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Thesis

Caracterización de personas con alto riesgo para el desarrollo de demencia mediante técnicas de neuroimagen

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Resumen

La demencia es un síndrome caracterizado por la pérdida de capacidades intelectuales que acaban afectando a las actividades diarias de las personas que lo sufren. Normalmente es un proceso crónico y progresivo cuyas consecuencias varían desde los estadios intermedios, donde empieza a afectar a ciertas funciones, hasta los estadios severos, donde la persona depende por completo de terceros para realizar las actividades diarias. Entre las muchas causas de demencia, como los cuerpos de Lewy o la demencia vascular, la enfermedad de Alzheimer (AD, por sus siglas en inglés) es la causa más común de demencia.

Como proceso neurodegenerativo, AD evoluciona lentamente y empeora a lo largo de los años. Más aún, la neuropatología asociada a AD podría empezar décadas antes de que se observen los primeros síntomas neuropsicológicos. La progresión de AD ha sido dividida en tres fases basadas en la evolución de ciertos aspectos neuropsicológicos: la fase sin deterioro cognitivo, donde algunas personas pueden llegar a reportar quejas subjetivas de memoria; la fase de deterioro cognitivo, donde las personas pueden realizar actividades diarias de manera independiente pero ciertos problemas cognitivos aparecen en actividades más complejas; la fase de demencia, donde el deterioro afecta gravemente a la vida diaria de las personas y éstas dejan de ser autosuficientes.

Durante décadas, la comunidad científica ha propuesto distintos modelos de progresión de AD y esta progresión ha sido caracterizada usando distintas técnicas, como la tomografía por emisión de positrones, la resonancia magnética, la electroencefalografía o la magnetoencefalografía. En los últimos años, ha habido un creciente interés por caracterizar las fases preclínicas de la enfermedad de Alzheimer. Este interés está motivado, en parte, por la necesidad de aplicar tratamientos o estrategias que ralenticen la progresión de la enfermedad antes de que se haya producido un deterioro severo de las capacidades cognitivas.

Así, la tesis que presento se engloba en el campo de la neurociencia de detección temprana del Alzheimer. El principal objetivo de la tesis es el estudio y caracterización del deterioro temprano, si hubiera alguno, debido a AD en una población joven y cognitivamente sana con un alto riesgo de desarrollar demencia. Esta tesis se compone de tres estudios que evalúan el efecto de este riesgo incrementado en distintas y esenciales características del cerebro:

- 1.- El primer estudio evalúa la conectividad funcional.
- 2.- El segundo estudio evalúa la conectividad estructural (o anatómica).
- 3.- El tercer estudio evalúa la relación entre conectividad funcional y estructural y sus posibles alteraciones.

Abstract

Dementia is a syndrome characterized by the loss of intellectual abilities to the extent that it interferes with daily life activities. It is usually a chronic and progressive process that ranges in severity from the mildest stage, when it is just beginning to affect a person's functioning, to the most severe stage, when the person must depend entirely on others for daily activities living. Among the different causes of dementia, such as Lewy body dementia or vascular dementia, Alzheimer's disease (AD) is the most common cause of dementia.

As a neurodegenerative process, AD develops slowly and gets worse over the years. Furthermore, the neuropathology associated with AD might start decades before the first neuropsychological symptoms are noticed. The AD continuum has been divided into three stages based on the evolution of different neuropsychological aspects: the cognitively unimpaired stage, where a subset of cognitively unimpaired individuals may report subjective cognitive decline; the mild cognitive impair stage, where the individuals perform daily life activities independently, but the cognitive difficulty may impact on the more complex activities of daily life; the dementia stage, where the impairment results in an evident functional impact on daily life and the individual is no longer entirely independent.

For decades, the scientific community has proposed AD progression models, and this progression has been characterized using different techniques, such as positron emission tomography, magnetic resonance imaging, electroencephalography, or magnetoencephalography. In recent years, there has been an increased interest in characterizing the preclinical stages of AD. This interest is partly motivated by applying treatments or strategies to slow the AD progression before severe cognitive abilities impairments are produced.

Thus, the thesis presented here is framed in this neuroscience field of early detection of AD. This thesis's main objective is to study and characterize early impairments, if any, due to AD in a young cognitively healthy population at increased risk of developing dementia. This thesis comprises three studies that evaluate the effect of this increased risk in different brain features:

- 1.- The first study evaluates the functional connectivity;
- 2.- The second study evaluates the structural (or anatomical) connectivity;
- 3.- The third study evaluates the association between functional and structural connectivity and its possible alterations.

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Acronyms

AAL	Automatic anatomical labeling
A β	Amyloid-beta
ACC	Anterior cingulate cortex
AD	Alzheimer's disease
ADC	Apparent diffusion coefficient
ADiff	Axial diffusivity
APOE	Apolipoprotein
BEM	Boundary element method
CBPT	Cluster-based permutation test
ciPLV	Corrected imaginary phase-locking value
CNS	Central nervous system
CSF	Cerebrospinal fluid
DMN	Default mode network
DTI	Diffusion tensor imaging
DWI	Diffusion-weighted imaging
E/I	Excitation/inhibition
EEG	Electroencephalography
FA	Fractional anisotropy
FC	Functional connectivity
FDR	False discovery rate
FH	Family history
fMRI	Functional magnetic resonance imaging
GM	Grey matter
HPI	Head position indicators
IC	Independent component
ICA	Independent component analysis
LCMV	Linear constraint minimum variance
NIA-AA	National Institute on Aging and Alzheimer's Association
NoS	Number of streamlines
MDiff	Mean diffusivity
MCI	Mild cognitive impairment
MEG	Magnetoencephalography
MIC	Maximal information coefficient
MINE	Maximal information-based nonparametric exploration
MLR	Multiple linear regression

MMSE	Mini-Mental State Examination
MNI	Montreal Neurological Institute
MRI	Magnetic resonance imaging
MSR	Magnetically shielded room
NFT	Neurofibrillary tangles
PCA	Principal component analysis
PLV	Phase-locking value
PNS	Peripheral nervous system
RDiff	Radial diffusivity
RFP	Radio-frequency pulse
ROI	Region of interest
RSN	Resting-state network
SCD	Subjective cognitive decline
SOBI	Second-order blind identification
SQUID	Superconducting quantum interference devices
T1WI	T1-weighted imaging
TE	Echo time
TIC	Total information coefficient
TR	Repetition time
tSSS	Spatiotemporal signal space separation method
WM	White matter

Chapter 1

Introduction

This thesis has been conducted in the framework of the national research project “*Cognitive and neurophysiological characteristics of people at high risk for developing dementia: a multidimensional approach.*” (PSI2015-68793-C3-1-R). This project's main objective is to characterize the preclinical stages of Alzheimer’s disease and, based on these results, define features for the early detection of Alzheimer’s disease. This project's participants are cognitively healthy people, young for the average onset of the disease, and different factors related to the development of Alzheimer’s disease (AD). This project is the first step of a longitudinal study, where the participants of the project will repeat all the procedures two years after the first time they participated.

In line with the project's global objective, the thesis's main objective is to characterize AD in its preclinical stages using neuroimaging techniques. Three different studies were designed regarding the human brain's three vital aspects: functional connectivity, structural connectivity, and the relationship between them. We hypothesized that early signs of “deterioration” due to Alzheimer’s disease might be present decades before the symptoms' onset, and these signs might be measured using state-of-the-art neuroimaging techniques.

This thesis can be conceptually divided into an introduction (Chapter 1), the studies performed (Chapters 2, 3, and 4), and a general conclusion (Chapter 5). The introduction contains the information necessary to put the thesis in context and for the reader to understand the studies and results presented. The introduction elaborates on the brain's anatomical characteristics and the cognitive brain functions, AD, and the neuroimaging techniques used in this thesis. Finally, the introduction summarizes the project's details, such as the number of participants or the battery of tests performed with each participant. The functional study, the structural study, and the study evaluating the association between both are presented in Chapters 2, 3, and 4, respectively. To conclude, Chapter 5 elaborates a general discussion gathering all the discussions and results presented in the thesis.

1.1. The brain

The brain is one of the most critical organs in the human body, and yet, it is the most “unknown” one. The literature estimates that this structure is composed of 10^{11} neurons and $1.8 - 3.2 * 10^{14}$ synapses. Although an adult's average brain weight is around 1.4 kg, 2% of the body weight, the brain consumes around 20% of the body's energy. This chapter introduces the brain's main anatomical concepts, vital for this thesis, and presents different theories about how high-level cognitive functions arise from the brain's anatomical structure.

1.1.1. Neuroanatomical bases

The nervous system is a highly complex structure of the human body responsible for voluntary and involuntary actions and responsible for transmitting signals between different parts of the body. The nervous system consists of two main parts: the central nervous system (CNS) and peripheral nervous system (PNS). The PNS is composed of nerves that connect the CNS to the rest of the body. The nerves that transmit information from the body to the CNS are called sensory or afferent nerves, while the nerves that transmit information from the CNS to the body are called motor or efferent nerves. The CNS is composed of the brain and the spinal cord. “Central” refers to the CNS function of integrating all the signals received from the body and responding to the different stimuli. The CNS controls various vital functions such as movement, breathing, emotions, or even the heart rate.

Figure 1. Elementary parts of the human brain.

Different anatomic classifications of the human brain.

The brain, the central part of the CNS, is the most complex organ in the human body. It is located inside the skull, covered by the meninges, namely the dura mater, the arachnoid mater, and the pia mater. As an elementary classification, the human brain is divided into the hindbrain, the midbrain, and the forebrain (Figure 1). The lower part of the human brain is called the hindbrain,

and it comprises the cerebellum, the brain stem, pons, and the medulla. The hindbrain regulates fundamental survival functions such as respiration or the heart rate. The midbrain, responsible for eye movement, and visual and auditory processing, is located above the hindbrain. Finally, the upper part of the brain is called the forebrain, and it is composed of the thalamus, hypothalamus, epithalamus, subthalamus, and cerebrum. The cerebrum is the central processing unit, it is related to the vast majority of high-level functions such as memory, language, judgment, among others, and it is the principal interest of this thesis.

Regarding the external structure, the cerebrum is a complex organ divided into two symmetrical hemispheres by the medial longitudinal fissure. Each of these hemispheres is again divided into four lobes: frontal, temporal, parietal, and occipital lobes. Regarding the internal structure, it is vital to describe first the principal “unit” of the brain: the neuron. A neuron is an electrically excitable cell responsible for processing and transmitting an electrochemical signal depending on the input received. An average neuron structure is divided into the cell body or soma, dendrites, axon, and synaptic terminals, all surrounded by a cell membrane (Figure 2). The soma, like other cells, contains the nucleus and other organelles. The dendrites, connected with preceding neurons by the synapses, receive the input from other neurons and transmit the signal to the axon through the cell body. The axon is the conduct responsible for transmitting the information from the soma to the next neuron. Most of the axons are surrounded by a lipid-rich substance, which increases the information transmission rate. On the opposite end of the soma, the axon divides into the axon terminals or synaptic boutons, which store neurotransmitters to transmit the information to the dendrites’ synapse of the posterior neuron.

