteria for aging-associated cognitive decline (AACD) (Levy et al., 1994) above 50 years of age were recruited from the Hospital Clinic de Barcelona and a primary health center (CAP Castellar del Vallès) in the area of Barcelona. Individuals complained of memory decline during at least one year and scored -1 SD below standardized age-matched norms in secondary memory tests. Despite their memory problems however, participants were free of dementia and depression

according to clinical and neuropsychological assessments (Supplementary Methods).

Apolipoprotein E

Genomic DNA was isolated from peripheral blood leukocytes. At the APOE locus, the polymorphism of the three common genetic variants, ε2, ε3 and ε4, due to Cys-Arg substitutions at amino acid positions 112 and 158 was analyzed. The polymerase chain reaction was used to amplify the alleles of the APOE gene as described elsewhere (Wenham et al., 1991). From the whole sample of twenty subjects, 9 were carriers of the ε4 variant (6 ε3/ε4, 2 ε2/ε4 and 1 ε4/ε4) and 11 were non-bearers (10 ε3/ε3 and 1 ε2/ε3).

MRI acquisition, base-line fMRI session and memory assessment

All scans were obtained on a GE Signa 1.5T (General Electric, Milwaukee, WI) (Supplementary Methods). For fMRI, we used a block design with alternating rest and experimental conditions (five blocks each). The task required encoding and learning of visually-presented face-name pairs. Before the fMRI session, subjects learnt 2 face-name pairs, which were used later as control stimuli (rest condition). During experimental condition subjects were presented 10 new face-name pairs that should be learnt during the scanning. The duration of each stimulus (face-name pair) was 2s and the inter-stimuli period was 1s. The whole experiment had a duration of 300s (30s per block, 150s for each condition). Following the fMRI session participants were assessed for recognition memory of the 10 face-name pairs learnt. For this purpose, both photographs and written names were given so that they could pair them. Only the stimuli used in the experimental blocks were presented during the associative memory task, and thus, only correct / incorrect face-name matches were recorded as responses. The maximum score for this task was 10 (all names correctly matched with the corresponding face).

rTMS

rTMS was applied during a rest period, between the first and second fMRI examinations. A MAGSTIM SUPER® stimulator and a double-cone coil were

used. The intensity of TMS pulses was set at 80% of motor threshold with the intersection of the double-cone coil positioned over the primary motor cortex. With this intensity we were to elicit visible twitch of the 1st dorsal interosseus muscle of the right hand in at least 5 out of 10 trials. For prefrontal cortex stimulation, the TMS coil was slightly diverted to the plane of the interhemispheric cissure and moved anteriorly approximately 5 cm to reach the prefrontal region billateraly. Ten rTMS trains lasting 10s each were delivered during a 5-minute period using a frequency of 5 Hz. Specifically, every 30s subjects were given 10s of TMS followed by a 20s rest period. The intersection of the double-cone coil was placed over the vertex in all patients.

Second fMRI session and memory assessment

Immediately after the rTMS, patients underwent a second fMRI examination. The time elapsed between the end of the first and the beginning of the second fMRI was 9.99 min ± 1.99 (SD). We used an equivalent 10 face-name pairs learning task that was counter-balanced with that administered during the first fMRI session. Once the scanning procedure was finished, participants were tested for memory recognition as in the base-line fMRI session (figure 1).

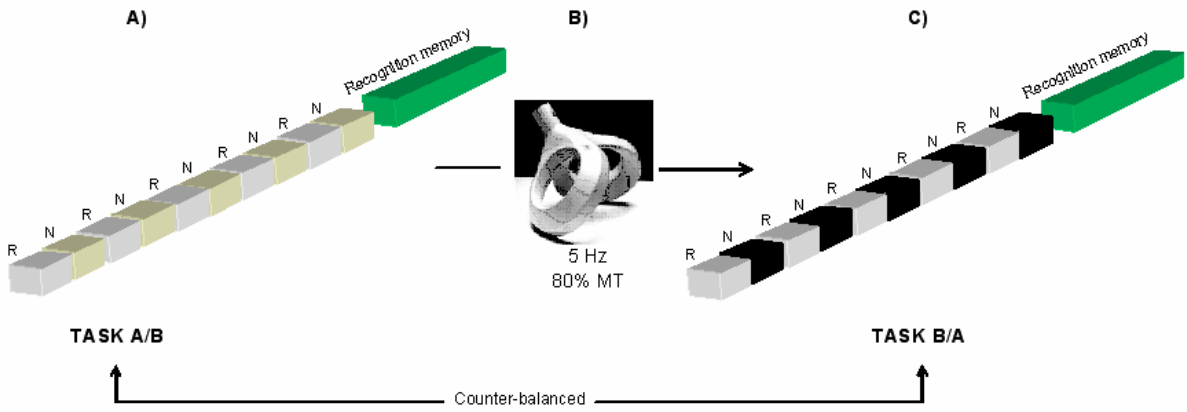

Figure 1. Experimental design: A) 1st. fMRI session: alternating blocks of 5 repeated (R) and 5 new (N) stimuli (face-name pairs). B) rTMS session was applied immediately after the recognition memory assessment outside the scanning. C) 2nd. fMRI session: the new stimuli (N) blocks showed equivalent pairs, while the repeated (R) remained the same learnt items. This was followed by a second memory assessment.

Statistical analysis

To analyze fMRI data we used SPM2 (Statistical Parametric Mapping) running in Matlab 6.5 (MathWorks) under the assumptions of the general linear model. For each fMRI, individual analyses were carried out for each subject to evaluate the increased activation observed during the experimental condition compared with that seen during the rest condition. Second-level (group) analyses were performed using the *two sample t-test* function for between genetic groups comparison and *paired t-test* for within group comparisons (base-line fMRI vs post rTMS fMRI for each group). Results were examined at the voxelwise threshold of $p < 0.001$ (uncorrected for multiple comparisons) and the $p < 0.05$ threshold on the extent of clusters. Demographical and cognitive data were analyzed with the statistical program SPSS v.14. Chi-squared test was used to compare gender distribution between groups. For continuous variables, assumptions for normality and homocedasticity were tested for all cognitive and demographical variables and parametric or non-parametric tests were used accordingly (Supplementary Methods). Student t-test, U-Mann Withney and chi-squared tests were used to compare cognitive and demographical variables between genetic groups. A two-way ANOVA was undertaken in order to examine whether rTMS exerted a distinct effect between genetic subgroups across the two memory examinations using genetic subgroup as the between-subject factor and moment of the memory evaluation (first or second fMRI) as within-subject factor.

Participants

Dementia was ruled out using a clinical and neuropsychological examination including measures of global cognitive function (MMSE $\geq$ 24), language (Token test), praxis (imitation and performance to command), gnosis (Poppelreuter's embedded figures and Luria's watches) and abstract reasoning (WAIS III Similarities subtest). Possible cases of major depression were excluded through a Hamilton Depression Scale cut-off score of 15. AACD individuals were restricted to those cases exhibiting impairments in memory domain since all of them scored -1 SD below standardized age-matched norms in at least one of the following memory tests: Rey Auditory Verbal Learning Test

(RAVLT) and Visual Reproduction of the Wechsler Memory Scale Revised (WMS-R).

MRI acquisition and processing

High-resolution T1-weighted images were acquired for anatomical identification with a FSPGR three-dimensional sequence (DICOM format, TR/TE = 12/5.2; TI 300 1 nex; FOV = 24 x 24 cm; 256 x 256 matrix). Whole-brain volumes were acquired in an axial plane yielding contiguous slices with slice thickness of 1 mm. Functional images were acquired using a T2* weighted gradient-echo planar imaging (TR = 2000 ms; TE = 40 ms; FOV = 24 x 24 cm; flip angle of 90º). Twenty axial slices were obtained for each brain volume with a slice thickness of 5 mm and a gap of 1.5 mm. Original fMR images registered in format GE-advanced (one two-dimensional file per slice) were organized into three-dimensional files (150 volumes per subject) by means of MRICro software (University of Nottingham, UK) and saved in ANALYZE 7.5 format compatible with SPM2. Following alignment along the anterior commissure-posterior comissure (AC-PC) line and realignement of the scans to remove the effects of head movement, images were transferred into a standardized coordinate system. Normalized images were smoothed with an isotropic Gaussian kernel (8 mm at full-width half-maximum).

Statistical analyses

Due to the small samples, normality and homocedasticity were specifically tested for all cognitive and demographical variables. Normality was tested using the Kolmogorov-Smirnov test. The following variables were normally distributed: age (K-S: 0.67, p=0.76), MMSE (K-S: 0.69, p=0.73), Vocabulary WAIS-III (K-S: 0.34, p=1), RAVLT (K-S: 0.39, p=0.69), VR-WMS-R (K-S: 0.28, p=0.78) and base-line (K-S: 0.75, p=0.63) and follow-up recognition memory (K-S: 0.55, p=0.93). However years of formal education was not normally distributed (K-S: 0.34, p<0.001). Homocedasticity was tested using the Levene test for the homogeneity of variances. The null hypothesis (homogeneity of variances) could not be rejected in any case with a probability of p<0.05: age (Levene: 0.07, p=0.94), MMSE (Levene: 0.91, p=0.35), Vocabulary WAIS-III (Levene: 0.02, p=0.9), RAVLT (Levene: 3.81, p=0.07), VR-WMS-R (Levene: 0.28,

p=0.78) and base-line (Levene: 0.38, p=0.55) and follow-up recognition memory (Levene: 0.25, p=0.63). Considering these observations, all comparisons were performed using parametric tests (Student's t test and ANOVA) except those concerning the years of education variable for comparing both genetic subgroups that were achieved using the U Mann-Whitney test. All tests were two-tailed and statistical significance was set at $p<0.05$.

Results

APOE ε4+ and ε4- groups were comparable in terms of age, gender distribution, years of formal education and cognitive status including verbal and visual memory assessments (table 1).

	ε4+ group (n=9)	**ε4- group (n=11)**	**Statistical values**	**P value**
Age	66.78 (9.70)	67.09 (9.69)	t=0.07	0.94
Years of formal education	6.11 (3.48)	7.45 (2.80)	U=38.5	0.41
Gender (M / W)	3 / 6	2 / 9	χ^2=0.61	0.44
Global cognition (MMSE)	26.33 (2.34)	26.64 (1.91)	t=0.31	0.75
Inferred IQ (Vocabulary WAIS-III)	50.89 (8.95)	51 (10.89)	t=0.02	0.98
Verbal memory (RAVLT)	7.73 (2.28)	6.25 (3.89)	t=1.04	0.31
Visual memory (VR-WMS-R)	10.88 (5.51)	10.33 (7.23)	t=0.16	0.88

Table 1. Demographic and global cognitive characteristics of the genetic groups. Values are given in mean (SD). MMSE: Mini-mental state examination. M: men, W: women. WAIS: Wechsler Adult Intelligence Scale. RAVLT: Rey-Auditory Verbal Learning test (delayed recall). VR-WMS-R: Visual Reproduction, Wechsler Memory Scale Revised (delayed recall). t= Student's t test. U= U Mann Wihtney test.

Base-line fMRI activations

The pattern of brain activity at baseline (before rTMS) during the encoding memory condition relative to the resting condition for the entire sample of subjects revealed a large cluster (highest significant voxel: Montreal

Neurological Institute coordinates (MNI)=-28, 8, 40; t=7.39; Brodmann area (BA)=44; number of voxels: 3736) of significant activations in the left hemisphere, including medial (MNI=-2, 6, 62; t=7.39; BA=6), inferior (MNI=-36, 16, 26; t=6.87; BA=44/46) and middle MNI=-24, 12, 42; t=5.76; BA=8) frontal gyri extending to the anterior cingulate cortex (MNI=-16, 14, 42; t=5.04, BA=32). A second cluster of activations was found in the left fusiform area (highest significant voxel for this cluster: MNI=-32, -48, -20; t=6.24; BA=36; number of voxels: 295). When considered separately, both groups activated significantly the middle left prefrontal cortex. The APOE ε4+ group also activated the inferior left prefrontal cortex and the left fusiform gyrus. However, no significant differences were observed when directly contrasting the fMRI patterns of both groups of patients at baseline (table 2 and figure 2a).

Before rTMS				
Group	**MNI (x,y,z)**	**Region**	**Voxel-level**	
ε3/ε3			t	P-value
	-48, 26, 28	L middle frontal gyrus (BA 9)	11.81	<0.001
ε4 carriers			t	P-value
	26, 6, 44	L middle frontal gyrus (BA 8)	6.29	<0.001
	36, 14,24	L inferior frontal gyrus (BA 44)	5.86	<0.001
	33, -48, -22	L fusiform gyrus (BA 37)	9.02	<0.001
After rTMS				
ε3/ε3			t	P-value
	34, -60, 40	R supramarginalis (BA 40)	10.05	<0.001
	36, -56, -24	R cerebellum	9.38	<0.001
	32, 0 54	R middle frontal gyrus (BA 6)	9.20	<0.001
	4, 38, 38	R/L mid frontal gyrus (BA 8)	8.95	<0.001
	-44, -68, -18	L cerebellum	9.78	<0.001
	-30,-56,36	L angularis (BA 39)	9.78	<0.001
	-42, 7, 32	L inferior frontal gyrus (BA 44)	8.22	<0.001
	-56, -44, -18	L inferior temporal gyrus (BA 20)	7.24	<0.001
	-10, 28, 0	L caudate	6.74	<0.001
ε4 carriers			t	P-value
	36, 26, 16	R inferior frontal gyrus (BA 45)	9.40	0.049
	6, 12, 8	R caudate	9.23	0.008
	26, 4, 36	R middle frontal gyrus (BA 9)	7.48	<0.001
	14, 34, 32	R/L medial frontal gyrus (BA 8)	6.85	0.011
	-10, 58, 28	L superior frontal gyrus (BA 9)	12.8	<0.001
	-40, 18, 20	L inferior frontal gyrus (BA 45)	10.76	<0.001
	-26, 38, 34	L middle frontal gyrus (BA 9)	10.12	<0.001
	-38, -40, 30	L supramarginalis (BA 40)	8.14	<0.001
	-24, -82, -6	L lingualis (BA 18)	7.98	<0.001

-26, 24, 0	L caudate	7.13	0.034
-28, -62, 38	L angularis (BA 39)	7.11	<0.001
-30, -16, 42	L inferior temporal gyrus (BA 20)	6.63	0.001
-22, -24, 14	L Thalamus	5.98	0.049

Table 2. Patterns of brain activity in the first and second fMRI for both genetic subgroups.

Behavioral effect of rTMS

As previously reported (Solé-Padullés et al., 2006), for the entire sample we observed an improvement of recognition memory scores during the second fMRI following rTMS (F=4.97, p<0.04). The interaction of group x time of evaluation was not significant, suggesting similar memory changes across genetic subgroups (table 3).

	PRE	POST	Statistic	P value
Whole sample	4.15 (2.94)	5.75 (2.99)	F=0.04	0.85.
ε4 carriers	4.22 (3.11)	5.67 (3.35)		
ε4 non-carriers	4.09 (2.95)	5.82 (2.82)		

Table 3. Measures of recognition memory, before (PRE) and after (POST) rTMS. Values are given in mean (ranges). F= interaction value of the two-factor ANOVA using genetic subgroup as the between subject factor and moment of the evaluation (PRE vs POST) as within subject factor.

rTMS effects on brain activity across genetic groups

Following rTMS, a more bilateral pattern of activation was observed for the whole sample during the second fMRI. The regions with increased brain activity during the encoding of face-name pairs in the second fMRI relative to the baseline in the right hemisphere included the middle (MNI=32, -2, 56; t=8.47, BA=6) and inferior frontal gyri (MNI=42, 20, 2; t=6.86, BA=47), the inferior parietal lobule (MNI=32, -58, 38; t=8.27, BA=40), the right caudate nucleus (MNI=10, 20, 2; t=6.95) and the fusiform gyrus (MNI=38, -58, -24; t=5.57, BA=36). In the left hemisphere, increased activity was evidenced in the inferior parietal lobule (MNI=-30, -60, 38; t=1.17, BA=40), the left caudate (MNI=-12, 26, 0; t=5.78), and the inferior temporal gyrus (MNI=-50, -54, -12; t=7.47, BA=37).

When the two genetic subgroups were studied separately, a similar (non-significantly different) bilateral pattern could be observed (table 2 and figure 2b). Visual inspections of these patterns showed that right middle and inferior frontal regions only activated within the APOE ε4+ group whereas right posterior activations were only observed among APOE ε3/ε3 individuals. In this regard, when baseline and post-rTMS fMRIs were compared within each group with paired t-test analyses, APOE ε4+ individuals displayed increases in fMRI BOLD signal in the second fMRI relative to the base-line scanning restricted to anterior cerebral regions including the inferior and the middle right frontal gyri. In contrast, ε3/ε3 recruited additional brain areas only in posterior regions bilaterally comprising the inferior parietal lobe and including the right supramarginalis and the left angular gyri (table 4 and figure 2c). No areas of increased activity in the first fMRI relative to the post rTMS fMRI were found in any group.

TMS-induced change in memory performance across the two fMRI sessions was included as a covariate in the paired t-test analyses comparing baseline versus post-rTMS activations within each genetic subgroup. For APOE ε4+ individuals, the right prefrontal regions additionally activated in the second fMRI were no longer significant, whereas for the APOE ε3/ε3 homozygous only the right supramarginalis survived statistical significance. These findings suggest that right prefrontal regions among ε4+ subjects and left inferior parietal areas in ε3/ε3 are associated with memory changes across the two fMRI assessments.