Without inputs, the neuron is in a ready state called resting membrane potential, with a negative potential of -70 mV because there are more positive ions outside than inside the neuron. When the neuron is perturbed, i.e., the dendrites receive a signal from another neuron, the neuron starts to depolarize. If the perturbation remains and the depolarization reaches a “threshold potential” around -55 mV, the membrane potential rises abruptly and then falls until it reaches the resting membrane potential. This process of depolarization and polarization is called “action potential,” and its amplitude and duration for an average neuron have been characterized (Figure 2). The action potential is sequentially transmitted along the axon until it reaches the synaptic bouton. The neurotransmitters are then released into the synaptic cleft (the space between the axon terminals and the dendrites), provoking a perturbation in the next neuron. The first neuron,

which starts the information flow, is called the *presynaptic neuron*, while the neuron that receives the information is called the *postsynaptic neuron*.

Figure 2. Neuron anatomy and action potentials.

Left panel: the structure of a neuron. The most common type of neuronal communication is presented, where the presynaptic neuron transmits information to the postsynaptic neuron. **Right panel:** the basis of neuronal communication, the action potential. Without stimuli, the neuron is in a steady state with a potential of -70 mV between the inside and outside of the membrane. When stimuli are received through the dendrites, the neuron starts to depolarize and, if the stimuli are strong enough, the membrane potential rises abruptly and then falls until it reaches the resting membrane potential. This process is transmitted along the axon to the terminal boutons. When it reaches the terminal boutons, they release neurotransmitters to the postsynaptic neuron's dendrites, and the process starts again.

The neurons' arrangement in the brain allows differentiating three different parts in the internal brain structure, namely the grey matter (GM), white matter (WM), and the cerebrospinal fluid (CSF). The CSF is a colorless liquid present in the ventricles, the brain's surroundings, and the spinal cord. CSF helps to maintain a constant pressure level within the skull, and it could absorb impacts in the case of head injuries such as concussions. In addition, CSF is involved in the clearance of metabolic waste products. WM refers to a deep (subcortical) brain tissue that contains most of the glial cells and the myelinated axons. These bulks of myelinated axons, known as tracts, support the communication between different brain areas. Depending on the areas that they connect, three kinds of tracts are defined: projection tracts extend between higher and lower brain areas, i.e., between subcortical areas and the GM; commissural tracts connect areas located in different hemispheres through the corpus callosum; association tracts connect areas located within the same hemisphere. Finally, GM is mostly composed of somas, dendrites, synapses, and glial cells, and it is distributed along the external surface of both hemispheres. This external layer of GM is also referred to as the cerebral cortex. The cerebral cortex shape is a convoluted surface where the peaks are called gyri, and the valleys are called sulci. The cerebral cortex comprises six layers numbered with roman numbers, where layer I is the outermost layer (adjacent to the pia mater), and layer VI is the deepest layer (in the interface with the WM). This division in layers is based on the GM physiology,

in which each layer is characterized by its types of neurons, their spatial distribution, and their connections to other layers.

Figure 3. The internal structure of the brain.

The neuronal arrangement allows differentiating three parts in the brain: the grey matter composed of the neuron's bodies or somas; the white matter composed of the neuron's axons; the CSF, a colorless liquid that helps to maintain a constant pressure level within the skull. Besides, the grey matter is classified into layers based on physiology, in which each layer is characterized by its types of neurons, the spatial distribution of the neurons, and its connections to other layers. The right panel presents Cajal's work, explicitly drawing the neuronal arrangement in the human motor cortex (Cajal, 1899).

How the brain addresses the known human high-level cognitive functions or the lobes' role has been an essential topic of discussion in neuroscience and is still in constant debate.

1.1.2. Mapping the cognitive brain functions

The clinical-anatomical correlation method allows brain functions to be inferred by studying the correspondence between clinical manifestations and lesion locations. This approach's validity depends on the level of sophistication of the methodology used for the patient's clinical characterization, the spatial resolution of the anatomical investigations, and the theoretical constructs and hypotheses being tested (e.g., psychological models) (Damasio & Damasio, 1989).

The localizationist view of the brain organization was the first clinic-anatomical model, dated from 1861. In that year, Paul Broca attended a lecture from Ernest Auburtin, who presented an interesting case of a patient conscious with the anterior brain exposed due to an open wound in the forehead. When Auburtin lightly pressed the anterior lobes with a spatula, the patient's speech *"suddenly terminated; a word that had been commenced was cut in two. The faculty of speech reappeared as soon as the compression ceased..."* (Auburtin, 1861). Later that year, Broca admitted to his service a 51-year-old man who had lost the use of speech since he was 30 years old. Broca found a lesion in the left inferior frontal gyrus in the autopsy and associated this speech loss with the lesion (Broca, 1861). Although it was not the first work to associate lesions with specific areas

(for example, Auburtin did it before), it was the cornerstone of localizationism. The localizationism asserted that each brain function is localized to a specific brain region. Thus, a severe lesion in a specific area provokes the loss of a particular cognitive function.

This perspective attracted criticism and a new (and opposite) perspective appeared: holism. The first author to criticize the theory was Jackson, arguing that localization of symptoms does not necessarily imply localization of functions. This argument was incorporated decades later by Henry Head, the leading representative of the holistic perspective (Head, 1920). As a general concept, the holistic approach considers any system's function to be the result of the system working as a whole, so that such function cannot be explained by decomposing the system into modules and studying them separately. This concept means that the brain functions result from different areas distributed over the whole brain, rather than being localized in particular regions or modules within the brain. In this sense, the localization of the brain damage is not so relevant since other regions may take over that area's functions. Instead, the extent of the brain lesions may predict the degree to which higher-order cognitive functions and mental states are disturbed.

As it usually happens in science, a compromising theory tried to overcome the two presented theories' problems: associationism. The first step to the development of this theory was the work of Wernicke. Also interested in language production, he investigated patients with aphasia different from Broca's one: these patients spoke fluently, but their speech made little sense; they had difficulty finding words and produced many non-words (neologisms) along with semantically related word substitutions. He found brain damage in the left hemisphere's posterior superior temporal gyrus in the autopsy of one patient. He concluded that this area of the brain must serve as a second "center" for language in the left hemisphere and the articulatory center in the frontal lobe identified a decade earlier by Paul Broca (Wernicke, 1874). In this sense, the cognitive function results from different brain areas working together (holism), but these areas are localized to specific brain regions (localizationism). In line with this theory, Alexander Luria suggested that one region in the brain can be involved in various higher-order cognitive functions. Most important, the same higher-order cognitive function may even recruit different regions in different instances, depending on the respective psychological and neuronal contexts. After decades of work, he defined a model of the brain functional organization where three functional units cooperate hierarchically to high-order cognitive functions (Lúriya, 1973): the first unit keeps the general cortical tone needed to activate the cerebral cortex, generating a state of alertness; the second unit is formed by the parietal, occipital, and temporal lobes, and is responsible for obtaining, processing, integrating, and

storing sensory information from the environment; the third unit is formed by the frontal lobe, which is in charge of the selection, planning, execution, and direction of a person's pattern of behavior, as well as its evaluation.

With this background and the evolution of various neuroimaging techniques in the past decades, a new theory has been defined, widely accepted across the scientific literature: network neuroscience. Under this theory, the brain is a complex system composed of large-scale networks of distributed and interconnected neuronal populations. These neurocognitive networks' interaction results in all kinds of body processes associated with the CNS, from the heart rate control to high-order cognitive functions such as memory or language. Each of the brain's neurocognitive networks is defined in terms of structure, function, and dynamics (Arbib, Erdi, & Szentagothai, 1997).

Structure refers to the anatomical connectivity patterns between brain areas that provide the structural bases for cognitive function. The structural connectivity is not limited to unidirectional connections from primary sensory to higher-level areas. Instead, a wide range of connections including ascend, descent, and "same-level" connections has been identified, allowing complex interactions between areas such feedback, feedforward, or parallel flow of information (Ledberg, Bressler, Ding, Coppola, & Nakamura, 2007; Petroni, Panzeri, Hilgetag, Kötter, & Young, 2001). The main classification of structural connections in the human brain is subcortical-cortical and cortico-cortical connections (not implying any difference of relevance between these two sub-groups).

Function refers to the co-activation of different areas to perform a cognitive task. Among all the possible combinations of anatomically related areas, some areas activate simultaneously to express a cognitive operation or function. At a local scale, i.e., neuron level, many studies have tried to infer functionality by testing the neurons' response to different stimuli (Mitzdorf, 1988; Shoham & Nagarajan, 2004). At a larger scale, the local function may only be interpreted in terms of its large-scale functional expression, i.e., a given local cortical area network may express activities related to more than one function. Its integrated functional expression may only be achieved when it works alongside interconnected areas within the large-scale network (Bressler & Tognoli, 2006).

Dynamics refers to the evolving patterns of interacting brain areas that express cognitive function in real-time. The activity in an area or node of the network is determined by the external time-varying inputs it receives from other nodes and its internal dynamics. In this line, the node output will be transmitted to the connected areas, influencing their activity. In this context, the brain has been considered a *quasi-stable* system, a system that operates in a specific state, but it

can flexibly change to another state in a matter of milliseconds (Bressler, 1999). This property would allow different brain areas co-activation in the execution of a particular cognitive operation, and it allows the deactivation of the areas when the operation is finished.

All the studies (and their hypotheses) presented in the thesis align with this neurocognitive network theory, mainly focusing on the structural and functional aspects of neurocognitive networks.

1.2. Alzheimer's disease

Dementia is a syndrome characterized by the loss of intellectual abilities to the extent that it interferes with daily life activities. It is usually a chronic and progressive process that ranges in severity from the mildest stage, when it is just beginning to affect a person's functioning, to the most severe stage, when the person must depend entirely on others for daily activities living. Among the different causes of dementia, such as Lewy body dementia or vascular dementia, Alzheimer's disease (AD) is the most common cause of dementia. AD is a neurodegenerative disease named after Dr. Alois Alzheimer. In 1906, Dr. Alzheimer noticed changes in the brain tissue of a woman who had died of an unusual mental illness. Her symptoms included memory loss, language problems, and unpredictable behavior. After she died, he examined her brain and found many abnormal clumps (now called amyloid plaques) and tangled bundles of fibers (now called tau tangles). Nowadays, the amyloid plaques and the tau tangles are considered the hallmarks of AD.

AD develops slowly and gets worse over the years, and the neuropathology might start decades before the first neuropsychological symptoms are noticed (Morris, 2005). Although different stages have been defined for AD, it is considered a continuum rather than three clinically different entities. The National Institute on Aging and Alzheimer's Association (NIA-AA) guidelines provide a formal description of different research stages to have a common framework to work with (Jack et al., 2018). From a neuropsychological perspective, the AD continuum is divided into three stages: cognitively unimpaired, mild cognitive impairment (MCI), and dementia due to AD (Figure 4). Cognitive performance may be abnormal in the cognitively unimpaired stage, based on population norms, but within the range expected for that individual. A subset of cognitively unimpaired individuals may report subjective cognitive decline (SCD) and demonstrate a slight decline in serial cognitive testing. The MCI stage is characterized by evidence of a decline in cognitive performance from the individual's baseline. The individuals in this stage perform daily life activities independently, but the cognitive difficulty may result in a noticeable but mild functional impact on

daily life's more complex activities. In the dementia stage, a progressive cognitive impairment that affects several cognitive domains is observed. This impairment results in an evident functional impact on daily life, and the individual is no longer entirely independent. The dementia stage may be subdivided into mild, moderate, and severe.

Figure 4. AD continuum.

Stages of the AD continuum defined by the National Institute on Aging and Alzheimer's Association. Reproduced from ("2020 Alzheimer's disease facts and figures," 2020).

AD might be divided into two subtypes based on the disease's age of onset: early-onset AD and late-onset AD. Early-onset AD accounts for approximately 1% to 6% of all cases, and the cause is genetic mutations that inevitably lead to early-onset AD development. These mutations are specifically mutations to the β -amyloid precursor protein, presenilin 1, and presenilin 2. On the other side, late-onset AD is believed to be a result of multiple factors (risk factors) rather than a single cause. The most significant risk factors for late-onset AD are age, apolipoprotein (*APOE*) gene, and having a family history of Alzheimer's ("2020 Alzheimer's disease facts and figures," 2020). AD prevalence increased significantly with the population age: 3% of people age 65-74, 17% of people aged 75-84, and 32% of people aged 85 or older have AD. Regarding *APOE*, everyone inherits one of three forms (alleles) of the *APOE* gene — e2, e3, or e4 — from each parent. Those who inherit one copy of the e4 form have about three times the risk of developing Alzheimer's compared with those with two copies of the e3 form, while those who inherit two copies of the e4 form have an eight- to 12-fold risk. Those with the e4 form are more likely to have beta-amyloid accumulation and AD at a younger. Finally, people with a family history of AD, i.e., first-degree relatives who develop AD, are more likely to develop AD, and the risk is higher if they have more than one first-degree relative with AD. The family history risk factor could be conceptualized as a composite factor reflecting the influence of known and unknown susceptibility genes and perhaps non-genetic risk factors that could be passed on through generations, and could even have direct effects on gene expression (Donix, Small, & Bookheimer, 2012).

All the risk factors above cannot be modified, but other risk factors related to lifestyle might reduce the risk of developing AD. The accepted lifestyle risk factors are education, social and cognitive engagement, and cardiovascular disease risk factors such as tobacco and diabetes.

1.2.1. Neuropathological hallmarks of AD

In the preclinical stage of AD, decades before the onset of symptoms, neuropathological processes start. In this section, we will focus on the neuropathologies and their associated biomarkers as listed in the NIA-AA guidelines (Jack et al., 2018), namely amyloid-beta ($A\beta$) accumulation, neurofibrillary tangles (NFT), and cortical atrophy. Other neuropathological processes are related to AD progression, such as cerebral amyloid angiopathy, granulovacuolar degeneration, and Hirano bodies and glial responses (Serrano-Pozo, Frosch, Masliah, & Hyman, 2011).

1.2.1.1. Amyloid-beta accumulation

β -amyloid precursor protein (APP) is a transmembrane protein encoded by a gene in chromosome 21 inside the neuron, and it is transported to the neuron membrane to be processed. The membrane process of APP can be amyloidogenic and non-amyloidogenic (Kang et al., 1987; Koo & Squazzo, 1994). In the non-amyloidogenic pathway, APP is sequentially cleaved in the membrane by two enzymes, namely α -secretase and γ -secretase, resulting in the generation of a long secreted form of APP and carboxy-terminal fragments (p3 and AICD50). The form of APP and p3 are soluble and non-toxic residues secreted outside the neuron. In the amyloidogenic pathway, APP is first cleaved by β -secretase resulting in the generation of a long secreted form of APP and a carboxy-terminal fragment called CTF99/CTF89. CTF99/CTF89 is further cleaved by γ -secretase resulting in the carboxy-terminal fragment AICD50 (kept inside the neuron) and an $A\beta$ fragment. The $A\beta$ fragment is released outside the neuron (Figure 5).

$A\beta$ is a small protein composed of from 39 to 43 amino acids, being the soluble $A\beta$ 40 and the insoluble $A\beta$ 42 the most common forms of $A\beta$. In a normal situation, there is a balance between the production and clearance of the extracellular $A\beta$. If this balance is compromised, increased amounts of soluble and insoluble $A\beta$ start to congregate outside the neuron, leading to $A\beta$ oligomers, fibrils, and eventually $A\beta$ plaques (Cline, Bicca, Viola, & Klein, 2018).

Spatio-temporal evolution of $A\beta$ is under intense discussion, but some authors have defined different phases of $A\beta$ accumulation (Thal, Rüb, Orantes, & Braak, 2002). In this work, the authors define five phases of $A\beta$ accumulation: (1) isocortical; (2) additional all cortical deposits (entorhinal

cortex, hippocampal formation, amygdala, insular, and cingulate cortices); (3) additional involvement of subcortical nuclei including the striatum, basal forebrain cholinergic nuclei, thalamus and hypothalamus, and white matter; (4) involvement of brainstem structures, including the red nucleus, substantia nigra, reticular formation of the medulla oblongata, superior and inferior colliculi; (5) additional amyloid deposits in the pons (reticular formation, raphe nuclei, locus ceruleus) and the molecular layer of the cerebellum Figure 5.

Figure 5. APP processing and A β accumulation.

On the top panel, the two main paths of APP processing are presented. In the amyloidogenic pathway, the APP is sequentially cleaved by β -secretase and γ -secretase. This processing results in non-soluble A β , which starts to aggregate into oligomers in the extracellular space and finally aggregating into the amyloid plaques. The accumulation of A β plaques is depicted at the bottom (Braak & Braak, 1991).

1.2.1.2. Neurofibrillary tangles

In neurons, the axons' transportation of essential biological materials is achieved through specialized filaments known as microtubules. Tau proteins are six soluble protein isoforms encoded by the tau gene in chromosome 17 that promote tubulin assembly into microtubules. Tau can

promote microtubule nucleation, growth, and bundling and significantly reduce microtubules' dynamic instability (Shahani & Brandt, 2002).

Tau is modified post-translationally in several ways in both healthy and pathological conditions, and we are focusing on alterations of tau phosphorylation because of its role in AD. Although the cause of tau hyperphosphorylation is not fully understood, this hyperphosphorylation reduces the tau binding affinity to microtubules, thus disrupting the structural organization and eventually provoking the collapse of the microtubules. Collapsing of the microtubule structure disrupts the transportation mechanism, provoking the degeneration and death of the neuron. Besides, when tau proteins de-attach from the microtubules, they accumulate in pre-tangles, then in filamentous aggregates (called *paired helical filaments* due to their shape), and finally, they assemble into NFTs (Figure 6). These NFTs were first described by Fuller (Fuller, 1907), a disciple of Alois Alzheimer, and it is one of the hallmarks of AD.

Figure 6. Tau hyperphosphorylation.

On the top panel, the formation of neurofibrillary tangles is presented. Inside the axons, tau fibrils (yellow) support microtubules' stabilization, structures needed for essential neuronal functions. Tau fibrils are hyperphosphorylated, provoking the separation of tau from the microtubules. These tau fibrils start to aggregate in oligomers, then in more prominent structures called *paired helical filaments*, and, finally, in NFTs. On the bottom panel, the accumulation of NFTs as defined by (Braak & Braak, 1991).

The spatiotemporal pattern of progression of NFTs has been divided into six stages summarized in entorhinal, limbic, and isocortical (Braak & Braak, 1991). The neurofibrillary degeneration starts in the medial temporal lobe (entorhinal cortex and hippocampus) and spreads

to the associative isocortex, relatively sparing the primary sensory, motor, and visual areas. This process described by Braak and Braak is presented in Figure 6.

1.2.1.3. Cortical atrophy

Although cortical atrophy is not specific for AD, it has been widely studied due to its correlation to the severity of cognitive impairment in AD patients. Besides, this brain atrophy is apparent in AD patients' brains in autopsies (Figure 7). The brain atrophy might start in the media temporal lobe and hippocampi, and it presents more widespread temporoparietal neocortical loss as the disease advances (Whitwell et al., 2008). This progression seems to match the NFT progression described in Section 1.2.1.2.

Figure 7. Cortical atrophy in AD.

Post-Mortem visualization of a healthy brain and an AD patient's brain. The AD brain (in a severe stage) presents mass loss, cortical thinning, and ventricles enlargement.

1.2.2. AD progression models

Since there are two main hallmarks of AD, there are two main models of AD progression: the amyloid hypothesis and the tau hypothesis. It is important to remark that none of them is entirely

accepted and present caveats and deficiencies. There is no hypothesis yet capable of explaining and being consistent with all the AD literature results.

The amyloid hypothesis proposes that the initiating event in AD is related to abnormal processing of A β peptide, leading to the formation of A β plaques in the brain and provoking NFTs formation and eventually cell death (Hardy & Higgins, 1992; Jack et al., 2010). This process occurs decades before the appearance of AD symptoms. Although it is unclear how A β causes neuronal loss and tangle formation, the peptide is known to disrupt calcium homeostasis and increase intraneuronal calcium concentrations. Thus, the APP may induce neurofibrillary tangle formation due to its ability to increase the concentration of intracellular calcium, leading to phosphorylation of tau and the formation of paired helical filaments first and NFTs later. Finally, the model summarizes the cascade of events as measured by classical biomarkers (Figure 8).

Figure 8. Evolution of AD biomarkers according to the amyloid hypothesis.

Evolution of the biomarkers associated with the hallmarks of AD according to the amyloid hypothesis. In this model, A β accumulation, NFTs formation, and cortical atrophy start to present abnormal magnitude years before the onset of clinical symptoms. Reproduced from (Jack et al., 2010).

On the other hand, the tau hypothesis states that tau lesions due to NFTs occur earlier than A β accumulation (Johnson et al., 2016). As mentioned before, hyperphosphorylated tau's aberrant presence induces mis-stabilization of actin, synaptic impairment, and defects in mitochondrial integrity. Therefore, tau pathology causes extensive damage in the cell, such as a transport system, cytoskeletal system, signaling system, and mitochondrial integrity. Although the transmission

mechanism of tau aggregates from cell to cell is unclear, the propagation evolution is defined by the five phases described in Section 1.2.1.2.