Group	**MNI coordinates (x,y,z)**	**Region**	**Voxel-level**	
			t	P-value
ε3/ε3	48,-56,44	R supramarginalis (BA 40)	7.23	<0.001
	-36,-64,36	L angluaris (BA 39)	6.66	<0.001
ε4 carriers	26,34,-6	R inferior frontal gyrus (BA 47)	8.65	<0.001

26,-6,44	R middle frontal gyrus (BA 8)	8.14	0.001

Table 4. Significant regional cerebral fMRI changes induced by rTMS. All listed regions reflect areas where brain activity was increased in the second fMRI examination (after rTMS) relative to the base-line fMRI.

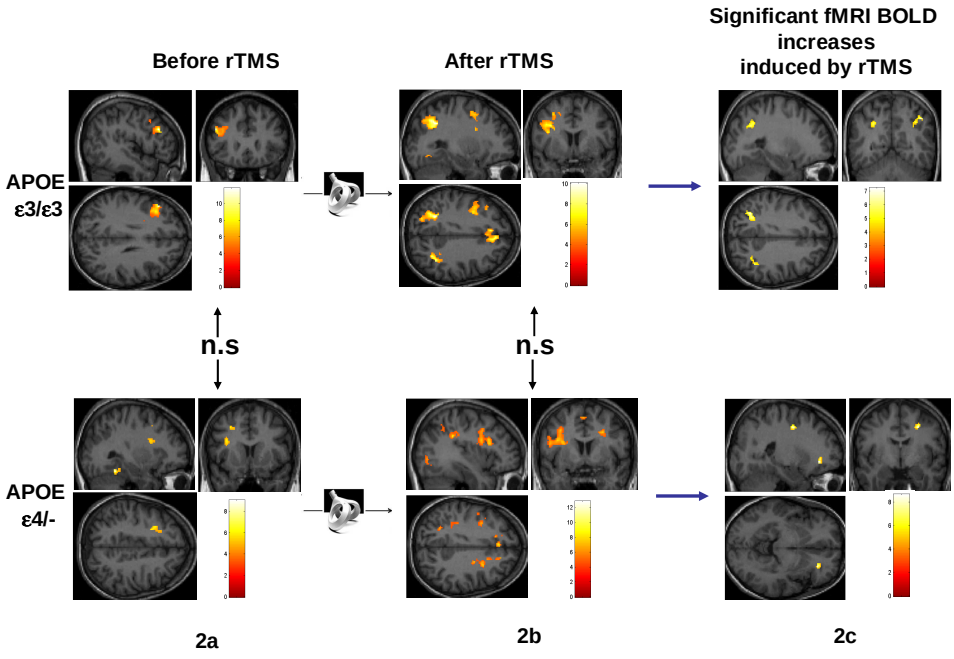

Figure 2a and **2b)** Patterns of brain activity before and after rTMS for the ε3 and ε4 groups. **2c)** Significant regions additionally activated for each group following rTMS session as compared to base-line fMRI.

Discussion

The main finding of the present study is that APOE status, a relevant genetic variation associated with cognitive and brain changes in the aged, differentially modulates the cerebral response to prefrontal rTMS. Present results offer first evidence of the influence of genetic factors onto the brain adaptive mechanisms to focal brain disruption as measured by functional neuroimaging.

Previous reports have been published demonstrating that rTMS is sensitive in reflecting phenotypic differences associated with genetic variations causative of several neurological conditions. For example, Abele et al. (1997) and Schwenkreis et al., (2002) used TMS to reveal changes in neuropyshiological measures such as motor evoked potentials, central motor

conduction time (CMCT) and intracortical facilitation, in patients with autosomal dominant cerebellar ataxia-I (spinocerebellar ataxia) differing by CAG trinucleotide expansion in distinct genes causative of this condition. Similarly, Winner et al. (2004) reported absent transcallosal inhibition in two patients with hereditary spastic paraplegia (HSP) 'complicated' with genetic linkage to chromosome 15q13-15 and thin corpus callosum on MRI assessments. Bönsch et al. (2003) showed that HSP patients with different mutations in the spastin gene differed in motor threshold and CMCT depending on the type of mutation present. Further, reduced intracortical inhibition and facilitation compared to controls was reported among sporadic amyotrophic lateral sclerosis patients as compared with those with similar functional disability but who were homozygous for the D90A superoxide dismutase-1 gene mutation (Turner et al., 2005).

Few TMS studies have been undertaken to date that correlate genotype-phenotype interactions of genetic variations naturally present in the general human population with known effect on CNS function or structure. Eichhammer et al. (2003) reported changes in intracortical inhibition induced by selective serotonin reuptake inhibitors (SSRIs) in healthy subjects that were more marked among l/l (long/long) homozygous for the biallelic polymorphism of the serotonin transporter gene (5-HTTLPR) as compared to individuals without the ll-genotype. According to former studies showing better response to serotonergic antidepressant drugs in patients with the C/C genotype of 5-HT1A receptor gene, greater improvements following 10-consecutive days of rTMS administration were also evidenced in such patients as compared to cases with a G allele (Zanardi et al., 2007). Finally, Keim et al. (2006) used TMS to determine the right first dorsal interosseus (FDI) cortical representation area before and after the performance of 30 min fine-motor exercises in healthy young subjects. These individuals differed in their genotypes for the val66met brain-derived neurotrophic factor gene (BDNF), a genetic variation previously associated with cognitive function and brain morphology (Savitz et al., 2006). The authors found that subjects homozygous for the Val allele showed a significant increase in mean FDI map area, MEP amplitude and map volume after training, which was not observed in Val/Met and Met/Met subjects. These

results suggest that TMS can reveal the effect of genetic variations associated with modification of use-dependent plasticity in the motor cortex.

In our study, two clear distinct patterns of brain activity emerged following rTMS: APOE ε3/ε3 subjects displayed increased activity in posterior cortical regions (including the inferior parietal lobe bilaterally), while these responses were absent in the APOE ε4+ group, who instead showed enhanced activity in right prefrontal regions. An increasing body of evidence shows that among non-demented elder, the presence of the APOE ε4 allele exerts a relevant effect on brain activity during cognitive processing as revealed by fMRI (Bookheimer et al., 2000; Bondi et al., 2005; Lind et al., 2006; Han et al., 2007; Bartrés-Faz et al., 2007). In the present report, the frontal brain regions with additional activations following rTMS in the ε4 group correspond to contralateral regions of those activated in the baseline fMRI, and thus reveal a more symmetrical pattern of frontal lobe activity during the second fMRI. Former studies with aged and young populations demonstrated that increased activity of prefrontal regions during semantic encoding correlates with the likelihood of eventual memory performance (Wagner et al., 1998; Stebbins et al., 2002) and that behavioral compensations in the elderly are frequently related to increased brain activity in the prefrontal cortex (Grady, 2007). Specifically, a recent study reported greater right frontal lobe recruitment during the learning of new verbal paired-associate encoding (versus fixation) among ε4 subjects than in non-carriers. This was interpreted as reflecting compensatory APOE ε4-related deficiencies associated with verbal episodic memory encoding and consolidation (Han et al., 2006). In our study, we found that when memory ameliorations from the first to the second fMRI examinations were included in the analyses, the areas of increased brain activation in the inferior and middle right frontal regions were no longer significant, indicating a relationship between both aspects. Thus, in combination with previous findings, our results suggest that the contralateral prefrontal changes induced by applying rTMS at frequencies able to enhance cortical excitability (Pascual-Leone et al., 1994; Maeda et al., 2000), could represent the mechanism underlying memory facilitation observed in the second fMRI in these individuals.

The fact that our ε4+ subjects recruited anterior areas but failed show additional activation induced by rTMS in parietal regions (as observed in the group lacking the risk factor allele) may be reflecting a more dysfunctional posterior cortex in the former cases. Abnormally low cerebral metabolic rates for glucose (CMRgl) in temporoparietal cortex is an established feature associated with the early diagnosis of Alzheimer's disease (Petrini et al., 2000). More recent evidence is also available indicating that a similar pattern of hypoactivity at rest can be observed even in cognitively normal middle-aged (Reiman et al., 2005) and young (Reiman et al., 2004) APOE ε4 carriers, possibly suggesting reduced activity or density of terminal neuronal fields innervating these areas or increased stages of histopathology (Reiman et al., 2004). In contrast, APOE ε3/ε3 recruited additional regions in the inferior parietal lobe bilaterally in the second fMRI. Previous reports have implicated the parietal lobe during learning of similar tasks on healthy elder subjects, individuals with mild cognitive impairment, and mild Alzheimer's disease patients (Sperling et al., 2003). Present findings suggest that in the context of a more preserved posterior cortex, these brain areas accounted for the memory changes across both evaluations in this group.

An obvious limitation of the present study is that we did not include equivalent genetic subgroups receiving sham stimulation. However, it seems unlikely that sham rTMS would exert differential effect in subjects depending on APOE status. Nonetheless, despite the use of equivalent-counterbalanced memory tasks, we cannot completely rule out that our results might partially be explained by a practice effect on memory encoding rather than as a consequence of rTMS. This assumption however is unlikely since we previously found no effects on cerebral activity or performance in an equivalent group of elders with the same design study but receiving sham stimulation (Solé-Padullés et al., 2006).

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Functional connectivity of the hippocampus in elderly with mild memory dysfunction carrying the *APOE* ε4 allele

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Abstract

The purpose of the present study was to evaluate functional connectivity of the hippocampus during a fMRI face–name learning task in a group of elders with mild memory impairment on the basis of the presence or absence of the *APOE* ε4 allele. Twelve ε4 carriers and 20 non-carriers with mild memory dysfunction and exhibiting equivalent performance in clinical evaluations of global cognitive function and memory were studied. Subjects underwent a fMRI session consisting of a face–name encoding memory task. Following scanning, subjects were asked to pair faces with their corresponding proper name. Functional connectivity of the hippocampus was measured by using coherence analysis to evaluate the activity of brain circuits related to memory encoding processes. In contrast to non-*APOE* ε4 allele bearers, *APOE* ε4 carriers showed enhanced connectivity with the anterior cingulate, inferior parietal/postcentral gyrus region and the caudate nucleus. Enhanced hippocampal connectivity with additional brain regions in *APOE* ε4 allele carriers during the performance of an associative memory task may reveal the existence of additional activity in the cortico-subcortical network engaged during memory encoding in subjects carrying this genetic variant.

Keywords: Apolipoprotein E; fMRI; Functional connectivity; Memory; Non-demented older adults

1. Introduction

The apolipoprotein E (*APOE*) ε4 allele is the major genetic risk factor for the prediction of Alzheimer disease (AD) (Saunders et al., 1993) particularly among subjects also exhibiting memory impairments (Petersen et al., 1995). Studies using PET have demonstrated that non-demented ε4 carriers present abnormalities resembling those observed in AD (Reiman et al., 2004; Small et al., 1995, 2000) in an allele dose-dependent manner (Reiman et al., 2005). The abnormal activations or cerebral metabolic rates can be observed at rest (Reiman et al., 2004; Scarmeas et al., 2003) and during cognitive efforts (Scarmeas et al., 2005) several decades before the age of onset of the disease.

Functional magnetic resonance (fMRI) studies parallel PET findings reporting differential patterns of brain activity associated with the presence of this genetic variant among the

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elderly. In this regard, increased activations in asymptomatic ε4 carriers in the left prefrontal region, bilateral orbitofrontal, superior temporal, and inferior and superior parietal regions, have been reported during the performance of a paired-associates learning and recall task (Bookheimer et al., 2000) as well as in the left parietal cortex during a letter fluency task (Smith et al., 2002). More recently, Bondi et al. (2005) found that among elders with normal cognitive function, carriers of the ε4 allele showed greater magnitude and extend of fMRI BOLD signal during picture learning relative to non-carriers in bilateral fusiform and medial frontal gyri, left inferior and middle frontal, right superior parietal, and right hippocampal and parahippocampal cortices. These findings were essentially replicated mainly for the left parahippocampal region using a verbal paired-associate learning task in a sample of younger subjects with both the *APOE* ε4 allele and positive history of dementia (Fleisher et al., 2005). The same group has reported right hemisphere overactivations in multiple regions among *APOE* ε4 positive elders using the same paired associate task (Han et al., 2007). Finally, Wishart et al. (2006) found increased activation among cognitively intact *APOE* ε3/ε4 relative to ε3/ε3 bearers in the medial frontal and parietal regions bilaterally as well as in the right prefrontal cortex during a two-back working memory task. Altogether, these results have been interpreted as supporting evidence that *APOE* ε4 subjects require increased cognitive work reflected by enhanced activity of supplementary brain regions as compared to non-carriers (Bondi et al., 2005; Bookheimer et al., 2000; Han et al., 2007; Smith et al., 2002). Conversely, decreased activation during a covert object naming experiment has also been reported in ε4 carriers at high risk for AD (Smith et al., 1999) with additional regional decreases at follow-up examinations (Smith et al., 2005). This decreased activity was found in occipital, inferotemporal and frontal regions. Other studies have further observed reduced activations in left inferior parietal lobe and bilaterally in the anterior cingulate region during a semantic encoding task (Lind et al., 2006) as well as in the right hippocampus and entorhinal cortex during the encoding of novel stimuli (Trivedi et al., 2006).

Differences in sample characteristics such as the age ranges of the subjects included, their cognitive status, and/or the presence of family history of AD may account for discrepancies in the literature. It has also been suggested (Smith et al., 1999; Smith et al., 2005) that increased versus decreased patterns of activity might reflect task differences. For example, the reported regions exhibiting changes in activity during learning or verbal fluency tasks as a function of the *APOE* ε4 variation were not observed in other cognitive demanding tasks irrespective of the cognitive effort required (Burggren et al., 2002). These latter observations suggest that distinct brain circuits are differently affected by the presence of the *APOE* ε4 when elderly subjects are confronted with cognitive tasks.

One way to evaluate how the *APOE* ε4 allele modulates brain networks related to memory processes using fMRI is by applying a multivariate approach. While univariate analyses such as those employed in the above-mentioned literature estimate regional activity, multivariate analyses evaluate interactions between regions, reflecting a dynamic aspect of the brain circuits. In this sense, functional connectivity, defined as statistical dependencies or correlations among neurophysiologic events (Friston, 2005), is one way to characterize such interactions. Exploratory functional connectivity analyses using patterns of covariance have been used in previous PET studies to demonstrate that relative to younger subjects, elderly individuals use different brain resources while maintaining a similar memory performance (Della-Maggiore et al., 2000; Grady et al., 2003). Additionally, it has been shown that the interactions between the hippocampus and cortical regions are altered in disorders affecting medial temporal lobe structures such as Alzheimer's disease (Grady et al., 2001).

Based on previous functional imaging studies demonstrating that the hippocampus is critically involved in declarative memory encoding (Schacter and Wagner, 1999), and previous fMRI data suggesting that the presence of the *APOE* ε4 allele may be associated with a particular network pattern of cortical and medial temporal lobe (MTL) activity during cognitive tasks, we sought to evaluate the functional connectivity of the hippocampus and other subcortical and cortical regions during a fMRI face–name learning task in a group of elders with mild memory impairment on the basis of the presence or absence of the *APOE* ε4 allele. To evaluate functional connectivity, we used coherence analysis, a method that measures the linear time-invariant relationship between two signals, which is invariant to interregional differences in the hemodynamic response function (HRF). For a mathematical description of coherence analysis see Sun et al. (2004). The coherence measure indicates the degree of linear association of the time series, between the seed region and all other brain voxels. This function is bounded by 0 and 1, where 0 indicates an absence of any linear relation, and 1 indicates that x (the seed region) can perfectly predict y (a selected voxel) in a linear fashion. Therefore, brain regions with a high coherence value can be considered as areas that belong to the same network structure (Müller et al., 2003). To our knowledge, this is the first study designed to evaluate the impact of the *APOE* genotype in brain activity using functional connectivity analysis.

2. Methods

2.1. Subjects

Thirty-two subjects above 50 years of age volunteered to participate in the study. Subjects were recruited from one primary health center in the area of Barcelona and the Alzheimer's disease and other cognitive disorders unit at the Neurology Service (Hospital Clínic of Barcelona). All participants reported memory complaints but did not meet criteria

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for dementia according to *Diagnostic and Statistical Manual* fourth edition (DSM-IV) criteria and a neuropsychological assessment including measures of global cognitive function (MMSE ≥ 24), language (Token Test, Boston Naming Test), praxis (imitation and performance to command), gnosis (Poppelreuter's embedded figures and Luria's watches) and abstract reasoning (WAIS III Similarities subtest). None of the participants suffered from other psychiatric or neurological disease based on medical evaluation. Moreover, possible cases of clinically depressive mood were ruled out through a Hamilton Depression Scale with a cut-off score of 15. Participants were assessed for secondary memory function using the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) verbal learning task (Morris et al., 1989). All patients scored in the low range (equal or below −1S.D.) in at least one of the three learning trials or in the long delayed free recall according to age-matched standardized norms. Measures of learning across the trial list administration (3rd trial minus 1st trial), total learning (sum of three trials) and delayed free recall (5 min) were obtained from the CERAD test. Genomic DNA was isolated from peripheral blood leukocytes and *APOE* ε2, ε3 and ε4 alleles were amplified using polimerase chain reaction (PCR) and restriction endonuclease HhaI as described elsewhere (Wenham et al., 1991). There were 12 carriers of the *APOE* ε4 allele (9 ε3/ε4, 2 ε2/ε4 and 1 ε4/ε4) and 20 non-ε4 carriers (15 ε3/ε3 and 5 ε2/ε3). Genetic groups were comparable in terms of age, cognitive status, gender distribution and educational attainment (Table 1).