Figure 9. Cascading network failure model.

Schematic view of the model relating large-scale brain networks, beta-amyloid, and tau across the AD spectrum. 1) Regions with tau depositions that may experience a processing overload. 2) Shift of the processing overload to the posterior DMN. 3) The posterior DMN becomes overloaded and begins to shift its processing load to other connectivity hubs. 4) This increased load leads to noisier synaptic activity, and alterations in APP processing occur to compensate. The altered APP processing eventually leads to amyloid plaque formation. 5) The uncompensated stress in the original system now leads to a rapid acceleration of the neurodegenerative process. Modified from (Jones et al., 2017).

Finally, we introduce a recent AD progression theory representing a trade-off between the two hypotheses discussed in this section: the cascading network failure model (Jones et al., 2016). This model is in line with this thesis's spirit and the thesis' author's ideas about brain functioning and AD progression. This model's basis is the brain's definition as a complex system where different subnetworks support human cognitive functions. Under this view, neurodegenerative diseases would affect the brain at a locally molecular level, and they would affect the interactions among these subnetworks (Seeley, Crawford, Zhou, Miller, & Greicius, 2009). In this framework, the model incorporates systems-level pathophysiology into existing molecular disease models: in the preclinical phase, regions with tau deposition start to experience a processing overload due to the degenerative tau process; these brain regions shift their preprocessing load to the posterior default mode network (DMN), a core processing hub; the posterior DMN becomes overloaded and begins to shift its processing load to other connectivity hubs; this increased load leads to noisier synaptic activity, and alterations in APP processing that eventually provokes the amyloid plaque formation; the inability to shift additional processing load, the uncompensated stress in the original system

now leads to a rapid acceleration of the previously indolent tau-associated neurodegenerative process (Figure 9). In this loop, local degeneration will provoke functional connectivity impairments that leads to more severe local degeneration. Under this view, functional connectivity patterns are significantly relevant since they not only reflect local degeneration but they are an active part of the neurodegeneration process.

1.3. Brain mapping and neuroimaging

As defined by Society for Brain Mapping and Therapeutics in 2013, brain mapping is the study of the anatomy and function of the brain and spinal cord through imaging, immunohistochemistry, molecular and optogenetics, stem cell, and cellular biology, engineering, neurophysiology, and nanotechnology. As a part of brain mapping, neuroimaging is the representation of structural anatomy, physiologic or metabolic capacities, or functions within the central nervous system through direct or indirect measures. Neuroimaging techniques can be divided into two major categories, namely, functional imaging techniques and structural imaging techniques.

Functional imaging techniques inspect the physiology, functional architecture, and dynamics of the human brain. Different techniques within this category are based on different physiological measures. The most used functional techniques are:

- Functional magnetic resonance imaging (fMRI), which measures the level of oxygen in blood;
- Positron emission tomography, which uses specific radioactive substances to measure different aspects of physiology and metabolism;
- Electroencephalography (EEG), which measures voltage fluctuation in the scalp resulting from the ionic currents produced by neurons;
- Magnetoencephalography (MEG), which measures magnetic fields outside the head also resulting from ionic currents produced by neurons. Due to the scope of this thesis, we will come into more detail in the MEG technique.

Functional imaging techniques can be classified depending on their temporal resolution, spatial resolution, and invasiveness. The temporal resolution defines the time between two images obtained due to either the technique's intrinsic limitations (as in positron emission tomography) or the time required for the second image to add new information (EEG). The spatial resolution is the minimum distance between two elements of an image to be distinguished. Finally, a technique's

invasiveness measures the discomfort caused to a subject and the risk associated with a technique. The temporal and spatial resolution and the invasiveness of the most used functional neuroimaging techniques are summarized in Figure 10.

Figure 10. Temporal and spatial resolution and invasiveness of functional neuroimaging techniques.

A visual summary of the temporal and spatial resolution and the invasiveness of the most used functional neuroimaging techniques. Reproduced from (Bruña, 2019).

Structural imaging techniques inspect different aspects of the anatomy and structure of the central nervous system. The main structural imaging techniques are computerized axial tomography, based on X-rays to obtain the measures, and magnetic resonance imaging (MRI), based on the different human body tissues' different magnetic properties. Due to the scope of this thesis, we will deepen the MRI technique.

1.3.1. Magnetoencephalography

Magnetoencephalography is one of the most recent functional imaging techniques when, in 1968, David Cohen obtained the first human MEG recording using a shielded room and a copper induction coil (Cohen, 1968). This first recording presented excessive magnetic noise, and it was not useful for further investigations. In 1972, David Cohen performed a new MEG recording using a better shielded-room and one superconducting quantum interference devices (SQUID) magnetometer (Cohen, 1972). In Cohen's own words, this new system for brain activity measurement "*shows promise both as a clinical and as a research tool for studying the brain.*" This first (and valid) MEG recording resembles the EEG recordings obtained until that time Figure 11.

Since then, MEG has been used to study the vast majority of neuropsychological disease, as schizophrenia (Siekmeier & Stufflebeam, 2010), autism (Marco, Hinkley, Hill, & Nagarajan, 2011), depression (Alamian et al., 2017), pre-surgical epilepsy (Lau, Yam, & Burneo, 2008) and, particularly relevant for this thesis, Alzheimer's disease (Engels et al., 2017). In fact, in our laboratory (Cognitive and Computational Neuroscience Laboratory), there is an entire line of investigation of normal aging and AD. In the lab, MEG activity has been characterized for all the stages of the AD continuum, namely AD (Fernández et al., 2002; Maestú et al., 2001), MCI (Bajo et al., 2010; Pusil et al., 2019), SCD (David López-Sanz, Garcés, et al., 2017; Serrano et al., 2020), and the population at risk such as first-degree relatives of AD (Ramírez-Toraño et al., 2021).

Figure 11. First MEG recordings.

In panel A, Cohen described the layout of the first MEG recording schematically. Panel B shows simultaneous MEG (top) and EEG recordings (bottom) from the same subject. Reproduced from (Cohen, 1972).

In summary, the MEG technique measures the magnetic field associated with the post-synaptic primary current of layer V pyramidal neurons (Murakami & Okada, 2006). Specifically, the pyramidal neurons in the sulci are oriented parallel to the scalp. This statement is based on the human brain morphology and the properties of the magnetic fields.

The magnetic field's strength produced by a single neuron is minuscule and impossible to measure outside the head with the current technology. Murakami and Okada (2006) estimated that to measure the brain activity with MEG, around 10,000 to 100,000 neurons must be active simultaneously with some specific spatial organization. The lack of myelin in the postsynaptic dendrites slows the signal transmission, easing the neural signals' temporal coherent sum. The layer V pyramidal neurons are arranged in parallel (Figure 12), allowing their magnetic fields' spatial sum. Finally, according to its physical properties, the magnetic field strength decrease with distance as $1/r^2$. This property constrains the possible orientations of the neurons with respect to the scalp. Magnetic fields originated in gyri will be strongly decreased at the MEG detector position, and only the ones that originated in sulci will be detected (Figure 12). Therefore, the frequency of the

oscillations observed by MEG (and EEG) is determined by these neuronal ensembles' activation and deactivation. The acquired signal is related to the envelope of the individual neurons' joint activity (Buzsáki, Anastassiou, & Koch, 2012). This relation between neuronal ensembles activation and the EEG signal acquired is shown in Figure 12.

Figure 12. The magnetic fields generated by the pyramidal neurons.

(A) The camera lucida reconstruction of a pyramidal neuron (Lubke, Markram, Frotscher, & Sakmann, 1996). (B) A schematic representation of the magnetic fields generated by the layer V pyramidal neurons in the sulci. Each little orange arrow represents a “single neuronal current,” while the big orange arrow represents the sum of all single neuron currents. The green circles represent the associated magnetic field. It is visually intuitive how only the sulci neurons produce a magnetic field that can be measured outside the head. (C) Simultaneously recorded local field potential traces from the superficial (‘surface’) and deep (‘depth’) layers of the motor cortex in an anesthetized cat and an intracellular trace from a layer V pyramidal neuron. Reproduced from (Buzsáki et al., 2012).

A SQUID is a very sensitive magnetometer developed in 1964 (Jaklevic, Lambe, Silver, & Mercereau, 1964), capable of measuring magnetic fields in the range of the fT. This sensitivity is only achieved when the SQUID behaves as a superconductor. The superconducting properties appear, in some materials, when their temperature is approaching absolute zero. In the case of MEG systems, liquid helium is applied, and, subsequently, a particular layout is needed to deal with the safety issues owing to this low-temperature working point. All the MEG data obtained for this thesis has been obtained using the Elekta Neuromag® System and, therefore, the specific layout presented in

this thesis is the Elekta Neuromag® System layout. A single SQUID sensor is composed of an actual SQUID and a pick-up coil. In this system, 306 SQUID sensors are placed in 102 sheets and arranged in a head-shaped detector array. The whole detector array is placed in a vacuum-insulated vessel filled with liquid Helium (Figure 13).

Figure 13. MEG detector array.

The head-shaped SQUID sensor array is placed inside a vacuum-insulated vessel filled with liquid Helium. The SQUID sensor array comprises 102 sheets with three SQUIDs each (one magnetometer and two gradiometers). Finally, a single SQUID sensor is presented. The green lines represent magnetic fields, and the yellow arrows represent the induced currents.

The adult brain human produces magnetic signals in the order of the pT, much smaller than the environmental magnetic noise and even smaller than other magnetic fields produced by the human body (Figure 14). The solution for the magnetic fields produced by the human body will be addressed later. Every MEG recording has to be performed in a magnetically shielded room (MSR) to cancel the environmental magnetic noise. An MSR mirrors the idea of a Faraday's cage but canceling magnetic fields. An MSR is composed of several layers of high magnetic permeability materials, in our case mu-metal, which offers a "low-resistant path" to the magnetic fields: magnetic fields will travel along the walls instead of going through the low-permeability inside of the room (Figure 14). This approach can attenuate around 40 dB of magnetic noise, even for low frequencies (Del Gratta, Pizzella, Tecchio, & Romani, 2001). Finally, the MSR is completed with high-conductivity layers that act as a Faraday's cage to isolate the interior from high-frequency electromagnetic fields.

Figure 14. Magnetic noise and the magnetically shielded room.

The left panel presents the strength of different magnetic fields produced by ordinary events. The top right panel presents a schematic view of the magnetic fields' behavior (green lines) and the MSR. The bottom right panel is a photo of the actual MEG and MSR used for this thesis (Cognitive and Computational Neuroscience Laboratory, Center for Biomedical Engineering, Madrid).

The MEG analysis is a complex process that can be conceptualized in four steps, namely, recording, preprocessing, source reconstruction, and analysis. In each step, the researcher must make some decisions based on the nature of the experiment or the population itself, the available resources, or the experiment's actual goal. This thesis aims to present a project related to AD more than a theoretical analysis of the different possibilities to perform a MEG study. Therefore, in the next subsections, we will focus on the process followed for this thesis. An overview of the complete process, from acquisition to analysis, is shown in Figure 15.

Figure 15. MEG workflow.

The upper box presents the preprocessing steps, from the MEG recording to the source reconstruction. The lower boxes show two elements of interest in this thesis: power spectrum analysis and functional connectivity analysis.

1.3.1.1. MEG recording process

Before the MEG recording itself, there is a setup process for the subject. First, the subject is told to dispose of any metallic object or any electromagnetic device. Two head position indicators (HPI) coils are placed in the subject's forehead and two in the subject's mastoids to track the head movements during the recording. Finally, two electrodes are placed above and below one eye, and two electrodes are placed above and below the heart to track blinks and continuously register the heartbeat. As we saw in Figure 14, eye movements and heartbeats produce magnetic "noise" that hides the brain activity. This noise must be controlled in the preprocessing step. When this preparation is over, the subject is introduced in the MSR.

The subject is accommodated on a seat and instructed to remain calm and still. The experiment designed for this project is comprised of four minutes of the eyes-closed resting state, a memory task based on remembering faces, four minutes of the eyes-opened resting state, four minutes of eyes-closed resting-state, another memory task also based on remembering faces, and a passive visualization of faces. The sampling rate for the MEG recordings, i.e., the number of

samples per second recorded, was set to 1000 Hz. Finally, an online band-pass filter between 0.1 Hz and 330 Hz was employed during the recording to avoid aliasing. We have used the second eyes-closed resting-state recording of each subject for all the work in this thesis.

1.3.1.2. MEG preprocessing

The preprocessing step's ultimate goal is to obtain segments of the MEG recordings free from “artifacts,” i.e., free from any signal but the subject’s brain activity. As we have mentioned, there are several magnetic noise sources: environmental noise, human magnetic noise, and electronic noise. Each of these noises is addressed differently.

Figure 16. tSSS model.

The signal recorded by the sensors is the sum of the magnetic fields produced inside the head (b_{in}) and outside the head (b_{out}). By exploiting the spatio-temporal characteristics of these magnetic fields, tSSS can remove the interfering signal b_{out} from the signal of interest b_{in} .

The first noise is the environmental noise that originates outside the MSR. Notwithstanding the MSR's isolating properties, attenuated magnetic fields break through the MSR, interfering with physiological brain recordings. The first preprocessing step is called the spatiotemporal signal space separation (tSSS) method (Taulu & Simola, 2006), and its objective is to model (and thus differentiate) the magnetic-field sources in the head and the outer head-space. To that end, tSSS defines two volumes and models the magnetic fields produced by the sources contained in them: the subject’s head (physiological sources) and the outer headspace (interfering sources). The space between these two volumes (that contains the sensor array) is defined as free of sources. (Figure 16). To define the two different magnetic fields, $B_{in}(\vec{r})$ and $B_{out}(\vec{r})$, tSSS exploits two assumptions: the magnetic fields generated inside of the head present a different spatial distribution than magnetic fields generated outside of the head; the inside components and the

external components are unrelated and independent. With these definitions, the magnetic field measured in a sensor-space position is $B(\vec{r}) = B_{in}(\vec{r}) + B_{out}(\vec{r})$, with $\vec{r} = (\theta, \varphi, r)$, and hence, the contribution of outer sources can be removed.

Figure 17. MEG artifacts and SOBI detection.

From left to right, the top panel presents heart artifacts highlighted in grey, eye artifacts highlighted in purple, muscle artifacts highlighted in green and electronic artifacts highlighted in orange. The bottom panel presents the original unmixed signals obtained using the SOBI method. The eye and heart components, the origin of the eye and heart artifacts, are correctly extracted from the brain signal.

After tSSS, each MEG recording was visually inspected to identify segments with artifacts, i.e., segments with noise from different origins (Figure 17). There are four kinds of artifacts in a MEG recording:

- Heart artifact. Artifacts originated from the electricity released in every heartbeat. These artifacts are generally observed in the sensors located in the left part of the head, specifically, the left temporal. These artifacts' main characteristic is its periodicity, presenting a sharp power spectrum with prominent peaks around 1-1.5 Hz (around 60-80 bpm) and the associated harmonics. The heart artifact is easy to detect since we use two electrodes to measure the electrocardiogram.
- Eye artifact. Artifacts originated from the eye movements and blinks. These artifacts are usually observed in the sensors located in the frontal part of the head. These artifacts present a gradual change of amplitude in several channels, generally as a v-shape wave. The eye artifact is easy to detect since we use two electrodes to measure the electrooculogram.
- Muscle artifact. Artifacts generated from any kind of movement of the body, from head movements to arm movements. By the magnetic field properties explained before, head movements are more severe than foot movements. These artifacts are not restricted to a concrete region. The muscle artifact presents an increase of amplitude and a high frequency.

- Electronic artifact. Failures and recalibrations in sensor electronics produce these artifacts.

These artifacts are not restricted to a concrete region. These artifacts present an abrupt change of amplitude.

To remove the heart and eye artifacts, an independent component analysis (ICA) was performed. This method assumes that the recorded data, i.e., the MEG recordings, is the product of an unknown mix of independent sources. This method aims to estimate the “mixing” matrix and to obtain the original unmixed signals. Different ICA approaches have been developed depending on the assumptions about the nature of the original signals and the “unmixing” strategy. In this thesis, the second-order blind identification (SOBI) method was used. The assumption in SOBI is that the original signals have a temporal structure with different autocorrelation functions (or power spectra) and are mutually uncorrelated. The diagonalization process in SOBI, i.e., the extraction of uncorrelated original signals, will use time-delayed data matrices to exploit the temporal assumption. Due to the above temporal and spectral characteristics of the heart and eye artifacts, the SOBI method can extract these independent artifact components (Figure 17).

Unfortunately, there is no reliable method to extract, cancel, or remove the muscle and electronics artifacts. In these cases, the segment is marked as “noise,” and it is removed from further analysis.

After this preprocessing step, we have obtained segments of clean MEG recordings. These clean segments were divided into four-second epochs that will be used for the subsequent analysis.

1.3.1.3. Source reconstruction

At this point in the pipeline, we have the basis to directly perform power spectrum analyses, evoked-response potential analyses, statistical analyses, or any planned analyses. Working with the clean data obtained from the sensors is known as working in sensor space, and it is a perfectly valid option. It has been the standard pipeline for many years, even nowadays (Puce & Hämäläinen, 2017). Nevertheless, working in sensor space has a significant caveat. The brain activity located in a concrete brain area is captured by several sensors close to that area, provoking spatial resolution loss, and the complication to link the obtained results to actual brain regions or processes. An alternative is to translate the information obtained in sensor space to different points of interest in the subject’s brain, a process known as source reconstruction. This translation is not straightforward, and several methods have been developed in the past decades. It is crucial to clarify that each method has its caveat, but the discussion of each method's advantages and disadvantages

is out of this thesis's scope. Nevertheless, all source reconstruction methods include the definition of the forward model and the inverse model.

Forward model

The forward model includes the number, position, and orientation of the current sources defined in the brain, the magnetic properties of the different elements (brain, skull, scalp, and air), the position and orientation of the sensor, and it defines the relation of the magnetic field produced by the sources and the magnetic field finally recorded by the sensors. As the reader may anticipate, different parameters are defined in the forward model, thereby giving rise to different forward models. The parameter selection entirely depends on the problem to be solved. As an illustrative example, MEG recordings are used to localize the epilepsy focus before surgery. In this case, the characterization of the whole brain activity is not needed. Only one current dipole is defined and placed at different brain positions to find the best fit for the actual epilepsy MEG recording.

In this thesis, referring to the definition of the sensor, only magnetometers have been used. The tSSS preprocessing reduces the data's dimensionality, provoking the magnetometers, and gradiometers' information to be highly redundant (Garcés, López-Sanz, Maestú, & Pereda, 2017). Referring to the head model, the subject's T1-MRI is segmented to obtain five different tissues, namely, cerebrospinal fluid, white matter, grey matter, skull, and scalp. With the current technology, the MEG spatial resolution is in the order of centimeters (Figure 10); therefore, two sources defined closer than the double of the spatial resolution would be indistinguishable. Considering this constraint, the source model is defined in Montreal Neurological Institute (MNI) space, utilizing a uniform three-dimensional grid with a spacing of 10 mm. The MNI template is commonly used in neuroscience obtained by averaging 152 T1-MRIs of healthy subjects (Mazziotta, Toga, Evans, Fox, & Lancaster, 1995). This source model is then linearly transformed into each participant's individual MRI using the subject's digitalized head shape. Finally, the forward model is solved with a boundary element method (BEM) of three isotropic and homogenous-conductivity elements (brain, skull, and scalp). The 3-BEM model has been proved to offer accurate performance (Stenroos, Hunold, & Haueisen, 2014).

Inverse model

Once the forward model is solved, i.e., the relation between the source activation and the sensor records, we want to solve the inverse process: the real data available from the sensor (the actual MEG recording), which sources were active? This one constitutes an inverse problem. In the typical case, and specifically in this thesis case, the inverse problem is ill-posed: the number of brain

sources is greater than the number of sensors. Thus, there is no unique solution to the problem. A series of constraints must be defined to solve ill-posed problems, and, again, different inverse models can be obtained depending on the constraints defined.

The linear constraint minimum variance (LCMV) beamforming technique is one of the most popular methods to deal with MEG's inverse problem (Van Veen, Van Drongelen, Yuchtman, & Suzuki, 1997). The LCMV beamforming technique is a spatial filtering method that independently reconstructs each source activity as a weighted sum of the MEG sensors' data. The filter for each source localization is estimated to suppress signals from other localizations using the minimum variance. The LCMV beamforming technique assumes that the sources are uncorrelated and the noise is uncorrelated from the sources. On the other hand, the LCMV beamforming technique makes no assumptions about the number of sources since each source is reconstructed independently. This technique has been proved to give relevant results in situations with distributed sources when they are not correlated, i.e., reconstructing the whole cortex's activity during resting state recordings (Hincapié et al., 2017).