2.2. fMRI session and memory assessment

The task used during the fMRI scanning session has been recently described elsewhere (Solé-Padullés et al., 2006). Briefly, we used a 10-block design paradigm with alternating 'repeated' and 'experimental' conditions (5 blocks each). The task began with a 'repeated' block consisting on the presentation of two face–name pairs that were learned before the fMRI session. Following this block, subjects were presented 10-face–name pairs previously unfamiliar to the subjects ('experimental' block). The same 'repeated' and 'experimental' blocks were thereafter presented in an alternating way until the total duration of the experiment was reached. The presentation time for each stimulus was 2 s and interstimuli period: 1 s. Each block had a duration of 30 s and thus the whole duration of the experiment was 300 s. During the 'repeated' blocks, subjects were asked to attend to the displayed face–name pairs even though they were already known. In the 'experimental' blocks subjects were given explicit instructions to try to remember which name was associated with which face for later testing. Following the fMRI session an associative memory procedure was used to test the performance of the subjects related to the face–name encoding task. For this purpose, subjects were shown 10 printed photographs as well as 10 written names and were instructed to pair each name with the corresponding face. Only the stimuli used in the 'experimental blocks' were presented during the associative memory task. The maximum score for this task was 10 (all names correctly matched with the corresponding face).

Table 1
Subject demographic and cognitive test data

	APOE ε4+ ($n = 12$)	*APOE* ε4− ($n = 20$)	P/χ^2 value
Age	67.66 (8.73)	66.0 (8.31)	0.605
Men (%)	25	25	1
MMSE	26.09 (2.16)	26.94 (1.76)	0.26
Years of education	8.60 (4.12)	6.91 (3.34)	0.24
CERAD learning	2.08 (1.83)	2.7 (1.42)	0.29
CERAD total	11.92 (4.52)	14.05 (4.05)	0.18
CERAD delayed	3.58 (2.64)	4.66 (1.66)	0.17
Token Test	11.38 (1.60)	12.13 (1.15)	0.20
BNT	51.63 (4.93)	48 (5.72)	0.62
Praxis errors	0	0	–
Poppelreuter	1	2	0.3
Similarities	8.40 (2.16)	9.18 (1.84)	0.35
HDS	3.38 (1.99)	3.88 (2.13)	0.63

Values are mean ± S.D. MMSE: mini-mental state examination. Poppelreuters: values represent number of cases performing a score below 10 in the two complex figures. Praxis error: percentage of cases unable to perform five basic ideomotor praxis for each genetic subgroup. Similarities: mean scaled score of WAIS similarities. BNT: Boston Naming Test. HDS: Hamilton Depression Scale. CERAD: Consortium to Establish a Registry for Alzheimer's Disease verbal learning task.

2.3. MRI acquisition and preprocessing

Scans were obtained on a GE Signa 1.5 T (General Electric, Milwaukee, WI). High-resolution whole-brain T1-weighted image were acquired for anatomical identification with a FSPGR three-dimensional sequence (TR/TE = 12/5.2; TI = 300 ms; NEX = 1; FOV = 24 cm × 24 cm; 256 × 256 matrix; slice thickness of 1.5 mm; voxel size = 0.94 mm × 0.94 mm × 1.5 mm). Functional images were acquired using a T2* weighted gradient-echo echo planar imaging (TR = 2000 ms; TE = 40 ms; FOV = 24 cm × 24 cm; flip angle of 90°; 64 × 64 matrix; 20 slice; slice thickness of 5 mm; gap of 1.5 mm; voxel size = 3.75 mm × 3.75 mm × 6.5 mm).

MRI data were processed in a SUN workstation using Solaris 8. The two-dimensional files were organized into volumetric three-dimensional files using MRICRO (http://www.sph.sc.edu/comd/rorden/mricro.html). The images were saved in ANALYZE 7.5 format, compatible with the SPM2 software (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, University College London, UK). In order to remove head movement effects, the 150 brain volumes acquired for each subject during the 300 s of duration of the experiment (TR = 2000 ms) were first realigned. The realignment of subsequent slices in a time series used a least-square approach to the first scan as a reference. Next, a single investigator (C S-P) blind to the carrier status of the subjects manually determined the anterior commissure for the mean functional image and

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the structural image, and all images were reoriented to the anterior–posterior commissure line. This process was first done with the functional images and in-plane anatomical images, and later with the high-resolution structural T1 images. All images were co-registered using mutual information defaults, and then normalized into a standardized coordinate system in two stages. First, the spatial normalization parameters of the high-resolution structural T1 images were estimated, then these parameters were applied to the fMRI EPI and anatomical images. For these steps, a trilinear interpolation was used. The normalized functional images were then smoothed with an isotropic Gaussian kernel (full width at half-maximum, FWHM = 8 mm) to create a local weighted average of the surrounding pixels. Smoothing in space enhances the signal-to-noise ratio of the data (Turner et al., 1998), increasing the validity of the subsequent statistical test across groups and compensating for possibly inexact normalization (Ashburner and Friston, 1999).

2.4. Statistical analysis

2.4.1. Behavioral data

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS for Windows v11.0, SPSS Inc., Chicago, USA). The Kolmogorov–Smirnov test was used to test whether cognitive and demographic variables followed a normal distribution. As they were normally distributed, we used a two-tailed *t*-test for group comparisons across *APOE* gene subgroups. The level of significance was established at $P < 0.05$.

2.4.2. fMRI data

2.4.2.1. Univariate analysis. Functional analyses were conducted to detect differences in cerebral activation between groups using SPM2. We first performed an 'experimental block' > 'repeated block' contrast separately for each genetic subgroup to obtain the pattern of brain activity reflecting the encoding of new face-pair associations. Subsequently, two one-sided comparisons were tested: (a) *APOE* ε4 carriers > *APOE* ε4 non-carriers; and (b) *APOE* ε4 non-carriers > *APOE* ε4 carriers for the same contrast ('experimental block' > 'repeated block') to study the brain regions that emerged differentially activated between the genetic groups. These univariate analyses were performed considering all voxels constituting the brain and results were interpreted at a voxel-level of $P < 0.001$ (uncorrected) considering only clusters at a corrected *P*-value < 0.05 (cluster extent (k) ≥ 10 voxels). The anatomical location of the activated cerebral regions was determined by the global maxima coordinates in the Montreal Neurological Institute (MNI) space. In addition, a region of interest (ROI) analysis was conducted to test for possible differences in hippocampal formation. This region was chosen a priori, based on previous findings that relate this structure to encoding memory processes (Schacter and Wagner, 1999). Hippocampal ROIs were manually drawn for each subject by a skilled operator (JM S-G), on multiple slices of the MRI displayed in three planes. The reliability of this procedure has been reported previously (Serra-Grabulosa et al., 2003). We used the convention that the ROI group comparison results should survive at the family wise error (FWE)-corrected voxel-level $P > 0.05$ threshold.

2.4.2.2. Functional connectivity. To study functional connectivity of the hippocampus, we used coherence analysis, a method that measures the linear time-invariant relationship between two signals (Sun et al., 2004). To identify networks of functional connectivity for the hippocampus, we generated coherence maps using the task-specific coherence between the reference voxels, or seed (see below), and all other regions in the brain. The method is outlined in four steps: (1) selection of a seed; (2) generation of condition-specific time series; (3) estimation of condition-specific coherence maps; (4) group analysis of condition-specific coherence maps. This analysis measures task-related interactions between regions and across groups.

(1) *Selecting the seed.* Based on the single-subject analysis of brain activity related to the encoding of new stimuli during the experimental conditions, we chose the left hippocampal formation as our seed, as we did not find consistent activity across all subjects in the right hippocampus. We identified the voxels with the most task-related activity by choosing the 10 significant voxels with the largest *F* values for each subject in the experimental versus repeated univariate contrast. It should be noted that the region we used as a seed for coherence analysis purposes, does not exactly match to group activations shown in Table 2. Instead, as explained above we chose a hippocampal area activated in each participant in the study with a *P*-value < 0.05 (uncorrected), which is a more adequate approach to perform coherence analysis (Sun et al., 2004).

(2) *Generating condition-specific time series.* To generate condition-specific time series, the data from every voxel were segmented into 30 s blocks (15 TRs) beginning with each cue. Each segment was mean-centered, windowed using a four-point split-cosine bell (Bloomfield, 1976), and concatenated with segments of the same condition. The windowing reduces spectral leakage from any discontinuities introduced by segmenting the time series. Each condition-specific time series had a total of 75 data points.

(3) *Estimating condition-specific coherence maps.* Coherence measures were computed using a fast Fourier transform algorithm implemented on Matlab 6.1 (http://www.mathworks.com). We used Welch's periodogram-averaging method to estimate the condition-specific coherence of the seed with all other voxels in the brain (using a 16-point discrete Fourier transform (DFT), Hanning window, and overlap of 8-points). We then generated coherence maps using the

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Table 2
Areas of significantly greater brain activity during encoding of new vs. repeated face–name pairs in the *APOE* ε4 carrier and non-carrier groups

MNI coordinates (*x*, *y*, *z*)	Region	Cluster size (cm^3)	Voxel-level	
			t	*P*-value
APOE ε4 carriers				
−26, −38, 0	L hippocampus	0.74	7.02	<0.000
−34, −50, 52	L inferior parietal lobe	1.29	6.85	<0.000
42, −54, −20	R fusiform gyrus	1.94	6.77	<0.000
−28, −66, 50	L superior parietal lobe	1.39	6.67	<0.000
−40, 12, 20	L inferior frontal lobe	1.70	6.24	<0.000
APOE ε4 non-carriers				
−44, 18, 30	L inferior frontal lobe	21.98	7.16	<0.000
−26, −72, 40	L superior occipital lobe	9.61	6.90	<0.000
−34, −68, −16	L fusiform gyrus	9.40	6.48	<0.000
2, −6, 32	R cingulate gyrus	2.74	6.16	<0.000
38, −56, −20	R fusiform gyrus	8.64	5.95	<0.000
4, 4, 62	R motor supplementary area	2.12	5.09	<0.000
4, −72, −32	R cerebellum	2.24	4.79	<0.000
−28, −30, 4	L hippocampus	1.30*	4.30	<0.000
50, −2, 40	R precentral gyrus	0.79	3.73	<0.000

MNI: Montreal Neurological Institute; L: left; R: right.
* Corrected cluster level > 0.05.

estimate of the band-averaged coherence for the low frequency band (0–0.15 Hz).

(4) *Group analysis of condition-specific coherence maps*. To identify differences in functional connectivity between groups, normalized coherence images were analyzed using a SPM2 group comparison. First, to normalize the images, we applied an arc-hyperbolic tangent transform to the coherency, as described in Rosenberg et al. (1989), so that the difference of the coherency magnitudes approach a zero-centered normal distribution. This transformation allows us to apply a parametric random-effects group analysis (a two-tailed, one-sample *t*-test) to determine regions with significantly different connectivity with the seed. We were only interested in regions with significant coherence with the seed during the encoding task, calculating the difference map between *APOE* ε4 carriers and non-carriers as follows: (a) *APOE* ε4 carriers > *APOE* ε4 non-carriers; and (b) *APOE* ε4 non-carriers > *APOE* ε4 carriers. Maps were thresholded at a corrected $P < 0.05$ cluster-level ($k \geq 10$) and uncorrected $P < 0.001$ voxel-level.

2.4.3. Voxel-based morphometry (VBM)

VBM was used to explore structural brain differences between ε4 carriers and non-ε4 carriers to determine whether observed fMRI results between genetic groups were influenced by differences in gray matter volume. VBM was performed following the main steps reported in Good et al. (2001) original description using SPM2, running in Matlab (MathWorks, Natick, MA). Briefly, an anatomical template was first created from the 32 subjects, so that each MRI was transformed into a standardized coordinate system. All the 32 structural images (in a native space) were then transformed to the same stereotactic space using the template created. The spatially normalized images were automatically partitioned into separate images representing probability maps for gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) using the combined pixel intensity and a priori knowledge approach integrated in SPM2. The normalized, segmented images were smoothed using an 8-mm FWHM isotropic Gaussian kernel. A separate gray matter template was created by averaging all the 32 smoothed normalized GM images. All the original images (in a native space) were segmented into gray and white matter images. The GM images extracted were normalized to the GM template and then segmented into gray and white matter. The GM images were further modulated by the Jacobian determinants derived from the spatial normalization step.

Whole-brain GM modulated images were compared using the following contrasts: (a) *APOE* ε4 carriers > *APOE* ε4 non-carriers; and (b) *APOE* ε4 non-carriers > *APOE* ε4 carriers. All results were at a voxel-level of $P < 0.001$ (uncorrected) considering only clusters at a corrected *P*-value < 0.05 ($k \geq 10$). Further analyses restricted to the hippocampi using the previously defined ROI were also undertaken and interpreted at voxel-level $P < 0.05$ family wise error (FWE) corrected.

The study was approved by the local ethics committee, and all the participants gave informed consent for their participation.

3. Results

3.1. Demographic and behavioral data

Genetic subgroups were comparable in terms of age, gender and educational attainment. The neuropsychologi-

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cal assessment, including the CERAD mean measures of learning and delayed recall, was comparable between groups (Table 1) as well as the frequency of cases being classified as memory impaired based upon the learning (and learning + delayed recall) or uniquely by the delayed recall (learning preserved) (*APOE* ε4 carriers = 10 and 2 cases, respectively, and *APOE* ε4 non-carriers = 16 and 4, respectively; $\chi^2 = 0.055$, $P < 0.82$). Performance on the associative memory task following fMRI was slightly but significantly lower in *APOE* ε4 carriers as compared to non-carriers (3.75 ± 3.1 and 5.85 ± 2.51 in ε4 carriers and ε4 non-carriers, respectively; $t = 2.09$, d.f. = 30, $P = 0.04$).

3.2. fMRI data

3.2.1. Univariate analysis

Whole-brain analyses were conducted to evaluate possible differences between *APOE* ε4 carriers and non-carriers. The separate analysis of the 'experimental block' > 'repeated block' contrast for each group is displayed in Table 2. Comparisons between the groups of the 'experimental block' > 'repeated block' contrast did not show any significant difference, either for *APOE* ε4 carriers > *APOE* ε4 non-carriers or for *APOE* ε4 non-carriers > *APOE* ε4 carriers. ROI analysis restricted to the hippocampi also did not reveal significant differences between genetic groups.

3.2.2. Functional connectivity: coherence analysis

For the *APOE* ε4 carriers > *APOE* ε4 non-carriers comparison, the coherence analysis showed that in ε4 carriers, left hippocampus activity had greater coherence with several areas in the ipsilateral hemisphere: the anterior cingulate (BA 32), an area which extended from the inferior parietal lobe (BA 40) to the postcentral gyrus (BA 3), and the caudate nucleus (Table 3 and Fig. 1). We did not find any region with significantly increased coherence with the seed in the *APOE* ε4 non-carriers > *APOE* ε4 comparison. A post hoc analysis was driven to evaluate the degree of coherence between the hippocampal seed and these regions. Results showed that ε4 and non-ε4 carriers have coherence activity between the seed and these regions, being greater in the ε4 than in the non-ε4 group for the anterior cingulate, the postcentral gyrus and the caudate nucleus, respectively (ε4: 0.50, 0.49, 0.55; non-ε4: 0.28, 0.27, 0.31).

Table 3
Areas of significantly coherent activity with left hippocampal seed on the *APOE* ε4 carriers > *APOE* ε4 non-carriers comparison

MNI coordinates (*x*, *y*, *z*)	Region	Cluster size (cm^3)	Voxel-level	
			t	*P*-value
−14, 26, 24	L anterior cingulate	1.92	5.28	<0.001
−32, −38, 50	L inferior parietal lobe	2.90	4.28	<0.001
−12, 12, 4	L caudate nucleus	1.86	4.22	<0.001

MNI: Montreal Neurological Institute; L: left; R: right.

3.3. Voxel-based morphometry

No significant differences in modulated GM volumes were observed for any brain region when *APOE* ε4 carriers were contrasted to non-bearers. These results suggest that fMRI findings are not attributable to volumetric differences between genetic subgroups.

4. Discussion

In the present study we found that during a face–name encoding task, older adults presenting with mild memory impairments and carrying the *APOE* ε4 allele exhibited enhanced activity in functionally connected cortical and subcortical structures to the left hippocampus compared to a clinically equivalent sample of non-*APOE* ε4 bearers. Hence, assessing interregional covariances of activity, present results are consistent with previous reports derived from univariate analyses supporting the observation that *APOE* ε4 subjects show particular recruitment of brain regions when confronted with cognitive tasks as compared to non-bearers (Bondi et al., 2005; Bookheimer et al., 2000; Han et al., 2007; Lind et al., 2006; Smith et al., 2002; Trivedi et al., 2006).

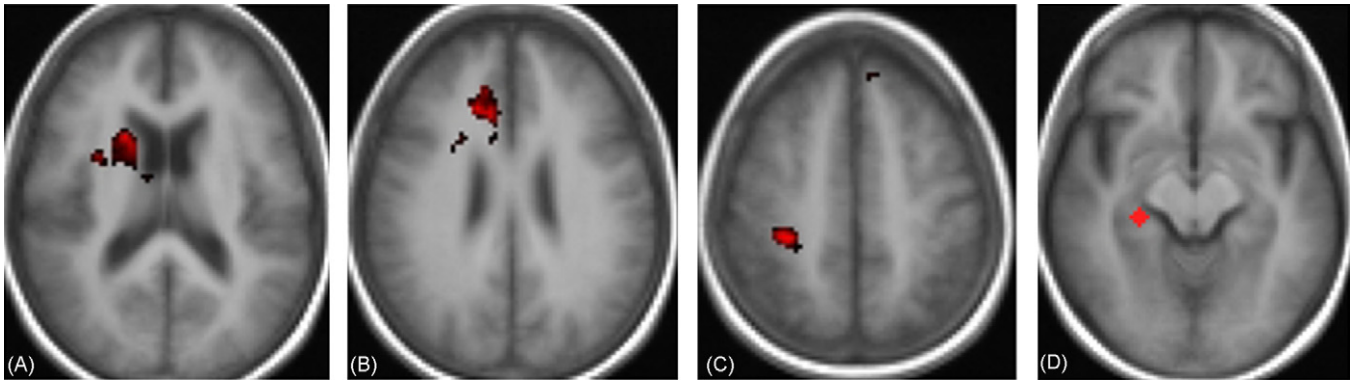

Fig. 1. Coherence maps showing (A) caudate nucleus, (B) anterior cingulate and (C) inferioparietal connectivity with (D) the left hippocampus seed (center at −28, −30, −14; subject 27). For precise localization of regions see Table 3.

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While univariate analyses did not show any differences between *APOE* ε4 carriers and non-carriers, coherence analysis detected greater functional connectivity among *APOE* ε4 carriers between the left hippocampus and regions in the ipsilateral hemisphere during memory encoding. Differences between univariate and multivariate analyses could be related to the fact that univariate statistical parametric maps measure the linear relation between the BOLD activities at each voxel, revealing differences in networks of activity. Conversely, the coherence map indicates the linear association between time-series of a "seed" (in this study the left hippocampus) and time-series of all other brain voxels (Sun et al., 2004), looking for associated modulations over time. In this regard, coherence analysis shows that the activity in the hippocampus is related to activity in other regions to a specific degree during the task. Therefore, results of both approaches should be interpreted as complementary and reflecting different mechanisms. On the other hand, it should be noted that we used axial plane acquisition for fMRI images, which is not optimal for hippocampal signal detection and could have contributed to the lack of group differences in the univariate analysis. Moreover, the interslice acquisition gap, needed to avoid that remainder magnetization of one slice could artifact next slice acquisition, might result in a loss of signal and, consequently, in a loss of statistical significance.

Coherence analysis results indicate that in the context of equivalent brain morphometry, an additional "effort" expressed in terms of brain activity of the hippocampal network was involved in supporting memory encoding. The additional activity found in the *APOE* ε4 carriers was located in the anterior cingulate, a region which extended from the inferior parietal region to the postcentral gyrus and the caudate nucleus. Similar regions were previously found to be active in non-demented elderly subjects during encoding process in a study using a similar design in the new versus repeated stimuli contrast (Sperling et al., 2003). Furthermore, these brain areas appear to conform to part of the underlying cortico-subcortical network responding to increased task complexity or difficulty in distinct memory tasks. For example, the anterior cingulate region was reported to be one of the brain regions showing increased activation with memory load during paired-associates learning (Gould et al., 2003) and activity in the left inferior parietal region has been also found to display functional connectivity with the hippocampus during semantic encoding in older adults (Sperling et al., 2003) and exhibit increased activity as a function of the memory load (Kirschen et al., 2005). Also, previous reports have indicated that the caudate nucleus is involved in processes such as maintenance of information (Gazzaley et al., 2004), increasing memory load (Zarahn et al., 2005) or manipulation of information (Lewis et al., 2004) during working memory tasks. Considering previous results and the present findings, it might be hypothesized, that the task we used in this study implicated relatively increased cognitive work for *APOE* ε4 carriers, which resulted in greater functional connectivity in a network of brain regions usually recruited as a result of increased cognitive demands.

Although our results may provide further evidence for the compensation hypothesis, the fact that the *APOE* ε4 group showed lower performance on the associative memory task following the fMRI session suggests that the use of additional activity is not sufficient to compensate for memory performance at a behavioral level. Thus, although *APOE* ε4 carriers and non-carriers did not differ in clinical evaluations of memory and global cognitive function, ε4 bearers exhibited reduced associative memory performance. One reason possibly accounting for the discrepancy between clinical and post-fMRI evaluations may be related to the nature and the difficulty of the memory task. Whereas the CERAD verbal learning task consists on learning one type of information (substantives, read both by the patient and aloud by the tester), the fMRI encoding task and subsequent evaluation included different visual and verbal stimuli (names and faces). In this sense, establishing an association between a name and a face may be particularly difficult since names are arbitrarily assigned; they were not conducive to forming easy associations (Sperling et al., 2003). The observation of reduced performance among *APOE* ε4 carriers on the fMRI memory task despite equivalent achievement in the clinical evaluations is in contrast to results presented in previous studies where ε4 carriers were able to perform at equivalent level during fMRI scanning by exhibiting increased brain activity (Bondi et al., 2005; Fleisher et al., 2005; Han et al., 2007; Lind et al., 2006; Trivedi et al., 2006). These inconsistencies are possibly related to the distinct clinical characteristics of the samples. Thus, whereas previous reports considered cognitively intact individuals, our patients exhibited memory complaints and scored −1S.D. in a secondary memory test. Hence, in our *APOE* ε4 patients, although a different pattern of hippocampal functional connectivity during an encoding task may support the existence of cerebral compensatory mechanisms, behavioral compensation may have been hampered by fundamental dysfunction of their memory systems.

In the present study, we did not find functional connectivity between left hippocampus and prefrontal regions. This may be related to the lower associative memory performance found in our ε4 subjects following the fMRI evaluation. In previous studies, prefrontal activity has been found in memory encoding and retrieval processes in aging (Rajah and D'Esposito, 2005), and it has been related to a compensatory mechanism to achieve a better memory performance in elderly subjects (Bookheimer et al., 2000; Cabeza et al., 2002; Gutchess et al., 2005). Moreover, Della-Maggiore et al. (2000) examined the effect of age on the hippocampus' functional connectivity in a short-term memory task. They found that performance in normal elderly, in relation to younger individuals, was related to a different hippocampal network, including middle cingulate gyrus, caudate nucleus and dorsolateral prefrontal cortex. The finding in our study that *APOE* ε4 carriers did not show enhanced connectivity between the

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left hippocampus and prefrontal regions despite increased connectivity with other brain regions may reflect 'attempted functional compensation' or spurious non-task-related activity (Rajah and D'Esposito, 2005) and might be related to the recognition memory differences between groups.

We would like to emphasize the advantage of using coherence analysis as a multivariate approach to examine differences between *APOE* ε4 carriers and non-carriers. In this sense, coherence analysis minimizes the influence of potential vascular confounds in interpretation of the data. For example, it has been shown that hemodynamic response in elderly subjects is highly variable (Handwerker et al., 2002). Vascular pathology characteristic of normal aging, such as ultrastructural changes in cerebral vessels due to atherosclerosis or increased tortuosity of cerebral vessels, can alter neurovascular coupling (D'Esposito et al., 2003), affecting the measured BOLD response and making the interpretation of group differences in task-related activation more difficult. Thus, the advantage of coherence analysis, in relation to univariate analysis, is that the former is not sensitive to changes in regional differences in the HRF (Sun et al., 2004).

The present study has several limitations related to the cognitive assessments and clinical characterization of our sample that should be addressed in future investigations. First, we did not assess the recognition memory performances of the CERAD test in our subjects. Recognition memory measures are necessary to distinguish between memory retrieval problems (poor delayed recall and intact recognition) and actual memory loss (poor delayed recall and recognition). Cued recall which is cognitively similar to recognition memory (since in any case subjects have to autogenerate cues at retrieval), has been proven to have a discriminative validity in differentiating MCI and AD from normal ageing and individuals with cognitive complaints as well as from 'stable' and 'converter' MCI groups at follow-up evaluations (Ivanoiu et al., 2005). Thus, since recognition or cued recall measures were not acquired in the present study, we cannot rule out that the nature of the memory disorder differs as a function of the *APOE* genotype, implying different risk for dementia in each group. Similarly, following the fMRI session we only obtained correct or incorrect answers based on the stimuli used in the 'experimental blocks' and did not include foils (i.e., stimuli not presented during the experimental blocks) making difficult to exclude that some proportion of the associative memory correct responses did not correspond to chance guesses.

The criteria used in the present study to define the cognitively impaired elderly do not correspond to that of mild cognitive impairment (MCI) entity (Petersen, 2004) frequently used in the cognitive ageing literature. We identified our subjects as those exhibiting cognitive complains and performing at least −1S.D. in a secondary memory test. By using this approach, some of our patients could be classified as exhibiting age-associated cognitive decline (AACD) proposed by the International Psychogeriatric Association, restricted to memory domain. Longitudinal studies have demonstrated that AACD is a relatively stable category overtime (Scönknecht et al., 2005) with rates of conversion to dementia similar to those individuals only exhibiting cognitive complaints (Visser et al., 2006). On the other hand, a proportion of our subjects felt below −1.5S.D. in the memory evaluation and could approach the neuropsychological definition of MCI of the amnesic subtype (a-MCI, since the other cognitive domains were required to be unaffected). Clearly, MCI individuals have high risk to develop dementia, particularly AD (Petersen, 2004). This observation highlights the possibility that our sample was indeed highly heterogeneous, including subjects at increased risk of AD and others representing cases more similar to the general elder population. In particular, in our sample 33.3% ($n=4$) of *APOE* ε4 allele carriers could be classified as MCI compared to 15% ($n=3$) of non-bearers, probably implying more risk for dementia in the former group. Finally, two patients in the *APOE* ε4 group were ε2/ε4. The conferred risk for dementia in individuals carrying this particular allelic combination is not well established in the literature. Some findings reported increased risk for AD in *APOE* ε2/ε4 (Scott et al., 1997) whereas others found the opposite results (Myers et al., 1996) depending on whether these subjects were grouped with ε4 carriers (ε3/ε4, ε4/ε4, ε2/ε4 genotypes) or as ε2 carriers (ε2/ε2, ε2/ε3, ε2/ε4 genotypes), respectively. We reanalyzed our connectivity data after excluding these two patients and observed that the main findings remained unchanged despite the level of significance was decreased at cluster-level (uncorrected $P<0.001$). Altogether, these observations strengthen the need to undertake further functional connectivity comparisons with more homogeneous cognitive and genetic grouping.

Another limitation relates to small sample size especially in the *APOE*4 group since this may have resulted in our inability to detect group differences on univariate analysis. Further, we only analyzed the experimental (novel) versus repeated encoding contrast, which is much less sensitive (but more specific) to task associated activity than looking at novel encoding alone (i.e. compared to baseline activity). Since we did not include a baseline activity block in our design (i.e. presentations of a bare cross hair) this may partly explain the lack of univariate findings yet significant connectivity findings. Further, since there were marginally albeit significant differences in task performance among genetic subgroups, it is difficult to determine whether the fMRI differences observed can be attributable to the *APOE* genotype or to more difficulty performing the task among ε4 carriers. Despite there is mounting evidence from previous fMRI experiments using univariant analyses indicating that *APOE* associates to differential patterns of brain activity during cognitive demands in the context of equivalent task performance (Bondi et al., 2005; Fleisher et al., 2005; Smith et al., 1999, 2002; Wishart et al., 2006), further research controlling for task difficulty is need to corroborate present findings regarding functional connectivity.

In summary, we found that *APOE* ε4 carrier subjects show a different pattern of brain connectivity as compared to non-

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carriers in a face–name encoding task. This could be related to a compensatory mechanism during the encoding process.

Disclosure statement

The authors reported no conflicts of interest.

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Brain structure and function related to cognitive reserve variables in normal aging, mild cognitive impairment and Alzheimer's disease

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Abstract

Cognitive reserve (CR) is the brain's capacity to cope with cerebral damage to minimize clinical manifestations. The 'passive model' considers head or brain measures as anatomical substrates of CR, whereas the 'active model' emphasizes the use of brain networks effectively. Sixteen healthy subjects, 12 amnestic mild cognitive impairment (a-MCI) and 16 cases with mild Alzheimer's disease (AD) were included to investigate the relationships between common proxies of CR and the cerebral measures considered in the 'passive' and 'active' models. CR proxies were: inferred premorbid IQ (WAIS Vocabulary test), an 'education-occupation' variable and a questionnaire of intellectual and social activities. MRI-derived whole brain volumes and brain activity by functional MRI during a visual encoding task were obtained. Among healthy elders, higher CR was related to larger brains and reduced activity during cognitive processing, suggesting more effective use of cerebral networks. In contrast, higher CR was associated with reduced brain volumes in MCI and AD and increased brain function in the latter, indicating more advanced neuropathology but that active compensatory mechanisms are still at work in higher CR patients. Overall, inverse relationships for healthy and pathological aging groups emerged between brain structure and function and CR proxies.

Keywords: Cognitive reserve, brain reserve, brain volumes, functional magnetic resonance imaging (fMRI), cognitive aging, mild cognitive impairment, Alzheimer's disease, recognition memory, compensation.

1. Introduction

Cognitive reserve (CR) refers to the hypothesized capacity of an adult brain to cope with brain pathology in order to minimize symptomatology (Stern, 2002). CR construct was proposed after having observed no direct relationships between brain damage severity and the clinical manifestation of symptoms. For instance, higher rates of Alzheimer's disease (AD) neuropathology at post mortem examinations were seen in individuals who were not clinically demented but possessed heavier brains and higher counts of large neurons (Katzman et al., 1988). More recent data is available indicating that a number of participants presenting with extensive AD-related neuropathology (Braak and Braak stage VI) and/or vascular damage were able to perform at a considerably high level in clinical and neuropsychological examinations, thus showing 'resistance to the clinical expression of neuropathology' (Snowdown, 2003).

Two hypotheses based on reserve mechanisms have been proposed to account for the abovementioned findings, one from a structural point of view and the other of a more functional fashion (Stern, 2002). At an anatomical level, the 'brain reserve capacity' (BRC) model ascertains that certain factors, such as the number of synapses and brain volume, confer a particular capacity to endure neuropathological processes. When considering dementia and pre-dementia conditions, BRC would be able to prolong the preclinical stage until a critical moment would be reached. From the moment of exceeding that threshold on, vulnerability to brain damage would be unavoidable and eventually, clinical and functional deficits would be evident (Satz, 1993). On the other hand, a more active or functional model of CR has been suggested by Stern (2002). According to this view, CR would be related to the ability to recruit brain networks in an effective way. Once pathological processes are beginning to occur, subjects would use alternative networks in order to perform a particular task successfully or to maintain one's clinical status within the normality, a process defined as compensation. The most frequently used proxies reflecting CR comprise educational/occupational attainment,

premorbid intelligence quotient, leisure, cognitive and mental stimulating activities (Valenzuela and Sachev, 2005).

Direct measures of brain (Katzman et al., 1998; Kidron et al., 1997; Coffey et al., 1999; Edland et al., 2002), head, or intracraneal size (Schofield et al., 1995; Jenkins et al., 2000; Tisserand et al., 2001; Mortimer et al., 2003; Wolf et al., 2004) been have been studied in the aging literature as surrogates of brain reserve. For instance, although negative findings have been reported (Edland et al., 2002; Jenkins et al., 2000), reduced intracranial volume or smaller head size by its own or in combination with low education may confer an increased risk for cognitive decline and dementia (Schofield et al., 1995; Schofield et al., 1997; Mortimer et al., 2003) including mild cognitive impairment (MCI) in old age (Wolf et al., 2004). Further, inconclusive reports have been published regarding the relationship between these variables and proxies of CR such as education in normal aging (Coffey et al., 1999; Edland et al., 2002; Tisserand et al., 2001) with inverse relationships between education and brain volumes in the case of established AD (Kidron et al., 1997).

Similarly, at a functional level, investigations mainly using positron emission tomography (PET), have been undertaken in the research of CR in healthy elders and AD. At rest studies in the latter condition found that education and intellectual and social life activities were inversely correlated with regional brain metabolic activity and/or cerebral blood flow mainly in temporal, parieto-temporal and parieto-occipital regions (Scarmeas et al., 2003a; Perneczky et al., 2006) also extending to other cortical and subcortical areas in other reports (Alexander et al., 1997). Further, reports of activation studies during cognitive tasks among demented and non-demented individuals found specific brain networks which were differentially activated depending on CR background (Scarmeas et al., 2003b; Scarmeas et al., 2004; Stern et al., 2005).

Despite its lower invasiveness, higher spatial and temporal resolution and the knowledge that increases and decreases of BOLD signal from functional magnetic resonance imaging (fMRI) studies reflect increases and decreases in neural activity

respectively (Logothetis et al., 2001; Shmuel et al., 2006), this technique has been less frequently applied to investigate CR. A number of reports have been published using this methodology to indicate that measures such as general fluid intelligence are related to variations in BOLD activity (reviewed in Jung and Haider, 2007). Further, studies in distinct pathological conditions (Chang et al., 2006; Cader et al., 2006; Ernst et al., 2002; Bartrés-Faz et al., 2006) have corroborated the capacity of this technique to reveal the usage of cerebral reserve mechanisms during cognitive tasks. However, only few investigations were specifically designed and interpreted in terms of the cognitive reserve theory. In this regard, Habeck et al. (2003) and Stern et al. (2003) found that the pattern of activation during a nonverbal recognition task in healthy young subjects was related to individual differences in CR variables. Yet, very scarce data is available in healthy elders or among AD patients.