1.3.1.4. Power spectral analysis

As we mentioned in chapter 1.3.1, MEG electrophysiological signals present different oscillatory patterns, and these patterns seem to be linked to biological or cognitive processes (Buzsáki, 2009). According to signal processing theory, any pure oscillatory signal can be characterized by its frequency, amplitude, and phase. The frequency measures the number of times the oscillation repeats itself in a second, the amplitude measures the height of the signal, and the phase (phase shift actually) measures the shift of the signal from an original sinusoidal function. Furthermore, the Fourier transform is a mathematical transform that allows decomposing any signal into its constituent frequencies with its associated amplitudes and phases. In this sense, MEG signals can be transformed from the original *time domain* to a new space called the *frequency domain*. The representation of a signal in the frequency domain is called the power spectrum, and thus, the study of the MEG signals' amplitude and frequency in the frequency domain is called power spectrum analysis.

The oscillatory patterns are classified according to certain frequency limits, and the frequency limits have been set based on empirical evidence (Klimesch, 1996). The name of the frequency bands, commonly known as "classical frequency bands," their limits and an average subject's power spectrum are presented in Figure 18.

This thesis estimates the most common measure associated with the power spectrum: the classical frequency bands' relative power. The relative power is calculated as the ratio between the power in each band and the total broadband power between 2 and 45 Hz, resulting in a value ranging from 0 to 1.

Figure 18. Classic frequency bands.

Left panel: an original MEG signal filtered into the classical bands. *Right panel:* the original MEG signal represented in the frequency domain (the signal's power spectrum). The different colors of the power spectrum corresponding to those used in the filtered signals on the left.

1.3.1.5. Functional connectivity

Functional connectivity (FC) in neuroimaging was defined for the first time as “*the temporal correlation of a neurophysiological index measured in different brain areas*” (Friston, Frith, Liddle, & Frackowiak, 1993). This definition captures the idea that changed the brain's study in the 20th century: different areas across the brain interact or collaborate to fulfill a specific task, and this collaboration is consistent in time. This paradigm discards the previous idea of specific isolated areas in charge of specific cognitive functions (as Broca area in charge of language (Broca, 1861)) and it presents the brain as a set of “networks,” each network involving specific brain areas, each of the networks associated with different cognitive functions. Besides, the original FC definition did not specify the concept of “*neurophysiological index*,” thus allowing different approaches to measuring FC. There are three main types of FC: linear synchronization, phase synchronization, and general synchronization (Pereda, Quiroga, & Bhattacharya, 2005). In this thesis, we focus on PS.

Besides the frequency (or the bandwidth in our case) and amplitude, any oscillation has associated a phase diagram. Phase synchronization models state that two synchronized oscillations will present a relation between their instantaneous frequencies, even when the oscillation's amplitude is not related (Pikovsky, Rosenblum, Kurths, & Hilborn, 2002). In Figure 12, when the phases of two time series are coupled, i.e., their activation and deactivation are somehow linked,

more times than expected for random unrelated events, those areas are considered synchronized and supposed to be engaged in the same task.

The signal phase, not just brain activity signals but any oscillatory signal, can only be correctly obtained if no other oscillators work in a different frequency interfering with the signal. This is due to the properties of the signal phase. The power of a mixed-signal from two oscillators is a linear addition while the addition of the phases is non-linear and, thus, the initial phase signal of each oscillator cannot be recovered. Therefore, to obtain the phase time series correctly, the signal must be filtered to separate the oscillators. The classical bands aforementioned are meant to be originated from independent oscillators, and, consequently, most of the studies in neuroscience filter the times series using this frequency limits. Leaving aside all the variations and redefinitions, the classic bands and their limits are: delta (2-4 Hz), theta (4-8 Hz), low alpha (8-10 Hz), high alpha (10-12 Hz), low beta (12-20 Hz), high beta (20-30 Hz) and gamma (30-45 Hz). The phase for each frequency band was estimated using the Hilbert transform (Rosenblum, Pikovsky, Kurths, Schäfer, & Tass, 2001). The Hilbert is a version of the original signal delayed exactly $\pi/2$ radians, or a quarter of an oscillation. Thus, the relation between the original signal and its Hilbert transform can be used to estimate the duration of a cycle or phase (Pereda et al., 2005).

Finally, the signal phases are used to obtain the “*neurophysiological index*.” This thesis uses the PLV (Lachaux, Rodriguez, Martinerie, & Varela, 1999). The basic intuition of PLV is that two signals are synchronized when the difference between the instantaneous phases (the phase at each time point) is bounded. Similarly, two series are not synchronized if their phase difference changes at each time point. Mathematically, this method represents the phase differences in polar coordinates with a vector for each time point. The vector for a time point phase difference has a unit amplitude, and the phase is equal to the phase difference. Then, the average of all these vectors is calculated. If the phase difference is bounded, the average vector will present a magnitude close to 1. On the other hand, if the phase difference spans over the whole polar graph, the average vector will have a magnitude close to 0. A visual example is presented in Figure 19.

Figure 19. Phase synchronization using PLV.

Top panel: the PLV of two desynchronized signals. *Bottom panel:* the PLV of two synchronized signals. On the left, the two signals and their instantaneous phases are overlaid. On the right, each instantaneous phase difference is plotted (blue circle) in a polar graph, and the average of these vectors (orange vector) is the PLV vector. The PLV value is equal to the norm of this vector.

Nevertheless, there are two caveats regarding the measure PLV: a positive bias and the zero-lag synchronization. In theory, PLV values range from 0 to 1, but this is in the perfect theoretical case where there are infinite samples of the two random distributions we are measuring. In a realistic scenario, we can only measure a PLV estimator ($\widehat{PLV}_{sample}$) from the N samples available and this estimator, although it is a consistent measure for phase synchrony, ranges from values higher than zero to 1 (Aydoore, Pantazis, & Leahy, 2013). The relevant properties of this estimator are:

$$\widehat{PLV}_{sample} \in [\varepsilon, 1], 0 < \varepsilon < 1 \quad (1)$$

$$E[\widehat{PLV}_{sample}] > PLV \quad (2)$$

$$\lim_{N \rightarrow \infty} \widehat{PLV}_{sample} = PLV \quad (3)$$

To deal with this problem, Aydore et al. (2013) defined a non-biased PLV^2 estimator:

$$\widehat{PLV}_{ub_sample}^2 \triangleq \frac{1}{N-1} (\widehat{PLV}_{sample}^2 \times N - 1) \quad (4)$$

On the other hand, there is the problem of zero-lag synchronization. The zero-lag synchronization occurs when the difference of phases between two signals is constant around 0. This zero-lag synchronization may be produced by real synchronization between two signals, by volume conduction or by source leakage. The functional relevance of whole-brain synchrony taking place with near-zero or zero-phase lag has been already described in the literature for the EEG (see., e.g., (Finger et al., 2016)). Volume conduction occurs when several sensors simultaneously pick up activity from the same source. Source leakage is a consequence of the data's low spatial resolution, provoking that the reconstructed source-space signals of two close sources share some activity. Due to the magnetic properties and the brain tissues properties, the propagation of the signals can be considered instantaneous and, therefore, the phase difference of the part of the signals related to such spurious connectivity must be zero (Nolte et al., 2004; Cornelis J. Stam, Nolte, & Daffertshofer, 2007). Since it is not straightforward to differentiate between real zero-lag synchronization and the one produces by these two undesirable effects, several measures insensitive to zero-lag synchronization have been defined. Related to PLV, Bruña et al. (2018) defined a zero-lag insensitive PLV measure called corrected imaginary PLV (ciPLV):

$$ciPLV_{i,j} = \frac{\frac{1}{T} \Im\{x_i \cdot x_j^T\}}{\sqrt{1 - \left(\frac{1}{T} \Re\{x_i \cdot x_j^T\}\right)^2}} \quad (5)$$

where x_i and x_j^T represent a signal and the transpose of another signal respectively, T is the length of the signal, $\Im\{\cdot\}$ represents the imaginary part and $\Re\{\cdot\}$ represents the real part.

1.3.2. Magnetic resonance imaging

The first milestone to MRI was the description and measurement of nuclear magnetic resonance in 1938 (Rabi, Zacharias, Millman, & Kusch, 1938), a physical property whereby a given

nucleus absorbs and re-emits electromagnetic radiation at a specific frequency. During the next decades, several groups studied and categorized the relaxation time of different materials and human tissues. Relaxation time is the delay of a nucleus to recovery to the original state from a high-energy state, i.e., when the nucleus has absorbed electromagnetic radiation. The material's physical properties mark this time under study, and it allows differentiating different human tissues, even pathological tissues, as tumorous cells (Damadian, 1971). During the 70s, several milestones reached by different research groups result in the design and build of Mark-1, the first clinically-used whole-body MRI scan. Among the milestone, we may highlight the first 3D nuclear magnetic resonance imaging using gradients (P. C. Lauterbur, 1973), the first cross-sectional image of a living mouse (Paul C. Lauterbur, 1974), and the development of “echo-planar imaging” technique capable of scanning in seconds rather than hours (Mansfield & Grannell, 1975). Nowadays, MRI technique is one of the most used imaging technique in the clinical routine. To understand the MRI technique's functioning, the best option is to deepen into the two concepts of its name separately: magnetic resonance and imaging.

The first concept is “magnetic resonance”: All atomic nuclei have an elemental physical property called spin, resulting from the sum of their elemental particles' spins. Due to the spin's quantic properties and values, if a nucleus presents an odd number of protons, an odd number of neutrons, or both, it behaves like a single particle with a non-zero spin. These non-zero-spin particles have associated an angular momentum and a magnetic moment. Although spin is an elementary property with no correspondence with classical physics, we consider these non-zero-spin particles as sphere magnets spinning around on their axis for explanatory purposes. When an intense and homogenous magnetic field is applied, all particles with magnetic moment under the magnetic field's effect align their axis to this intense magnetic field (Figure 20). The majority of them will align with the magnetic field orientation (parallel), and some will align with the opposite orientation (anti-parallel). This difference in alignments results in a net or macroscopic magnetization pointing in the main magnetic field's direction.

In 1897, the mathematical physicist Joseph Larmor demonstrated that, under the influence of an external magnetic field, particles with magnetic moment precess synchronized in phase around the applied magnetic field's direction with an angular frequency directly proportional to the strength of the applied field times a constant. The relationship between the strength of the magnetic field applied and the Larmor's equation defines the precession frequency: $f_0 = \gamma B_0$, where f_0 is the precession frequency, B_0 is the strength of the externally applied field, and γ is the gyromagnetic

ratio, a constant specific to each specific nucleus or particle. In this situation, magnetic resonance, vital for MRI, appears: particles absorb and release electromagnetic energy at the precession frequency. Therefore, when another external radio-frequency pulse (RFP) at the precession frequency is applied, the absorption of energy will alter their alignment from the direction of the main magnetic field to the direction opposite the main magnetic field. The particles will spin in synchrony (Figure 20). When the external RFP is suppressed with this same principle, the particles return to the magnetic field alignment releasing the absorbed energy at the precession frequency and desynchronizing their phases.

Figure 20. Physical principles of MRI.