Even when considering previous findings, there is still little or inexistent data of how functional and structural brain measures are distinctly related to CR variables in the same samples of individuals. Since previous reports have found positive evidences for a relationship between accounts of education, occupation, or premorbid IQ variables and both morphologic brain measurements and patterns of brain activity, an issue of interest would be to determine the effects of these relationships in the same sample of individuals that differ according to their clinical status. Specifically, very scarce data (Wolf et al., 2004) addressed these questions in patients presenting high risk conditions for AD such as MCI. Thus, the aim of the present report was to investigate the correlations between the main proxies for CR, brain activity (by means of fMRI) and cerebral structural characteristics among healthy elders, patients diagnosed as having MCI and mild AD patients.

2. Method

2.1. Subjects

Forty-four subjects older than 65, who provided written informed consent (or their relatives in AD cases) were enrolled in the study. The whole sample comprised 16

healthy elders, 12 amnestic MCI (a-MCI) cases and 16 mild AD patients. Participants were selected from Alzheimer's disease and other cognitive disorders unit, at the Neurology Service, Hospital Clinic of Barcelona, and from a primary care health centre in the area of Barcelona (CAP Castellar del Vallès). All subjects underwent clinical and neuropsychological evaluations. The diagnostic procedures employed to classify individuals into the abovementioned groups have been described elsewhere (Rami et al., *in press*). Briefly, healthy individuals did not meet criteria for dementia, and did not present cognitive complaints. Further, they did not exhibit cognitive performance below 1.5SD in a secondary memory test or in any other test comprised in neuropsychological examinations of language, praxis, gnosis and abstract reasoning (Rami et al., *in press*). Amnestic MCI was diagnosed according to the modified Petersen et al. criteria (2001) and two additional criteria, similar to Lopez et al. (2003): 1) memory decline according to clinical judgment and preferably corroborated by an informant, 2) impaired memory function for age and education 3) preserved general cognitive function, 4) intact activities of daily living and 5) non demented; 6) the memory impairment had to be of the episodic memory type defined by 1.5 SD below the control group mean, taking into account age and educational level, and 7) absence of psychiatric or medical causes accounting for these memory problems. A previously validated normative Spanish test: the Delayed Text Memory Test (Pena-Casanova et al., 1997) was used as an episodic memory test for determining a 1.5 SD cut-off below the mean, taking into account age and educational level. Probable AD diagnosis was established by an interdisciplinary clinical committee formed by two neurologists and one neuropsychologist. DSM-IV and NINCDS-ADRDA criteria were applied taking into account clinical and objective functional and neuropsychological results. All AD patients included were mild AD (Global Deterioration Scale-4 stage). Atypical AD variants with non-significant episodic memory impairment were excluded from the study.

2.2. Proxies of cognitive reserve

Three main proxies reflecting those commonly used in the CR literature were defined. The first one was the Wechsler Adult Intelligence Scale 3rd version (WAIS-III) Vocabulary subtest, administered as a measure reflecting premorbid IQ (Lezak et al.,

2004). A second CR variable was defined as ‘education-ocupation’ and included quantifications of both educational and occupational attainment. This measure was coded as in a previous study (Staff et al., 2004) using ordinal values as follows: 0=no formal education, 1=primary school, 2=secondary education and 3=superior or university education and as regards occupation; 0=non-qualified manual, 1=qualified manual, 2=qualified non-manual or technician, 3=professional (university degree required), 4=manager or director (university degree required). The final value was obtained by adding the education and occupation values (range 0 to 7). Finally, a third proxy taken into account as an aim to consider other relevant variables related to CR (Scarmeas, 2007) including recordings of lifetime occupations in leisure and cognitively stimulating activities (reading, writing, music playing, painting) as well as physical (sports, daily walking) and social life (participation in social activities or groups, associations, voluntary work). These measures were gathered into a customized questionnaire with scores ranging from 0 to 19, the greater the score indicating increased CR. The questionnaire was administered directly to the subject participating in the study with the presence of their relatives in the case of patients to ensure the validity of the data provided.

2.3. Magnetic Resonance Imaging Acquisition

All 44 subjects underwent structural and functional MRI examinations. Scans were obtained on a GE Signa 1.5T (General Electric, Milwaukee, Wisconsin). High-resolution T1-weighted images were acquired for anatomical identification with a Fast Spoiled Gradient-Recalled Echo three-dimensional sequence (Digital Imaging and Communications in Medicine) format: Repetition time [TR]/Echo time [TE] = 12/5.2, Inversion time = 300, Number of Exitations = 1, FOV = 24 3 24 cm, 256 3 256 matrix). Whole-brain volumes were acquired in an axial plane yielding contiguous slices with slice thickness of 1.5 mm. Functional images were acquired using a T2*-weighted gradient echo planar imaging (TR = 2000 ms, TE = 40 ms, FOV = 24 3 24 cm, flip angle of 90). Twenty axial slices were obtained for each brain volume with a slice thickness of 5 mm and a gap of 1.5 mm.

2.4. Structural MRI

Statistical Parametric Mapping (SPM2) running in Matlab 6.5. was used to analyze structural brain images. A single investigator performed the prior manual steps in image preparation (anterior–posterior commissure line determination and image reorienting). Following segmentation, (which was performed against the T1 template (MNI) implemented in SPM) of the three tissue compartments, a measure of whole brain volume was obtained in mm^3 by adding the gray and white matter volumes. This measure was then corrected for whole intracranial volume ([gray+white]/[gray+white+CSF]). We used brain volumes in our study instead of head size or intracraneal volumes previously employed to reflect premorbid brain status (Schofield et al., 1995; Schofield et al., 1997; Mortimer et al., 2003; Wolf et al., 2004; Edland et al., 2002; Jenkins et al., 2000) because we did not aim to determine a risk for AD or a-MCI associated with these variables but instead we were interested in directly investigating the correlations between CR proxies and brain integrity (or otherwise atrophy) for each subject relative to their overall head size.

2.5. fMRI procedure and memory recognition assessment

Original magnetic resonance images registered in format GE-advanced (1 two-dimensional file per slice) were organized into three-dimensional files (150 volumes per subject) by means of MRICro software (University of Nottingham, UK) and saved in ANALYZE 7.5 format compatible with SPM2. Following alignment along the anterior commissure-posterior commissure line and realignment of the scans to remove the effects of head movement, images were transferred into a standardized coordinate system. Normalized images were smoothed with an isotropic Gaussian kernel (full width half maximum) of 8 mm. As a cognitive experiment, we used a 15-block design task with alternating “repeated”, “rest” and ‘‘experimental’’ conditions (5 blocks each) presented in an alternating way until the total duration of the experiment (15 blocks, 30s. per block, 450 s) was reached. The presentation time was 2s. and the interstimuli period 1 s. The task started with a repeated block, which consisted of the projection of one single photograph. The same picture was used for all the ‘repeated’ blocks. Following this

condition and before the “experimental” block, a ‘blank’ block (presentations of a bare cross hair) was inserted. As regards the experimental task *per se*, subjects had to focus on fifty non-emotional photographs for a later recall test, as they were told before the scanning. Pictures were outdoor images showing a person or a group of people doing or performing different activities or landscape images. For each ‘experimental’ block, ten novel pictures were presented. Following the scanning, subjects underwent a 2 forced-choice task where they were given two photographs each time and they had to decide which one had been previously displayed in the fMRI session. The maximum score for recognition memory was 50.

2.6. Data analysis

The Statistical Package for Social Sciences (SPSS v.14.0) was used to investigate group differences in demographic, clinical and CR measures by means of ANOVA, and chi-square tests when appropriate. Partial correlations (adjusting for age and gender) of whole brain volumes and CR measures were performed separately for each clinical group. To analyze fMRI data, individual analyses were performed using SPM2 for each subject to compare brain activity observed during the ‘experimental’ condition compared with that seen during the ‘repeated’ condition (contrast: experimental>repeated). Second level (group) regression analyses (adjusted for age, gender, and post-fMRI recognition memory performance) were further undertaken for each clinical group to analyze how brain activity and CR variables were related. In these analyses, CR proxies (considered conjointly and separately) were entered as independent variables and brain activity during cognitive performance was the dependent variable. fMRI results were interpreted if they attained both voxelwise threshold of $p<0.001$ (uncorrected) and at $p<0.05$ (corrected) threshold on the extent of clusters. Only clusters containing more than 20 contiguous voxels were considered. This study was approved by the local ethics committee.

3. Results

Demographic variables, recognition memory and values for CR proxies are given in table 1. Groups were statistically comparable for both age and gender and, as expected, controls outperformed AD and a-MCI participants in recognition memory following fMRI. As regards CR proxies, measures for the Vocabulary WAIS-III subtest and for 'education-occupation' were also significantly higher for healthy elders. Our CR questionnaire correlated positively with Vocabulary and 'education-ocupation' in the whole sample (r=0.62, p<0.0001; r=0.64, p<0.0001, respectively).

	CONTROLS (n=16)	**a-MCI (n=12)**	**Mild AD (n=16)**	**F / χ^2**	**Post hoc (Scheffe)**
Age	73.31 (4.90) [CI 70.7÷75.9]	74.25 (6.18) [CI 70.3÷78.2]	76.50 (5.80) [CI 73.7÷76.4]	1.36	n.s.
Gender (M/F)[a]	5/11	2/10	5/11	0.94	n.s.
Recognition memory	43.37 (5.21) [CI 40.6÷46.2]	36.25 (6.88) [CI 31.9÷38.1]	26.31 (5.35) [CI 32.4÷38.1]	35.43	C>MCI** C>AD*** MCI>AD***
COGNITIVE RESERVE (CR)					
Vocabulary WAIS-III	43.56 (9.06) [CI 38.7÷48.4]	30.08 (7.99) [CI 25.0÷35.2]	29.75 (14.54) [22.0÷37.5]	7.64	C>MCI* C>AD**
Education-occupation	4.56 (1.86) [CI 3.1÷5.6]	2.42 (2.02) [CI 1.6÷3.7]	2.75 (2.21) [CI 1.6÷3.9]	4.79	C>MCI*
CR Questionnaire	9.00 (4.24) [CI 6.7÷11.3]	5.67 (3.82) [CI 3.2÷8.1]	5.56 (3.93) [CI 3.5÷7.7]	3.63	n.s.

Table 1. Demographic characteristics and CR variables across the three studied groups. Values are given in means (standard deviations); n.s. non-signifficant; *p<0.05; ** p<0.01; *** p<0.005; [a] M, male; F, female. WAIS-III: Wechsler Adult Intelligence Scale 3rd version. Vocabulary rates are given in direct scores. CI: Confidence Interval for the mean (Confidence Level CL=96%).

3.1. CR and brain structure

Results for partial correlations for each clinical group indicated that there were negative correlations between the Vocabulary test score and whole brain volume both for a-MCI and AD groups. In contrast, a positive relationship was observed for control subjects for this variable although the results did not reach statistical significance (figure 1). However, significant positive correlations reaching statistical significance were observed between 'education-occupation' and brain volumes for healthy subjects ($r=0.59$, $p<0.03$).

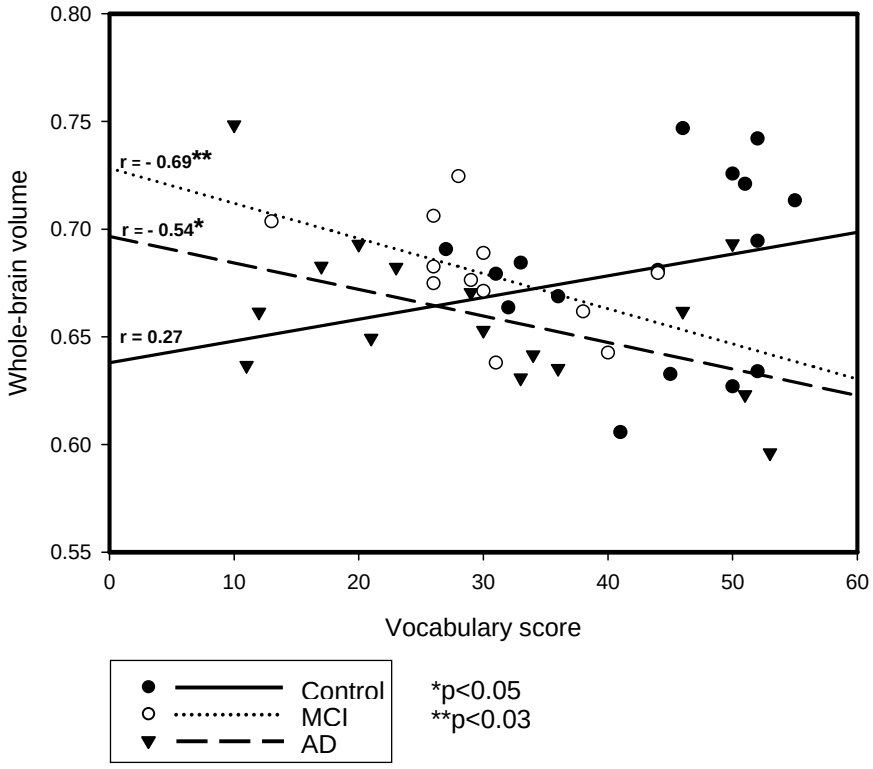

Figure 1. Scatterplot displaying partial correlations between whole-brain measures and Vocabulary scores for each sample group. r values and significances are given after partial correlations have been adjusted for age and gender.

3.2. CR and brain function

For clinically normal and demented participants opposite brain activation patterns during fMRI were observed as a function of CR. On the one hand, among healthy subjects, higher global CR (all CR variables considered simultaneously in the analysis) was related to decreased activations in several cortical and subcortical regions including the frontal lobe bilaterally, the left parahippocampal cortex, and the cerebellum. When CR proxies were considered separately, the composite variable of literacy rates and occupational attainment was the one variable emerging significant from this analysis (table 2). No positive correlations were observed in this analysis.

On the other hand, results from AD patients reflected a direct relationship between brain activity and CR. In these patients, we found positive slopes for the multiple regression analyses between the CR questionnaire score and brain function in the right anterior and posterior cingulate cortices as well as in the left superior temporal gyrus (table 2 and figure 2). In contrast to what was observed among control subjects, no negative correlations were found in this analysis. No significant correlations (either positive or negative) were seen between CR variability and brain activity in the a-MCI group.

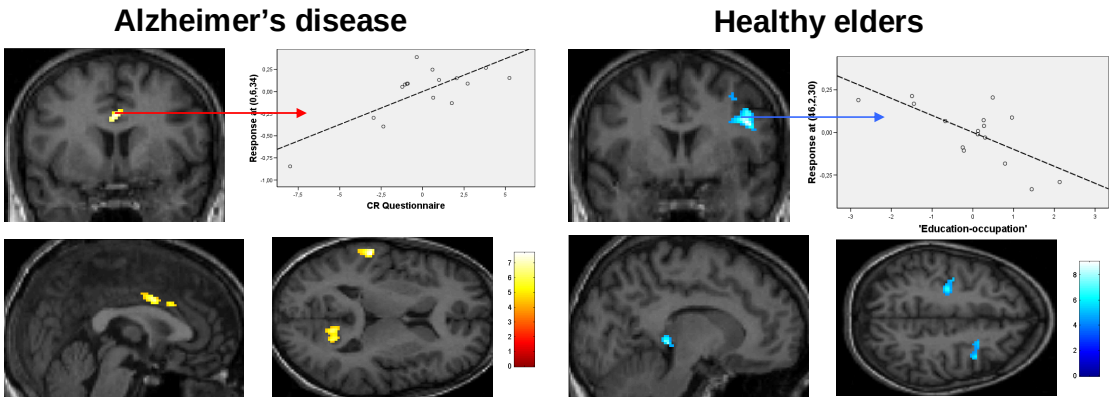

Figure 2. Brain areas showing positive (in hot colours) and negative (in winter colours) correlations with CR questionnaire in Alzheimer's disease patients and total CR in healthy controls. Scatterplots for the regions with greater activity associated with CR variables are also depicted. For a precise localization of the cerebral regions, see table 2.

CONTROLS	**CR proxies**	**Correlation**	**t value**	**Talairach coordinates [x, y, z]**	**Region (BA)**	**Num. voxels**
	Total CR	Negative	9.03	[46,2,30]	R Inferior frontal gyrus (BA44)	196
			8.71	[-14,-36,-2]	L ParaHPC gyrus (BA30)	102
			7.95	[-30,-18,50]	L Precentral gyrus (BA4)	146
			6.09	[-40,-62,-32]	L Cerebellum	82
			6.09	[24,12,44]	R Mid frontal gyrus (BA8)	98
	Education-occupational attainment	Negative	8.10	[46,2,30]	R Inferior frontal gyrus (BA44)	133
			8.04	[-14,-36,-2]	L ParaHPC gyrus	75
			6.80	[0,-48,-24]	Cerebellum	101
MILD AD	**CR proxies**	**Correlation**	**t value**	**Talairach coordinates [x, y, z]**	**Region (BA)**	**Num. voxels**
	CR questionnaire	Positive	6.82	[0,6,34]	Anterior cingulate (BA24)	90
			6.29	[18,-60,6]	R Posterior cingulate (BA23/31)	125
			7.69*	[-64,-28,8]	L Superior temporal gyrus	77

Table 2. Brain regions showing significant relationships between CR proxies and brain activity in healthy elders and mild AD cases. All results are displayed after regressions were adjusted for age, gender and memory recognition performance following fMRI. Total CR: all CR variables entered simultaneously in the multiple regression analysis; R= right, L= left, ParaHPC= parahippocampal, *= $p<0.08$ cluster level (corrected).

4. Discussion

In the present study we gathered a number of CR indicators as an attempt to examine their relationships with brain structure and function in three aging conditions: normal aging, a-MCI and mild AD. Overall and as it is discussed below, results from healthy elders compared to clinical samples reflect an inverse effect of CR measures both on brain function and structure. Specifically, among healthy elders, higher CR was associated with larger brains and increased efficiency (as reflected by reduced brain activity) during cognitive performance, whereas among cognitively impaired elders the opposite pattern was observed. Present results thus, suggest reverted relationships between the passive (brain structure) and active (brain activity) correlates of cognitive reserve measures between normal ageing as compared to conditions where a threshold implying clinical manifestations has been exceeded.

In our sample of healthy elders, we found a positive significant correlation between 'educational-occupational' attainment and whole-brain volume. At first glance, these results may appear counterintuitive as regards to the cerebral reserve hypothesis. In this sense, a study by Coffey and colleagues (1999) investigating the association between education and brain measures in a large sample of non-demented elders found results in the opposite direction (more brain atrophy for those more educated) suggesting that the more educated individuals possessed larger reserve (in that they were able to remain clinically healthy despite a more advanced age-related brain atrophy). In that study however, the possible presence of cognitive impairment was solely excluded by a general cognitive measure (MMSE >24) probably including a proportion of individuals with incipient dysfunction such as MCI cases. On the other hand, an increasing body of literature has been published evidencing positive associations between behavioral measures similar to the CR proxies used here and cerebral volumes. For example, highly qualified/skilled occupation such as being a professional musician is related to increased gray matter volumes in particular brain areas (Gaser and Schlaug, 2003). Further, Haier and colleagues (2005) found a direct relationship between gray matter volumes and full-

scale IQ WAIS scores among healthy older adults (mean age=59), and Colom et al. (2006) evidenced positive correlations between measures of the general intelligence factor (including the Vocabulary subtest of the WAIS) and increased gray matter volume throughout the brain. Finally, higher education has also been associated with enlarged brain or head size in normal elders (Edland et al., 2002; Tisserand et al., 2001). Altogether previous and present findings may suggest that when subtle putative cases of clinical impairment (such as MCI) are ruled out, higher rates of CR in normal elders are related to increased levels of cerebral reserve as reflected by MRI volumetric measurements. Thus, according to our results, aging *per se* would not revert the association between CR and structural brain integrity observed in former studies of younger individuals until a critical clinical threshold has been overcame (see discussion below for MCI and mild AD groups).

Regarding the fMRI results among healthy elders, a negative association emerged between CR and brain activity during a memory encoding task. In our study, healthy elders with reduced CR recruited additional regions to perform a visual memory task. These regions included mainly inferior frontal lobe areas, left parahippocampal cortex and the cerebellum. All these brain regions have been previously found to show activity during memory tasks among healthy elders and were associated with compensatory responses. In this regard, the cerebellum was identified as a region within a network showing enhanced response to increased memory demands (Gould et al., 2003). Further, mounting evidence indicates that the frontal lobes have an important compensatory role during aging in a variety of cognitive tasks (reviewed in Grady, 2007). The compensatory role of the prefrontal cortex could be associated with a reduced activity in the parahippocampus (Gutchess et al., 2005), despite this latter region has also been suggested to serve the purpose of functional compensation in other learning tasks (van deer Ven et al., 2006). Only few previous studies using functional neuroimaging procedures have included samples of elder participants and interpreted their findings in the terms of CR hypotheses. Scarmeas et al. (2003b) used PET to demonstrate CR-related differential success in coping with age-associated changes in cognition. In their study, the authors found positive correlations between brain activity

and CR variables during a visual memory encoding task in young individuals whereas more brain regions including parietal, frontal and medial and lateral temporal lobe areas were negatively correlated among the elder. Similarly, using PET and a nonverbal recognition memory task, Stern et al., (2005), identified an 'age-related' topography whose change in expression varied from a low to a titrated demand as a function of CR variables within each group. These findings reflecting the differential usage by the elders of a network normally used by the younger partners were interpreted as providing evidence of neural compensation, as the altered network is used to compensate for the inability to recruit the healthy (young) brain's responses to increased task difficulty. Finally, Springer et al. (2005) used fMRI to identify brain networks recruited during encoding and recognition episodic memory tasks whose activity was correlated with years of education in elder and young participants. Among the elder, they found that bilateral frontal activity was associated with more education whereas posterior medial temporal lobe showed the opposite pattern. On the whole all these studies reflect that CR relates to individual differences in how tasks are processed and that the reorganization of brain function in old participants is associated with maintained cognitive function into old age. In contrast to previous reports, we only observed negative correlations between brain activity and 'educational-occupational' attainment among our healthy elder subjects. These differences may be related to methodological approaches (i.e. comparing young vs old and comparing distinct levels of task difficulty) and the methods used to analyze data (i.e. univariate in ours vs multivariate in Stern et al. (2005) and Springer et al. (2005)). However, as previous reports, our findings are in accordance with the CR in the sense that while they can not prove that CR is related to greater capacity (since there were no correlations between increased CR and enhanced cognitive performance) they suggest the use of more efficient brain networks among healthy elders with high CR (less activation for the same performance) (Stern, 2007).

In a-MCI and mild AD significant negative correlations were observed between whole brain volume and CR, specifically with the WAIS Vocabulary test. According to the reserve capacity hypothesis, the findings would suggest that at particular level of clinical severity (all MCI of the amnestic type and all AD GDS=4), those patients with increased

background in terms of intellectual attainment do in fact, exhibit a more advanced neuropathological process as reflected by increased atrophy of their brains relative to their intracranial volume sizes. Similar findings were previously reported in AD samples (Kidron et al., 1997). Our results provide first evidence that a similar relationship can be evidenced in very early stages of the disease (a-MCI). Previous research in AD considering CR variables such as reading, education and occupation have demonstrated faster decline after dementia onset (reflecting more advanced brain pathology) for patients with higher CR (Wilson et al., 2000; Stern et al., 1999; Scarmeas et al., 2006). Since present findings reflect the same directionality between CR and brain atrophy measurements for AD and a-MCI and since this latter condition has been demonstrated to correspond to high risk (Petersen et al., 2001) or very incipient AD (Morris et al., 2001), it would be of interest to determine in further longitudinal studies whether among these patients higher rates of CR is also related to faster declines and/or higher rates of conversion to dementia.

Considering the fMRI findings, a positive correlation between CR level and activity in anterior and posterior cingulate cortices as well as in the lateral temporal lobe regions emerged in mild AD cases, an opposite pattern to that observed among healthy elders. When we analyzed the impact of CR background on recognition memory performance, no particular variable was related to higher memory scores, indicating that despite increased brain damage (as reflected by reduced cerebral volumes), mild AD patients with higher CR were able to perform the task at an equivalent level to those with lower CR scores. In this regard, overactivation among these patients may reflect more efficient use of brain networks resulting in a behavioral compensation (i.e. maintainance of behavioral performance as compared to less atrophied patients with low CR). Despite less frequently studied as compared to healthy subjects, there is evidence that AD patients can also recruit brain regions to support performance both in memory and non-memory tasks (Grady, 2007). Specifically, a network including middle fronto-parietal and lateral temporal areas similar to those found in the present study were associated with successful encoding and retrieval in mild AD cases during a visual learning task (Gould et al., 2006). Another report found additional recruitment of the anterior cingulate region

among early AD patients as compared to controls during an episodic working memory task, a region usually recruited during semantic memory tasks. The authors suggested these increased activations observed in their AD cases could be reflecting a residual capacity of their premorbid cerebral reserve (Starr et al., 2005). Present results add further evidence to previous findings suggesting that CR status may be one of the variables explaining such compensatory mechanisms formerly shown among mild AD cases.

Several limitations should be considered in our study. An issue to bear in mind is the small sample sizes. Although our samples are comparable to many fMRI studies, a larger sample would have been desirable, especially in the MCI subgroup. The reduced number of subjects studied might explain the lack of significant results for the fMRI experiment in this group. A further aspect to consider is that the fMRI memory task did not entail the same level of difficulty for all participants both within and between clinical groups, an aspect that by itself could lead to differential brain activity. Some previous studies (e.g. Habeck et al., 2003; Stern et al., 2003; Stern et al., 2005) adjusted the task difficulty so that all tested individuals performed at an equivalent level. Instead, we used recognition memory as a covariate in all analyses as a measure of control for this variable. However, no correlations were observed between memory performance and CR variables in any group, suggesting as in previous report (Stern et al., 2003) that it is unlikely that the relationship observed between fMRI and CR is an artifact of correlations between cognitive performance. A further limitation refers to the statistical approach. In the present report we did not analyze data using a multivariate approach to reveal networks underlying task performance during cognitive tasks. In exchange, we employed a General Linear Model (GLM) analysis only reflecting correlations between CR measures and task-related activations. Previous fMRI reports in similar samples have found complementary findings using both methodologies (Stern et al., 2003; Habeck et al., 2003). Thus, future studies using multivariate approaches such as functional connectivity may reveal dynamic associations between CR variables and brain activity among distinct clinical groups.

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Disclosure statement

There is no conflict of interest for any author concerning this manuscript.

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4. GENERAL DISCUSSION

Results derived from the present thesis identify environmental and genetic factors that significantly modulate brain structure and function in elders with and without cognitive impairment. Many other 'extrinsic' and 'intrinsic' factors as well as their interactions unexplored here remain to be tested in further studies for a better understanding of the biological and environmental variables that influence brain integrity in our seniors. Thus, this work might be seen as a first attempt to show that an integrative approach using distinct techniques (from brain imaging to cerebral stimulation and molecular genetics) is suitable to investigate brain-behavior relationships in this population. In the following paragraphs, a general discussion of the results of each study is presented.

Our first study evidenced viability of rTMS to transiently facilitate recognition memory in a sample of elders with memory complaints, a cognitive effect that co-occurred with brain activity changes. Specifically, right frontal regions where over recruited in those participants receiving real stimulation, being these areas responsible for a more symmetric pattern of prefrontal brain activity. Since lateralization of PFC is reduced during aging (Cabeza, 2002) increased bilaterality exhibited in our first study could be counteracting age-related cognitive impairment and be thus considered compensatory. Displaying a bilateral pattern of PFC has been related to better performance in older adults in verbal working memory (Reuter-Lorenz, 2000) and behavioral compensations in the elderly are frequently related to increased brain activity in the PFC (Grady, 2007). However, the mechanism mediating brain activity and memory improvement remains unclear (Grady et al., 2006). The transient recovery of PFC symmetry would resemble young brain activity patterns and therefore with a better cognitive performance, consistent with prior studies (Wagner et al., 1998; Stebbins et al., 2002). The fact that right PFC is more activated can be indicating increased attentional function and task difficulty, as demonstrated in previously (Sunaert et al., 2000).

In a second study, the effects of rTMS observed previously were investigated conditionally to the APOE genotype of the participants. Since APOE represents the most clearly genetic factor influencing brain function in the elder, it was hypothesised that this biological variable would modulate the effects of an external brain stimulation. The results

of this study evidenced that in subjects carrying the ε4 allele, brain activity in the PFC was increased and this was again accompanied by memory amelioration. Specifically, the frontal brain regions with additional activations following rTMS within this genetic group were the contralateral regions found in baseline fMRI, revealing a more symmetrical pattern of frontal lobe activity during the second fMRI. Nonetheless, ε3 carriers exhibited a more posterior brain pattern following rTMS session and they also improved memory recognition score. As a consequence, present investigation evidenced for the first time how a biological variable can distinctly modulate brain response to the effect of magnetic stimulation in elders. Since previous studies showed a reduced metabolic response in parieto-temporal regions among carriers of the ε4 variant revealing incipient neuropathogical changes (Reiman et al., 2001, 2005; Small et al., 1995, 2000) it was concluded that the response of APOE carriers to rTMS was mediated by those less damaged regions (frontal areas).

Due to the differential brain activation effects observed in the second study as a function of the APOE genotype, in the third investigation we moved forward and deeply to investigate the patterns of functional connectivity in elders differing by this genetic variation. The results of this study evidenced that during a visual encoding task the hippocampus of the bearers of this allelic variant exhibited increased connectivity with the ipsilateral caudate, anterior cingulate and inferior parietal areas as compared to non-carrriers. Thus, this report indicated that additional brain connections conforming an extra network within the left hippocampus of ε4 elders is required in order to perform an associative memory task. Compared to present findings, the results from the rTMS x APOE study indicated that the increased brain activity observed in frontal areas in ε4 carriers could be considered as an 'efficient compensatory mechanism' since it resulted into cognitive amelioration. On the other hand, in the present investigation the additional activations in the network comprising the left hippocampus seems to show non-efficient compensation, since it indicated extra cognitive effort when compared to non ε4 carriers but non-equivalent task performance. Thus, while the different pattern of hippocampal connection in ε4 subjects may point to compensatory brain mechanisms, a compensation in terms of behaviour performance (memory encoding) would be hampered by basic dysfunctions in their memory systems, since they represent a population with memory impairment. Altogether, the second study of this thesis seems to indicate that overactivations in prefrontal areas found in elders bearing the APOE ε4 variant are

translated into increased cognitive performance. This is however not seen when taking into account the extra activations observed within the hippocampal network. Further studies with stimulation techniques and functional connectivity of prefrontal-hippocampal regions should help to determine how this particular genetic variant influences the interactions of these two relevant brain areas as well as its translation into cognition.

The last study of this thesis focused on the investigation of extrinsic variables that have been referred to as cognitive reserve (CR) and how these indicators influenced brain activity and structure in elders with and without cognitive impairment. In this report, we observed how healthy elders with lower CR needed to recruit additional brain areas to perform a visual recognition memory task. This effect may be seen as comparable to the ε4's less efficient brain activity evidenced in the previous studies of this thesis. Therefore, we have showed how biological and environmental variables are related to brain efficiency in terms of amount of brain activity recruited during the performance of cognitive tasks or the patterns of brain connectivity. On the other hand, when dealing with pathological aging, our data derived from mild AD patients agreed the CR hypothesis described by Katzman et al. (1993). In this sense, we proved that despite equivalent clinical level, those AD with higher CR had more brain damage (less grey matter) and this may be a proof that there is an individual threshold of tolerating brain damage that may be exceeded later by higher CR subjects, or in other words, they would require more brain damage to manifest dementia symptoms. Besides, these subjects recruited more brain areas, indicating that they were still able to activate brain regions and perform at the same level as those AD with lower CR level and therefore less structural brain atrophy. In this sense, we could speak of compensation as defined by Stern (2005). Apart from results derived from an AD sample, we were the first to prove that MCI subjects with higher CR had a similar pattern of brain damage. The same directionality between CR and brain atrophy measurements for AD and MCI should be taken into account considering that this latter condition has been described as high risk to dementia (Petersen et al., 2001) or very incipient AD (Morris et al., 2001). In this regard, since longitudinal studies have shown that among AD cases, those with higher levels of CR experience a more accelerated decline overtime (i.e. reflecting more advanced neuropathological state), our data indicate that this could also be true for MCI. Thus, further longitudinal studies should be addressed to investigate if CR is among the variables influencing the clinical course of MCI.

In summary, the whole body of results derived from this thesis evidences that there are extrinsic and intrinsic variables able to modulate brain function in the elderly population with memory complaints but also in cases of demented patients. Intrinsic or biological variables would be genetic background like the presence or absence of the ε4 allele, which would be affecting MTL connections when dealing with memory tasks and would also be enhancing a more anterior pattern when brain response is modified by high frequency rTMS. Extrinsic or environmental factors would be linked with CR proxies, like education and occupational attainment, continuing education, leisure and intellectual stimulating activities; issues that would offer a protection against brain damage and would prevent that in case brain damage was present, it was needed a greater amount of it to be manifested clinically.

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5. CONCLUSIONS

Below are described the general conclusions corresponding to each of the four studies presented in the present thesis:

1) A single session of high frequency rTMS is able to transiently modulate brain function and increase learning ability in elders with aging-associated cognitive decline. Since memory improvements were associated with an increased activity of the right prefrontal cortex following magnetic stimulation, we concluded that rTMS facilitated the activation of compensatory brain mechanisms previously reported in functional neuroimaging studies of elders.

2) APOE ε4 carriers exhibited a more anterior pattern of brain response after rTMS as compared to ε3 carriers, who showed increased brain activity in posterior regions. This result offers first evidence that the genetic background of the subjects modulates the brain response to external stimulation. Previous studies showed decreased glucose metabolism in parieto-temporal areas in non-demented ε4 carriers, evidencing incipient neuropathological changes in these regions. Thus, our data also seem to reveal that brain responses to magnetic stimulation are mainly mediated by brain regions which are probably more preserved.