In a normal situation, the nuclei are randomly oriented. Therefore, on a macroscopic scale, the net magnetization is zero. When an external magnetic field is applied, the vast majority of nuclei align to this magnetic field with the same orientation (parallel) and some with the opposite orientation (anti-parallel). For explanatory purposes, the nuclei are overlaid on the bottom panel, and in that position, a Cartesian system has been defined. When the external magnetic field is applied (left), a net magnetization results with the same magnetic field orientation. According to *Larmor's equation*, when another RFP is applied with a specific

frequency, the energy is absorbed by the nuclei, provoking the synchronization of their precession phase and the appearance of a transversal net magnetization (M_{xy}).

The excess of energy supplied by the RFP will be released through spin-lattice relaxation and spin-spin relaxation. The spin-lattice relaxation is the mechanism of energy release to the medium surrounding the nucleus. The rate of relaxation, or energy release, depends on the surrounding material properties and its molecular structure, i.e., the medium's ability to absorb the nucleus's excess energy. The mean spin-lattice relaxation time of a material is termed T1 time, and it is characteristic of each material. The spin-spin relaxation is produced by the phase desynchronization of the magnetic moments at the microscopic scale, where the magnetic moments randomly influence each other. The mean spin-spin relaxation time is termed T2 time.

The second term to address is “imaging,” in other words, how to encode the magnetic resonance measures to build a graphical representation. As a general concept, MRI scanners measure relaxation times at different spatial grid points and assign intensity values to these points to obtain a magnetic resonance visual representation. This measurement and encoding is performed using two different magnetic fields, B_0 and B_1 , different types of RFP and a set of coils to measure the relaxation times. Naturally, there is a computational process to transform this information into the final MRI.

Although this is not the only option, the classical structural MRI technique exploits the presented properties with hydrogen atoms. 65% of the human body is made up of water, i.e., it is made up mostly of hydrogen. Thus, it is present in most human body tissues. Besides, hydrogen has a single proton in its nucleus. According to *Larmor's equation*, the resonance frequency of hydrogen will depend on the applied magnetic field B_0 . The standard B_0 values for clinical applications are 1.5 T and 3 T, and considering that the gyromagnetic ratio of hydrogen is $\gamma = 42.58 \text{ MHz/Tesla}$, the hydrogen resonance frequency is 83.87 MHz when a 1.5 T magnetic field is applied or 127.74 MHz when a 3 T magnetic field is applied. If we define, for the sake of clarity, theoretical axes in Cartesian coordinates, B_0 is applied along the z-axis (Figure 21). The second magnetic field, B_1 , is added to the homogenous B_0 to encode the spatial information. In opposition to the homogenous magnetic field B_0 , B_1 presents less strength and a linear variation in the three axes, provoking a linear variation in the total magnetic field amplitude. This linear variation changes both the resonance frequency and phase as a function of position (following *Larmor's equation*, stronger magnetic fields result in higher resonance frequencies). Adjusting the RFP frequency, we can stimulate and measure the particles' relaxation times at locations of interest.

Figure 21. Gradient magnetic fields in MRI.

According to Larmor's theory, there is a linear relationship between the magnetic field's strength applied to a particle and its precession frequency. When a gradient magnetic field is applied along the human body, the particles' frequency will differ depending on their position. This concept is exploited to encode MRI information spatially.

In the end, MRI scanners measure and encode the relaxation phenomena (spin-lattice and spin-spin relaxation) at different locations. To visualize this process, let us define two magnetic fields in the previously defined Cartesian axes, namely, the longitudinal magnetic field, M_z . And the transverse magnetic field, M_{xy} . When the RFP is applied, nuclei align themselves perpendicular to B_0 , thus decreasing M_z and increasing M_{xy} . Moreover, the process is reversed when the RFP is suppressed (Figure 22). T1 relaxation time is defined as the time-lapse that takes M_z to recover to the 63% of its initial value. Concurrently, when the RFP is suppressed, the precession phase of different nuclei desynchronized, thus decreasing M_{xy} without increasing M_z (Figure 22). T2 relaxation time is defined as the time-lapse that takes M_{xy} to dissipate the 63% of its initial value. As we mentioned, T1 and T2 relaxation times depend on the material properties and the molecular structure, and, therefore, different human body tissues present different relaxation times (Figure 22). MRI scanners use precise RFP sequences that exploit this difference in relaxation times to build the MRI.

Figure 22. Longitudinal and transverse relaxation times.

When the RFP is suppressed, the particles realign with B_0 emitting the excess of energy by spin-lattice and spin-spin relaxation. On top, the spin-lattice relaxation. The excited nuclei lose energy to the tissue, thus decreasing the precession angle and increasing the longitudinal magnetization (M_z). The time needed for each tissue to reach 63% of the original longitudinal magnetization is called the *T1 relaxation time*. In the right panel, the T1 relaxation time of each tissue in the brain is presented. On the bottom panel, the spin-spin relaxation. The precession phase of different nuclei goes out of sync, thus decreasing the transversal magnetization (M_{xy}) without increasing the longitudinal magnetization (M_z). The time needed for each tissue to emit 37% of the absorbed magnetization is called the *T2 relaxation time*. In the right panel, the T2 relaxation time of each tissue in the brain is presented.

MRI scanners only measure the transversal magnetization M_{xy} . Selecting proper RFP sequences, the brain tissues may be distinguished due to their specific T1 and T2 relaxation times. To define two vital concepts, echo time (TE) and repetition time (TR), we construct a simple RFP sequence (Figure 23). In this sequence, we emit an RFP that flips the nuclei into the XY-plane, obtaining a peek in transversal magnetization measures. After a specific time, before the tissues recover all the longitudinal magnetization (T1 relaxation time), we emit another RFP that flips the nuclei 180° , i.e., the opposite orientation of B_0 . With this new RFP, there will be two alterations in the transversal magnetization: once when the nuclei are flipped and a second one when the nuclei return to the original position. When the nuclei return to the original position, and transversal magnetization is the maximum, TE is called. TE occurs at the double of time between the first 90° RFP and the second 180° RFP, and it is actually when the MRI signal is acquired. After a certain time,

the whole process is repeated. This time is called TR. As we see in Figure 23, different TE and TR use allows obtaining different contrast for tissue intensities resulting in different MRI modalities. Among the vast types of MRI modalities, we focus on two used for this thesis: T1-weighted imaging and Diffusion-weighted imaging (DWI).

Figure 23. RFP sequence.

On the top panel, a timing diagram of a traditional RFP. The repetition time (TR) and the echo time (TE) characterize and allow for different MRI modalities. An example of how different TRs and TEs produce different MRI modalities is presented in the middle and bottom panel.

1.3.2.1. T1-weighted images

T1-weighted image (T1WI) is one of the most used MRI modalities. This type of image is obtained using the RFP sequence called spin-echo with a short TR and a short TE. Spin-echo sequences maximize the difference in the T1 relaxation times of the brain tissues. T1WI presents the dark intensities for CSF, grey intensities for GM, and WM's bright intensities (Figure 24).

Figure 24. T1-weighted image.

On the top panel, T1 and T2 relaxation times for each brain tissue are presented. To obtain T1-weighted images, the RFP sequence is characterized by a short TR and a short TE. In this kind of MRI, WM would be the brightest tissue, GM would have intermediate intensities, and CSF would be the darkest. On the bottom panel, an example of a T1WI of a brain from different points of view.

Anatomical MRI modalities have been widely used to characterize the AD continuum. The essential anatomical MRI measure for AD characterization is the cortex volume. The atrophy, i.e., loss of volume and thickness, of the GM is part of the normal and healthy aging process. Nevertheless, the rate of atrophy is significantly accelerated by AD, and it presents a specific spatiotemporal pattern. Hippocampi, followed by the amygdala, present atrophy in the preclinical

stages of AD (Coupé, Manjón, Lanuza, & Catheline, 2019). The atrophy later evolves to the temporal lobes and the medial parietal lobes in AD's middle stages. Finally, the frontal lobes show atrophy in the later AD stages (Scahill, Schott, Stevens, Rossor, & Fox, 2002).

1.3.2.2. Diffusion-weighted images

When a group of molecules is released in a fluid with no boundaries, each molecule's trajectory presents a random pattern known as *Brownian motion*. This random motion is provoked by each molecule's random interaction with the others, resulting in an overall null linear and angular momentum in the fluid. The movement of the molecules over time is called molecular *diffusion*. On the contrary, if the fluid is constrained (as the blood in the veins), the molecular diffusion is restricted. Thus, a linear and angular momentum appears. Based on this concept, the DWI modality is constructed.

Before the DWI sequence, B_0 The RFP has been applied, i.e., the nuclei of a particular position have the same precession frequency, and their phases are synchronized. At that moment, the DWI sequence is applied. First, a new gradient is applied at this location, provoking the nuclei's frequency at this position to change. The new gradient is suppressed (leaving only B_0) so the precession frequency will be again the same for the nuclei, but they will be dephased. Finally, the gradient is applied with the inverse orientation an equal time lapse. There are two possible outcomes when the final gradient is suppressed: if the nuclei have not moved between gradients, they will be in phase again, and a strong RFP signal will be measured; if the nuclei have moved between gradients, their phases will be desynchronized because the second gradient applied would be different from the first, and no RFP signal will be measured. For the sake of clarity, this process is visually presented in Figure 25. DWI's main parameters are the *b-value* (or *b-factor*), the diffusion directions, and the apparent diffusivity map (D). Based on the previous work of (Stejskal & Tanner, 1965), Le Bihan derived a new formula (Le Bihan & Breton, 1985) to estimate the signal attenuation using those parameters:

$$S_i = S_0 e^{-b_i \cdot D_i} \quad (6)$$

where S_0 is the signal intensity before the gradient application and S_i is the signal after the gradient with intensity b_i is applied. The b-value reflects the strength and timing of the gradients used to generate diffusion-weighted images and. The diffusion directions refer to the gradients' orientation and define how many movement patterns can be measured. The apparent diffusivity map is an

estimate comparing the intensity changes between S_0 and S_i . Moreover, if six or more directions are estimated, the directionality of diffusivity can be estimated and written as a diffusivity tensor:

$$D_i = \frac{\ln(S_i) - \ln(S_0)}{b_i} \quad (7)$$

$$D = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix} \quad (8)$$

The estimation of the diffusivity tensor and its information extraction arise a sub-modality of DWI: the diffusion tensor imaging (DTI).

Figure 25. DWI principles.

On the top panel, the DWI principle is represented. First, three nuclei are placed between two coils, and their phases are synchronized due to the external field B_0 . When a gradient is applied, their frequencies and phases decouple depending on their position concerning the gradient. When a second gradient with the same strength and duration (but in the opposite direction) is applied, the nuclei that have stayed in their

position will return to the original situation. In turn, the nuclei that have moved would present a phase different from the original position. The more the number of nuclei that have moved, the lesser signal will be measured. Three examples of DWI with different b-values (0, 1000, and 3000 s/mm²) are presented on the bottom panel.