3) Older adults presenting with mild memory impairments and carrying the APOE ε4 allele exhibited enhanced activity in functionally connected cortical and subcortical structures to the left hippocampus compared to a clinically equivalent sample of non-APOE ε4 bearers in a face-name memory task. However, behaviourally these individuals performed poorer. We conclude that MTL structures are less efficient in the latter subjects and therefore increased connectivity with other brain regions is needed as a compensatory mechanism, even though it may not be behaviourally successful.

4) Healthy elders with higher cognitive reserve have larger brain volumes and use brain networks more efficiently when performing a memory task. Conversely, mild Alzheimer's disease patients with higher level of cognitive reserve exhibit increased brain atrophy but recruited more brain regions than patients with lower cognitive

reserve, performing at the same level behaviourally. Results regarding MCI patients were similar in terms of the structural analyses. Thus, we conclude that among elders with memory impairment and initial stages of dementia, cognitive reserve is related to brain integrity and allows to use brain networks more efficiently during cognitive efforts.

4. SUMMARY OF THE THESIS (Resum de la tesi)

'Funció i estructura cerebral en l'envelliment amb i sense afectació cognitiva'

Introducció

L'envelliment cognitiu fa referència a una sèrie de canvis relacionats amb l'edat que afecten les diverses funcions cognitives. S'han descrit almenys tres patrons de canvis: declivis al llarg de la vida, declivis que tenen lloc en fases avançades i relativa estabilitat al llarg de la vida. Hi ha una certa especificitat en quant a quines funcions cognitives es veuen afectades i quines romanen més o menys estables. Així, els mecanismes bàsics de processament de la informació (velocitat de processament, memòria de treball, codificació d'informació a memòria episòdica) tendeixen a declinar durant la vida adulta. Sembla que la velocitat de processament seria la variable més afectada abans dels 60 anys. Estudis longitudinals també han evidenciat un més ràpid declivi en edats molt avançades o al voltant de 3 anys abans de la mort (veure Hedden i Gabrieli, 2004 per una revisió).

Per altra banda, tasques que impliquen una pràctica no solen declinar amb l'edat o ho fan en edats molt avançades. La memòria a curt termini seria un exemple d'una funció que declinaria més tard. Mesures de vocabulari i coneixement semàntic també es mostren estables, fet que indica que l'experiència conduiria al coneixement i la sabiesa de la que sovint es relaciona amb l'envelliment (Baltes et al., 1995). De la mateixa manera, la memòria implícita i autobiogràfica així com el processament de les emocions i l'atribució d'estats mentals a altres individus (teoria de la ment) tampoc declinen al llarg de la vida (Fromholt et al., 2003; La Voie i Light, 1994). En resum, els estudis que s'han realitzat fins ara conclouen que hi ha una certa estabilitat de les habilitats que han requerit pràctica constant pel seu aprenentatge i de processos automàtics, amb una certa alteració d'aquells tipus de memòria que requereixen formació de noves connexions, tals com el record de fets nous o recents. Precisament els canvis en memòria episòdica han estat llargament estudiats i s'ha conclòs que aquestes dificultats podrien estar lligades a dèficits tant en la codificació com en la recuperació d'informació. S'ha proposat una menor capacitat d'iniciar espontàniament les estratègies de codificació o d'organitzar degudament el material pel seu aprenentatge.

La recerca dels canvis en funcions cognitives durant l'envelliment no es pot separar dels canvis neurobiològics que s'hi acompanyen. Estudis post-mortem han confirmat una disminució en el volum de la substància gris en cervells envellits al ser comparats amb joves (Haug i Eggers, 1991; Resnick et al., 2003). Aquests serien deguts a una menor densitat sinàptica (Terry, 2000), la qual presenta una especificitat regional. Així, les regions de l'escorça prefrontal i estructures del lòbul temporal medial es veurien especialment afectades, mentre que regions occipitals romandrien relativament estables (Raz et al., 2004). Aquesta especificitat regional ha portat a diferenciar entre dos sistemes neurofisiològics: sistema frontobasal i del lòbul temporal medial (LTM). Els circuits frontals es veuen afectats per la disminució dels nivells de dopamina, noradrenalina i serotonina que s'acompanyen amb l'edat. A més, durant la vida adulta el lòbul frontal és el que pateix una major reducció volumètrica, sent aquesta d'aproximadament un 5% per dècada a partir dels 20 anys (Raz et al., 2003). El volum de l'estriat també decreix amb l'edat (aproximadament un 3% per dècada, Gunning-Dixon et al., 1998). Les connexions entre aquestes estructures subcorticals i el còrtex prefrontal es veurien per tant afectades durant l'envelliment i això s'ha relacionat amb una major dificultat en funcions executives, memòria de treball i velocitat de processament de la informació. Per altra banda, la importància de l'hipocamp i altres estructures del LTM en la memòria declarativa fa que el LTM sigui d'un especial interès en l'envelliment (Erickson i Barnes, 2003). Aquesta perspectiva pren la malaltia d'Alzheimer (MA) com a exemple. El volum de l'escorça entorrinal, una estructura que pertany al LTM, ja comença a declinar fins i tot 10 anys abans de que es faci el diagnòstic clínic de la malaltia (Killiany et al., 2000; Dickerson et al., 2001).

L'alteració cognitiva lleu (ACL, de l'anglès Mild Cognitive Impairment) fou descrita com a entitat clínica per Petersen et al. (1999). Els criteris d'inclusió dels ACL de tipus amnèsic inclouen persones no demenciades amb queixes subjectives de memòria objectivables mitjançant proves neuropsicològiques (1.5 desviació estàndard per sota dels barems que correspondrien per edat), manteniment de la funció cognitiva global i de les Activitats de la Vida Diària (AVD) (Petersen et al., 2004). Sembla que les persones amb ACL evolucionarien amb major probabilitat cap a MA, amb una progressió anual d'entre 10-15% (Petersen, 1999). Així com s'ha demostrat en la MA, en pacients amb ACL també s'ha vist una reducció del volum i un major hipometabolisme de l'escorça entorrinal quan es compara amb gent gran preservada (Small et al., 2002; Rusinek et al., 2003). Per tant, les dades semblen indicar que l'atròfia d'aquesta estructura seria un millor predictor de

progressió a MA que la reducció en el volum de l'hipocamp. Malgrat tot, els resultats derivats dels molts estudis realitzats amb ACL no semblen convergir, fet que ha fet sorgir un gran nombre de dubtes i controvèrsies referents a aquesta entitat. Alguns estudis han equiparat l'ACL a un valor de 0.5 en el CDR (Clinical Dementia Rating), instrument no diagnòstic que pot indicar tant casos d'ACL com de MA inicial. A més, s'han descrit subtipus clínics d'ACL (ACL amb afectació d'un sol domini cognitiu: memòria o no i ACL de diversos dominis cognitius que pot incloure o no afectació de memòria) (López et al., 2006).

El diagnòstic definitiu de la MA només pot establir-se quan es confirma una afectació neuropatològica (cabdells neurofibrilars intraneuronals, acumulació de proteïna β-amiloide i pèrdua i disfunció sinàptica), de tal manera que només pot ser realitzat pot-mortem, tot i que actualment les tècniques de neuroimatge in vivo permeten mostrar un patró de distribució de neuropatologia i per tant ja poden facilitar un primer diagnòstic de la malaltia (Small et al., 2006). Els cabdells neurofibrilars semblen originar-se en el LTM, afectant primerament les funcions mnèsiques, es van agrupant en el còrtex temporal inferior així com cingulat posterior en una primera fase inicial, fins que s'extenen a nivell parieto-temporal i còrtex prefrontal, estructures implicades en la percepció, atenció i llenguatge (Braak i Braak, 1996).

A nivell funcional, durant l'envelliment hi ha una pèrdua de l'asimetria en l'activitat de l'escorça prefrontal. Aquesta ha estat una de les troballes més consistents dels estudis de neuroimatge funcional realitzats entre joves adults i adults envellits i s'ha descrit com a model HAROLD (de l'anglès Hemispheric Asymmetry Reduction in Old Adults) (Cabeza, 2001). En l'envelliment patològic (ACL i MA) no s'han trobat patrons d'activació bilateral tals com els descrits en l'envelliment normal. Estudis realitzats amb Tomografia per Emissió de Positrons (TEP) indiquen una reducció del metabolisme en regions parieto-temporals i del cingulat posterior en estadis inicials de MA i en àrees frontals en fases més avançades de la malaltia (veure Mosconi et al., 2006a per una revisió sobre el tema). En el cas de l'ACL, s'ha demostrat que aquest patró d'hipometabolisme també està present en pacients de tipus amnèsic i que posteriorment evolucionaran cap a MA (Arnaiz et al., 2001; Chetelat et al., 2003; Drzezga et al., 2003; Anchisi et al., 2005; Mosconi et al., 2005). Per altra banda, els estudis amb ressonància magnètica funcional (RMf) senyalen que en aquests pacients es donaria una sobreactivació en regions del gir parahicocampal durant la

codificació d'una tasca de memòria, fet que representaria un intent de compensació pel dany neuropatològic (Dickerson et al., 2004).

Objectius de la tesi

L'interès general d'aquest projecte de tesi doctoral es centra en l'estudi dels patrons d'activació cerebral subjacents a l'envelliment cognitiu, tant en condicions clíniques normals com patològiques (Alteració cognitiva relacionada amb l'edat, alteració cognitiva lleu o Alzheimer inicial). Per aquest motiu hem empleat la tècnica de la RMf i hem estudiat com diferents variables intrínseques i extrínseques als individus estudiats influeixen la seva activació cerebral.

En un primer estudi ens vam plantejar l'estudi de les relacions entre el cervell i conducta en subjectes envellits amb queixes subjectives de memòria, segons criteris de Levy (1994). Així, el primer objectiu era estudiar com una tècnica capaç de modificar l'excitabilitat cortical de forma transitòria, l'estimulació magnètica transcranial (EMT) podia modular l'activació cerebral observada amb RMf i de retruc influir l'execució d'una tasca d'aprenentatge.

Posteriorment, després d'haver observat un efecte facilitador de l'EMT en el rendiment en memòria d'aquest subjectes vam voler tenir en compte com el fet de ser portador de la variant ε4 del gen de l'apolipoproteïna E (APOE), podria modular l'activació cerebral després d'una sessió d'EMT. Aquest al.lel s'ha relacionat amb disfuncions de tipus tant metabòlic com d'activació cerebral durant la realització de tasques cognitives, similars als observats en la MA, per tant l'objectiu d'aquest estudi era veure com dos factors, un intrínsec (ser portador de ε4) i una altre extrínsec (EMT), ambdós amb un efecte conegut en l'activació cerebral s'influïen mútuament per modular els patrons funcionals i com això afectava en últim terme l'execució en memòria.

Seguint amb l'estudi de les diferències funcionals entre portadors ε4 i no ε4, ens va interessar determinar els patrons de connectivitat cerebral de l'hipocamp en aquests dos grups de subjectes, amb queixes de memòria, durant l'execució d'una tasca de memòria associativa.

Finalment, després d'haver observat les diferents graus de manifestacions clíniques en subjectes amb una neuropatologia equiparable o les diferències en patrons d'activació causades per un emergent patologia cerebral vam considerar l'estudi d'aquestes diferències individuals (descrites com a factors de reserva cognitiva) per investigar els seus efectes en la funció i estructura cerebral de subjectes envellits pertanyents a diferents categories clíniques (envelliment normal, ACL i MA).

En resum, els objectius específics de la tesi es podrien concretar en els següents punts:

1) Estudiar els efectes de l'Estimulació Magnètica Transcranial (EMT) en l'activitat cerebral i el rendiment cognitiu durant una prova d'aprenentatge visual en una mostra de pacients envellits amb queixes de memòria.
2) Estudiar els efectes de la interacció entre l'EMT i el genotip de l'APOE en una mostra de pacients amb queixes de memòria.
3) Estudiar com el fet de tenir un determinat genotip del polimorfisme de l'APOE podria afectar els patrons d'activitat i connectivitat cerebrals mentre es realitza una tasca d'aprenentatge en pacients amb alteració cognitiva relacionada amb l'edat.
4) Estudiar la influència de les variables de reserva cognitiva en l'estructura cerebral per tal de provar la hipòtesi de la reserva cerebral, així com investigar com un determinat nivell de reserva cognitiva pot influenciar els patrons d'activitat cerebral per tal d'explorar els models actius (compensació) en l'envelliment normal, ACL i MA inicial.

Metodologia

La present tesi consisteix en quatre estudis, els quals han requerit diferents mètodes i tècniques àmpliament emprades en neurociències. Per començar, una exhaustiva avaluació neuropsicològica ha estat essencial per un adequat diagnòstic i selecció de la mostra o mostres que haurien de participar en els diferents estudis. A més, les tècniques de neuroimatge funcional escollides (RMf) han estat necessàries per investigar com el cervell treballa, a partir de la resposta hemodinàmica d'aquest. La tècnica de l'EMT també ha estat emprada entre dues sessions de RMf en dos dels tres estudis per tal d'observar els seus efectes en l'activació cerebral i la funció cognitiva en gent gran amb queixes de memòria.

Així mateix, la tècnica multivariant de l'anàlisi de la coherència es va utilitzar per identificar xarxes de connectivitat funcional amb l'hipocamp. Finalment, variables de reserva cognitiva han estat mesurades i posades en relació amb patrons d'activitat i estructura cerebrals en mostres d'envelliment patològic (ACL i MA) i no patològic (envelliment normal) de cara a estudiar en quina mesura determinada càrrega de reserva cognitiva pot afectar la manera en com treballa el cervell i el grau d'atròfia d'aquest en les diferents condicions clíniques d'envelliment normal i patològic mencionades anteriorment.

Resultats

En el primer estudi vam demostrar una millora en memòria associativa (mesurada per l'aprenentatge en l'associació cares-noms durant una sessió de RMf) només en aquells subjectes majors de 50 anys amb queixes subjectives de memòria que rebien una sessió d'EMT a alta freqüència (n=20). Tanmateix, en un altre grup de participants amb les mateixes característiques cognitives però sense ser subjectes a un sessió real d'EMT, és a dir el grup placebo (n=20), no s'evidenciava cap canvi significatiu. La interacció entre les condicions d'EMT (real vs. placebo) i sessions d'aprenentatge dins la RMf (abans vs. després de la sessió EMT) fou mesurada amb una ANOVA de mesures repetides, resultant aquesta significativa ($F = 7.15$, $df = 1$, $p=0.01$). A més, aquesta millora en el rendiment en memòria es va acompanyar d'una addicional activació cerebral en regions frontals dretes i bilaterals posteriors en la sessió de RMf després d'haver estat adminsitrada l'EMT (girs frontals mig i inferior drets, coordenades de Talairach [42,40,14] i [52,20,-4], $t=5.12$, $p=0.001$ i $t=5.99$, $p<0.001$, respectivament; així com gir occipital superior bilateral, coordenades de Talairach [-36,-62,34] i [38,-52,40], $t=7.40$, $p<0.001$ i $t=6.01$, $p<0.001$, respectivament). Aquest estudi demostra per tant, que l'EMT a alta freqüència és capaç de produir un efecte transitori i positiu en l'activitat cerebral i cognició en una mostra de pacients amb queixes de memòria.

En un segon estudi vam investigar l'efecte del polimorfisme APOE sobre l'activació cerebral i el rendiment cognitiu en els 20 pacients en els quals en l'estudi anterior s'havia estimulat amb EMT. Nou casos eren portadors de la variant ε4 del gen de l'APOE i la resta no portadors. Tot i ser comparables en edat, distribució de gènere i nivell cognitiu global (MMSE), inclòs en proves específiques de memòria, les anàlisi realitzades amb una t de mesures repetides van demostrar que només aquells pacients portadors de l'al.lel ε4

milloraven significativament l'execució en la mencionada tasca respecte els no portadors (t=3.04, p=0.02). El més rellevant però, va ser observar que els subjectes poradors de l'al·lel ε4, a més a més activaven més regions corticals prefrontals dretes en la sessió de RMf immediatament a l'aplicació de l'EMT (gir frontal mig, coordenades de Talairach [30,2,37], t=9.47, p=0.001 i gir frontal inferior, coordenades de Talairach [36,25,-10], t=10.15, p=0.036), mentre que els subjectes no portadors activaven exclusivament regions de l'escorça posterior (gir supramarginalis dret, coordenades de Talairach [48,-56,44], t=7.23, p=0.001 i gir lingual esquerre, coordenades de Talairach [-36,-64,36], t=6.66, p=0.036). Aquests resultats indiquen que la càrrega genètica en relació al gen de l'APOE modula la resposta cerebral davant l'administració d'una sessió d'EMT.

En un tercer estudi vam avaluar la connectivitat funcional de l'hipocamp durant una tasca d'aprenentatge associatiu nom-cara en subjectes majors de 50 anys amb queixes subjectives de memòria, separant dos grups segons el gen de l'apolipoproteïna E (portadors de l'al.lel ε4 versus no portadors). Per tal d'analitzar la connectivitat funcional vam utilitzar el mètode de l'anlàlisi de la coherència, el qual mesura la relació entre dues senyals indicant el grau d'associació lineal temporal entre una regió origen (anomenada *seed* o llavor) i altres àrees del cervell. Aquesta funció seria propera a 0 amb absència de relació lineal o propera a 1, mostrant que la regió '*x*' (seed) prediu perfectament '*y*' (un determinat voxel d'activació). Així, regions cerebrals amb un gran valor de coherència poden ser considerades com àrees que pertanyen a la mateixa xarxa neuronal (Müller et al., 2003). L'estudi emprant aquest mètode va evidenciar que l'hipocamp esquerre dels subjectes portadors de l'al.lel ε4 presentava una major coherència amb diverses àrees de l'hemisferi ipsilateral: cingulat anterior (àrea de Brodmann 32, t=5.28, p<0.001), una àrea que s'extenia des del parietal inferior (àrea 40) al gir postcentral (àrea 3, t=4.28, p<0.001) i el nucli caudat (t=4.22, p<0.001). Aquest estudi aporta noves evidències de que els subjectes amb la variant ε4 tenen un patró d'activació particular respecte els no portadors, quan s'enfronten a determinades tasques cognitives (Bookheimer et al, 2000; Smith et al, 2002; Bondi et al., 2005; Lind et al., 2006; Han et al., 2007; Trivedi et al., 2006). D'aquesta manera, un "esforç" addicional expressat en termes d'activació cerebral de les connexions hipocàmpiques és requerida per una codificació mnèsica, la qual no obstant no dóna lloc a una execució conductual equivalent als subjectes no portadors de ε4 (els subjectes portadors d'aquesta variant alèl·lica varen rendir pitjor en la prova d'aprenentatge en el context de la RMf).

Finalment, la influència de variables de reserva cognitiva (RC) com l'escolaritat, la ocupació, la intel·ligència premòrbida, la participació en activitats d'oci, socials i educació continuada, en l'estructura i funcionament cerebral, queda palesa en el nostre tercer estudi. En concret, vam estudiar com aquests factors interactuen amb l'envelliment i els possibles processos patològics cerebrals per donar lloc a un determinat funcionament cognitiu. Quaranta-quatre subjectes majors de 60 anys (16 sense cap tipus de deteriorament cognitiu, 12 amb ACL i 16 amb criteris de MA lleu) van ser seleccionats i per realitzar una tasca de memòria visual en el context d'una sessió de RMf. L'aprenentatge fou avaluat mitjançant un test de reconeixement visual immediatament després de la finalització de la RMf. Un primer anàlisi realitzat entre variables de RC i grau d'atròfia cerebral (mesurat pel volum cerebral) va demostrar correlacions negatives significatives entre un índex d'intel·ligència premòrbida (Subtest Vocabulari, WAIS III) i el volum cerebral per ambdós grups d'alteració cognitiva ($r=-0.69$, $p<0.03$ pel grup ACL i $r=-0.54$, $p<0.05$ pel grup amb MA), contrastant amb una correlació positiva entre aquests mateixos factors en el grup sense patologia (controls). Els anàlisis van demostrar correlacions negatives entre el nivell de RC en el grup sense deteriorament cognitiu i l'activació cerebral en regions frontals, temporals i cerebel (gir frontal inferior dret [46,2,30], $t=9.03$, $p<0.0005$; gir frontal mig dret [24,12,44], $t=6.09$, $p=0.022$; gir parahipocàmpic esquerre [-14,-36,-2], $t=8.71$, $p=0.018$; cerebel [-40,-62,-32], $t=6.09$, $p=0.046$; gir precentral esquerre [-30,-18,50], $t=7.95$, $p=0.003$). En canvi, les correlacions amb el grup de MA van ser positives; és a dir el nivell de RC en aquest grup estava directament relacionat amb l'activitat cerebral en el cingulat anterior i posterior drets ([0,6,34], $t=6.82$, $p=0.045$ i [18,-60,6], $t=6.29$, $p=0.01$, respectivament) així com una en el gir temporal superior esquerre, tot i que en aquest darrer cas no arribava a ser estadísticament significatiu ([-64,-28,8], $t=7.69$, $p=0.08$).

Discussió general

El nostre primer estudi demostrava la viabilitat de l'EMT per facilitar l'execució en aprenentatge associatiu de forma transitòria en una mostra d'adults amb queixes de memòria, efecte que succeïa a la vegada que els canvis en patrons d'activació cerebral. Específicament, regions frontals dretes foren addicionalment activades en aquells subjectes que rebien una estimulació real, sent aquestes responsables d'un patró d'activació prefrontal més simètric. Degut a que la lateralització del lòbul prefrontal es veu reduïda durant l'envelliment (Cabeza, 2002), un increment de bilateralitat mostrat en aquest estudi podria

estar afrontant el dèficit cognitiu en l'envelliment i per tant podria ser considerat compensatori. El fet de mostrar un patró bilateral del lòbul prefrontal s'ha vist relacionat amb una millor execució cognitiva en adults en tasques de memòria de treball (Reuter-Lorenz, 2000). A més, compensacions a nivell conductual en l'envelliment s'han associat freqüentment amb un increment d'activitat del lòbul prefrontal (Grady, 2007). Tot i que el mecanisme que estaria mediant la funció cerebral i la millora en memòria és poc clar (Grady et al., 2006), la recuperació transitòria d'aquesta simetria frontal s'assemblaria als patrons d'activitat que tenen els joves adults i a la vegada amb una millora en memòria (Wagner et al., 1998; Stebbins et al., 2002). El fet de que el lòbul prefrontal dret presenti una major activitat després de la sessió d'estimulació podria estar indicant un increment de la funció atencional i de la dificultat en la tasca, tal i com s'ha observat anteriorment (Sunaert et al., 2000).

En un segon estudi, quan vam introduir la variable genètica ε4 de l'al.lel de l'apolipoproteïna E, vam trobar un efecte diferencial de l'EMT. En aquells subjectes amb l'al.lel ε4, l'activitat cerebral en l'escorça prefrontal es va veure incrementada i a més va ser acompanyada d'una millora cognitiva. Aquelles regions frontals que oferien una activació addicional després de la sessió d'EMT eren les àrees contralaterals activades en la primera RMf, fet que mostra un patró de major simetria frontal després de l'estimulació. Per contra, que en els subjectes portadors de la variant ε3 van presentar un patró d'activació més posterior després de la sessió real d'EMT, millorant també a nivell conductual. En aquest estudi es va posar de manifest com una variable biològica podia modular la resposta cerebral en l'envelliment de forma diferencial davant l'efecte de l'estimualció magnètica. Estudis previs han mostrat una reducció del metabolisme de la glucosa en àrees parieto-temporals en subjectes sans portadors d'ε4 (Reiman et al., 2001, 2005; Small et al., 1995, 2000), posant de manifest canvis neuropatològics incipients en aquestes regions. Per tant, les nostres dades també semblen revelar que la resposta cerebral després d'una sessió d'EMT està mediatitzada per aquelles regions probablement més preservades.

A més de les troballes cognitives i funcionals en els individus portadors de l'ε4 derivades de l'estudis anterior, també vam observar que l'hipocamp dels subjectes adults amb queixes de memòria i amb aquesta variant al.lèlica desenvolupa connexions cerebrals diferents a les dels no portadors per tal d'efectuar una tasca de memòria associativa. El caudat, cingulat anterior i parietal inferior ipsilaterals foren les regions que conformaven

una xarxa addicional amb l'hipocamp esquerre dels pacients ε4. En l'estudi anterior es va observar un augment d'activitat cerebral degut a l'efecte de l'EMT que va ser considerada eficient, degut a que es derivava en una millora cognitiva; però en aquest cas l'activitat extra només observada en els subjectes portadors de la variant genètica de risc sembla mostrar una compensació no eficaç, ja que denota un major esforç cognitiu quan es compara amb els no portadors però amb pitjor execució conductual. Per tant, si bé el diferent patró de connexió de l'hipocamp en pacients ε4 podria indicar l'existència de mecanismes cerebrals compensatoris, una compensació a nivell conductual es veuria dificultada per disfuncions bàsiques en els seus sistemes de memòria, ja que es tracta de població amb un afectació mnèsica. Per tant, sembla que les sobreactivacions frontals observades en el segon estudi en subjectes portadors de la variant ε4 es traduirien en una millora cognitiva. No succeeix el mateix però quan es té en compte l'activació addicional derivada de circuits hipocàmpics. Estudis futurs emprant tècniques d'estimulació i de connectivitat funcional de xarxes prefrontals-hipocàmpiques haurien d'ajudar a determinar com aquesta variant genètica influencia les interaccions entre ambdues regions i com això afecta la cognició.

Finalment, quan s'introdueixen variables extrínseques, definides com a indicadors de reserva cognitiva (RC), l'activitat i estructura cerebral també es va veure influenciada. Hem evidenciat com en l'envelliment sa amb nivells baixos de RC es necessita activar més regions cerebrals per tal d'efectuar una tasca de memòria visual al mateix nivell que altres subjectes amb major RC, mostrant així un patró similar d'activitat cerebral menys eficient amb els subjectes ε4. Per tant, tant variables biològiques com ambientals es relacionen amb l'eficàcia cerebral en termes de quantitat d'activitat cerebral requerida durant tasques cognitives així com els patrons de connectivitat cerebral. En el cas dels subjectes sans amb alta RC, aquests activen menys regions cerebrals durant la realització de la tasca però rendeixen de forma similar als de baixa reserva denotant major efectivitat en el processament cognitiu. Probablement aquesta major efectivitat està en part relacionada amb un cervell més preservat anatòmicament, ja que en aquest grup de persones sanes hi ha correlacions positives entre la RC i els volums cerebrals.

D'altra banda, en el cas dels pacients amb MA lleu, les correlacions negatives entre la RC i l'activació cerebral durant les tasques cognitives també apunten cap a un cervell amb més capacitat de processar informació ja que no cal perdre de vista que en aquest cas, els pacients amb més RC presenten majors atròfies cerebrals. Així, tot i presentar

probablement un procés neuropatològic més avançat, mitjançant activacions superiors el cervell d'aquests pacients permet rendir comparativament als pacients amb menys reserva (no hi havia diferències en la prova de memòria). En aquest sentit, es podria parlar de compensació tal i com va ser descrita per Stern (2005). Aquestes dades, obtingudes per primera vegada emprant RMf, també recolzen les hipòtesi 'clàssiques' de la RC descrites per Katzman i col·laboradors (1993), indicant que hi hauria un llindar individual de tolerància al dany neuropatològic el qual és sobrepassat més tard per subjectes amb alta RC. En altres paraules, es requeriria un major dany cerebral per manifestar símptomes clínics en subjectes amb alta RC. A banda dels resultats derivats de la mostra de MA, vam ser els primers en evidenciar que els subjectes amb alteració cognitiva lleu (ACL) amb major RC presentaven un patró de dany cerebral similar. Aquesta mateixa direccionalitat en les relacions entre RC i dany cerebral estructural trobats en MA i ACL s'hauria de tenir en especial consideració ja que aquesta última condició ha estat descrita com d'alt risc per demència (Petersen et al., 2001) o MA incipient (Morris et al., 2001). Degut a que els estudis longitudinals han demostrat que entre els casos de MA, aquells amb major RC experimenten un declivi cognitiu més accelerat (per tant reflectint un estat neuropatològic més avançat; ex. Scarmeas et al., 2006), les nostres dades indiquen que això també podria ser aplicat a l'ACL. Així, s'haurien d'adreçar més estudis longitudinals per tal d'investigar si la RC pot ser considerada una variable més a tenir en compte en el curs clínic evolutiu de l'ACL.

Totes les troballes derivades d'aquesta tesi evidencien que hi ha factors extrínsecs i intrínsecs capaços de modular la funció cerebral en la població envellida amb queixes de memòria però també en casos de pacients amb demència. Variables intrínseques o biològiques a tenir en consideració seria l'aportació genètica, com la presència o absència de l'al·lel ε4, fet que podria estar afectant les connexions dels lòbul temporal medial (LTM) necessàries per afrontar tasques de memòria així com prioritzant un patró d'activació cerebral més anterior quan la resposta cerebral es veu modificada per l'EMT a alta freqüència. Els factors extrínsecs o ambientals per contra, estarien lligats a variables de RC com l'educació, ocupació, formació contínua, activitats d'oci i intel·lectuals estimulants; aspectes que conferirien una protecció contra el dany cerebral i, en cas de que aquest estigués present, se'n faria necessària una major quantitat per tal de que es manifestés clínicament.

Conclusions

1) Una sessió d'EMT a alta freqüència permet modular la funció cerebral i millorar l'aprenentatge de forma transitòria en subjectes envellits amb queixes de memòria relacionades amb l'edat. Degut a que una millora en memòria fou associada amb un increment d'activitat en l'escorça prefrontal dreta després de l'estimulació, vam concloure que l'EMT facilitava l'activació de mecanismes cerebrals compensatoris prèviament descrits en estudis de neuroimatge funcional en l'envelliment.

2) Subjectes portadors de la variant ε4 del gen APOE mostraven majors activacions de regions frontals dretes en resposta a la EMT comparats amb els portadors de la variant ε3, els quals presentaven un increment d'activitat cerebral posterior. Aquest resultat mostra una primera evidència que l'aportació genètica modula la resposta cerebral davant l'estimulació externa, probablement reflectint una major facilitat de resposta per part del cervell en les regions amb menor afectació neuropatològica.

3) Pacients amb alteració en memòria i presència de l'APOE ε4 mostraven una major activitat en estructures corticals i subcorticals que estableixen una connexió amb l'hipocamp en una tasca de memòria associativa nom-cara, quan eren comparats amb individus clínicament semblants però sense l'al.lel ε4. Es conclou que les estructures del LTM són menys eficients en els primers i que per tant una major connectivitat amb altres regions cerebrals és necessària com a mecanisme compensatori, encara que no s'arribi a assolir una execució en memòria tan satisfactòria com els subjectes no ε4.

4) Adults sans amb alta RC tenen un major volum cerebral i utilitzen les xarxes cerebrals de forma més eficaç davant una tasca de memòria. Per contra, pacients amb MA inicial amb alta RC presenten major atròfia cerebral però activen més regions que els pacients MA amb menor RC, aconseguint ambdós grups nivells equivalents d'execució en memòria. Els resultats derivats de pacients ACL són similars als individus amb MA en termes dels anàlisis estructurals. Es conclou que en els subjectes amb alteració en memòria i estadis inicials de demència, la RC es relaciona amb la integritat

estructural cerebral i permet utilitzar els circuits cerebrals més eficaçment davant esforços cognitius.

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ANNEX

Qüestionari sobre variables relacionades amb la reserva cognitiva

Nom i cognoms:

Any de naixement:

Data de l'exploració:

EDUCACIÓ / CULTURA

1. Anys totals d'escolarització formal:

0. no esc. / 1. primaris (fins 8 anys) / 2. secundaris (8-12) /3. superior (>12)

2. Tipus d'escola al que anava (encerclar): pública / privada religiosa / laica

3. Li han comentat alguna vegada que li va costar molt o bé li va costar molt poc aprendre a llegir o a escriure?

0. li va costar / 1. normal / 2. li va costar poc

4. Del 0 al 10 quina creu que era la seva mitjana de notes durant l'etapa d'escolaritat?

5. Actualment està realitzant algun curs en alguna escola o universitat?. Anotar-ho.

0. no / 1. sí

6. Els seus pares tenien estudis? A casa de petita hi havia un ambient 'culte' (aficions literàries, artístiques, musicals)?

0. no / 1. algun d'ells amb estudis normal / 2. Algun d'ells amb estudis superiors

7. Ha après algun idioma? (apart del català o castellà?)

0. cap / 1. algun coneixement / 2. bon coneixement d'un idioma / 3. bon coneixement de 2 o + idiomes estrangers

ACTIVITAT PROFESSIONAL

1. Quan estava actiu laboralment quina era la seva professió o professions? Anotar-les.

0. no qualificat manual / 1. qualificat manual / 2. qualificat no manual, secretariat o tècnic (requereix formació específica no superior) / 3. professional (requereix estudis superiors) / 4. directiu

ACTIVITAT INTELECTUAL I D'OCI

1. En la seva infantesa i joventut llegia habitualment? Si respon que sí intentar estimar el nombre d'hores setmanals:

0. no habitualment o esporàdicament / 1. habitualment (> 3h/setm)

2. Actualment llegeix? Si respon que sí intentar estimar el nombre d'hores setmanals:

0. no habitualment o esporàdicament / 1. habitualment (> 3h/setm)

3. Ha après a tocar algun instrument musical?

Si respon que sí preguntar: Quin?

Va estudiar música al conservatori o acadèmia o el va aprendre a tocar 'd'oïdes'?

0. No / 1. N'ha après i actualment també el sabria tocar

4. Vol destacar algun altre tipus d'activitat que consideri 'cognitiva' o 'intel·lectual' que hagi desenvolupat al llarg de la seva vida (ex. escriure poesies, novel·la...)?

5. Vol destacar algun altre activitat d'oci que hagi practicat al llarg de la seva vida?

ACTIVITAT FÍSICA

1. Durant la seva infantesa i joventut practicava esport? Si respon que sí, anotar quina era i intentar quantificar la mitjana d'hores setmanals:

1. Sí / 0. No

2. Actualment practica alguna activitat esportiva (incloure caminar habitualment)? Si respon que sí, anotar quina és i intentar quantificar la mitjana d'hores setmanals:

1. Sí / 0. No

ACTIVITATS SOCIALS

1. Durant la seva vida ha tingut molts amics?

0. pocs / 1. normal / 2. molts

2. Com definiria el grau d'implicació en d'activitats socials (inclou sortir amb amics, participar en associacions, tasques de voluntariat etc.) que ha realitzat al llarg de la vida?

0. baix / 1. normal / 2. alt

3. Actualment quin és el seu grau d'implicació en activitats socials?

0. baix / 1. normal / 2. alt