Diffusion tensor image

While DWI offers information about the total diffusion, DTI complements it by adding the directionality of diffusion. DTI technique characterizes the magnitude, the degree of anisotropy, and the orientation of water diffusion. This information and the *prior* knowledge of the brain tissues characteristics allow testing the white matter integrity.

As previously mentioned, water molecules in a fluid with no boundaries will present the same diffusion in all directions, i.e., isotropic diffusion. This is the case of CSF, where particles freely move, and the signal recovery is meager. On the other hand, white matter presents several barriers for particles' movement, such as the cellular membranes, myelin of the axon, and the axons' packing. In this case, particles' movement is constrained to the extracellular space along the axon's longitudinal direction, thus presenting great diffusion values in some directions and almost none in others, i.e., presenting an anisotropic diffusion (Figure 26). The tensor can be decomposed into matrixes of eigenvectors and eigenvalues. These matrixes allow representing the tensor of each voxel as an ellipsoid, where the axes would be defined by the eigenvectors and the radius of each axis would be defined by the eigenvalues Figure 26. Several quantitative analyses can be done regarding the diffusivity, the anisotropy, and the fiber tract trajectories in the white matter using the eigenvalues. In this thesis, the metrics used are fractional anisotropy (FA), mean diffusivity (MDiff), axial diffusivity (ADiff), radial diffusivity (RDiff), and the number of streamlines (NoS).

Figure 26. Water diffusion in different brain tissues.

Water diffusion and its associated anisotropy in two different brain tissues. On the top panel, the water freely moves in CSF. Therefore, it has an isotropic movement, and the eigenvalues associated with this movement are the same. In the WM, the water movement is constrained by the myelinated axons, presenting an anisotropic movement. In this case, the eigenvalue associated with the axons' direction is higher than the other eigenvalues.

FA quantifies the tensor's anisotropy ranging from 0 to 1, where 0 is a perfect isotropic tensor, and 1 is a diffusion restricted to a single axis. MDiff measures the overall diffusivity of a voxel regardless of the orientation. To complete this measure, ADiff quantifies the diffusivity along the ellipsoid's principal axis while RDiff quantifies the diffusion perpendicular to the principal diffusion axis. NoS is based on the tractography technique, and it measures the strength of the white matter connection between areas of interest. A streamline is a 3D trajectory constrained or directed by the anisotropy information obtained for each voxel. The formulae of the DTI quantitative measures are presented in Table 1. These measures have been used along the AD continuum, from the early stages (Barbara B. Bendlin et al., 2010; Gold, Powell, Andersen, & Smith, 2010), MCI (Cherubini et

al., 2010; Teipel et al., 2010), and proper AD stage (Mayo et al., 2019; Nir et al., 2013). Previous to the estimation of quantitative measure, a preprocessing step must be performed on the DWIs.

Table 1. DTI quantitative measures.

Eigenvalue decomposition	$D = E \cdot \Lambda \cdot E^{-1}$	
	where eigenvalues, $\Lambda = \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix}$	(9)
	eigenvectors, $E = \begin{bmatrix} e_{1x} & e_{2x} & e_{3x} \\ e_{1y} & e_{2y} & e_{3y} \\ e_{1z} & e_{2z} & e_{3z} \end{bmatrix}$	
Fractional anisotropy	$FA = \sqrt{\frac{3 \left((\lambda_1 - \bar{\lambda})^2 + (\lambda_2 - \bar{\lambda})^2 + (\lambda_3 - \bar{\lambda})^2 \right)}{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}$ where $\bar{\lambda} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$	(10)
Mean diffusivity	$MDiff = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$	(11)
Axial diffusivity	$ADiff = \lambda_1$	(12)
Radial diffusivity	$RDiff = \frac{\lambda_2 + \lambda_3}{2}$	(13)

The DTI quantitative measures characterize different aspects of the diffusion and anisotropy of a DWI. The table presents the measures used in this thesis.

1.4. Project details

In this section, we present the technical details of the different tests performed in this project. Specifically, we will focus on the participants' demographic characteristics, the neuropsychological battery used, the DNA genotype test, the MEG recording and preprocessing parameters, and the parameters of MRI acquisition preprocessing.

1.4.1. Participants

Two hundred and sixty-two healthy older adults were recruited from local hospitals, via advertisements placed at the Fulbright alumni association, in the “Asociación Española de Ingenieros de Telecomunicación Delegación de Madrid,” as well as in public media. We used the following exclusion criteria for this project:

- History of psychiatric, neurological disorders, or drug consumption that could affect MEG activity.

- Evidence of infection, infarction, or focal lesions in a T2-weighted Magnetic Resonance Image (MRI) scan.

- Alcoholism or chronic use of anxiolytics, neuroleptics, narcotics, anticonvulsants, or sedative-hypnotics;

- Being younger than 50 years old. The project's main objective is to characterize early signs of AD in cognitively healthy people, decades before the onset of symptoms. Nevertheless, if the population is too young, two problems may arise: the AD process may not have started or, if the process has started, it is so subtle to be detected. For that reason, the time windows of observation are critical, and thus, the age constraint.

- Mini-Mental State Examination score below 27. The Mini-mental State Examination is a neuropsychological test that ranges from 0 to 30 used for grading the cognitive impairment of patients (Folstein, Folstein, & McHugh, 1975).

- Subjective cognitive complaints. Over the last years, it has been suggested that subjective cognitive complaints might be an early sign of cognitive impairment due to AD (Jessen et al., 2014). To be sure of studying early signs of AD before any neuropsychological symptoms, people with subjective cognitive complaints may not participate.

All participants signed informed consent. The “Hospital Clínico San Carlos” Ethics Committee approved this study, and the procedure was performed following internationally accepted guidelines and regulations. For this thesis, the population was divided into first-degree relatives of a patient with AD and controls. First-degree relatives were defined as being direct descendants or siblings of a patient with AD. Relatives of AD patients were required to provide a medical report indicating the patient's diagnosis following the *Nacional Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association* Criteria (McKhann et al., 1984). The demographic characteristics of the sample are displayed in Table 2.

Table 2. Demographic characteristics of the participants.

	First-degree relatives	Controls	Dropouts
N	166	88	8
Age	58,70 ± 7,88	63,70 ± 9,10	
Sex	63 M / 103 F	39 M / 49 F	
<i>APOE</i> ε4 carriers	59 (+) / 95 (-)	16(+) / 67 (-)	17
Years of education	15,58 ± 5,01	16,03 ± 5,29	

The table summarizes relevant characteristics of the population, divided into first-degree relatives and controls. The third column, dropouts, indicate the number of participants who quit the project during the evaluation process (second row) or the number of participants who did not perform a specific test (fifth row). M = Male; F = Female; (+) = Carrier; (-) = Non-carrier.

1.4.2. Neuropsychological tests

Cognitive functions were assessed by administering a neuropsychological battery widely used in the literature and medical settings. The neuropsychological assessment is performed in one hour and a half by a specialist neuropsychologist. The battery includes the Mini-Mental State Examination (MMSE) (Folstein et al., 1975); the Geriatric Depression Scale (Marty, Pepin, June, & Segal, 2010); the Montreal Cognitive Assessment (Nasreddine et al., 2005); the Rey Complex Figure Test (Jerskey, 2018); the Wechsler Adult Intelligence Scale III (Wechsler, 1997); the F-A-S test, which is a subset of the Neurosensory Center Comprehensive Examination for Aphasia (Spreen, O., & Benton, 1977); the COOP/WONCA test (Nelson et al., 1987). This battery was defined to characterize with different (and maybe overlapping) tests a range of memory components, namely, auditory memory, immediate memory, delayed memory, visual memory, and visual working memory. In addition, other possible measures of interest, but not directly related to memory, were acquired such as the Geriatric Depression Scale or the COOP/WONCA test, which measures the patient's subjective well-being.

1.4.3. *APOE* genotype test

DNA was extracted from whole-blood samples of the participants of this study. As previously described in (Cuesta et al., 2015), the *APOE* haplotype was determined by analyzing SNPs rs7412 and rs429358 genotypes with TaqMan assays using an Applied Biosystems 7500 Fast Real-Time PCR machine (Applied Biosystems, Foster City, CA). A genotyping call rate over 90% per plate, sample controls for each genotype, and negative sample controls were included in each assay. Three well-differentiated genotyping clusters for each SNP were required to validate results. Intra and inter-plate duplicates of several DNA samples were included.

1.4.4. Magnetoencephalography data

To summarize Section 1.3.1.1 and Section 1.3.1.2, four minutes of eyes-closed resting-state MEG activity was recorded from each participant at the Center for Biomedical Technology (Madrid, Spain) with an Elekta Vectorview (Elekta AB, Stockholm, Sweden) 306-sensor system. Two sets of bipolar electrodes were used to record the ocular and cardiac activity. Four head position indication (HPI) coils were placed on the participants' scalp, two on the forehead, and two on the mastoids. The HPI coil position, together with around 200 points of the participant's head shape, was digitized using a Fastrack Polhemus system (Polhemus, Colchester, VT, USA). During the recording, these coils were fed to determine the head position for the MEG helmet continuously. During the recordings, subjects sat inside a shielded room and were instructed to keep still and relax.

Data was filtered (0.1 to 330 Hz) online and digitized with a sampling rate of 1000 Hz. The spatiotemporal expansion of the signal space separation (tSSS) method (Taulu & Simola, 2006), implemented by Neuromag Software (MaxFilter version 2.2, correlation 0.90, time window 10s), was used to remove external noise and to compensate for head movements inside the MEG scanner.

MEG recordings were preprocessed by a MEG expert using Fieldtrip (Oostenveld et al., 2011). The continuous data were segmented into non-overlapping artifact-free segments of four seconds. Only subjects with at least 20 segments were kept for further analysis. Due to the high redundancy of the data after applying the tSSS (Garcés et al., 2017), only data proceeding from the magnetometers were used for the subsequent analysis.

We carried out the source reconstruction using the T1-weighted MRI of each subject. We defined the source model in the MNI space, using a uniform three-dimensional grid with a spacing of 10 mm, which we then linearly transformed into the individual MRI of each participant. The individual images were segmented using SPM12 (Penny, Friston, Ashburner, Kiebel, & Nichols, 2007), and the resulting brain mask was used to build a set of individual single-shell realistic head models. Finally, this head model, together with the individual source model, was used to generate a lead field using a modified spherical solution (Nolte, 2003).

Time series of each source location were obtained using Linearly Constrained Minimum Variance beamformers (Van Veen et al., 1997) as an inverse model. The beamformers were calculated using the covariance matrix of each classical frequency band. A Finite Impulse Response

filter of order 1800 designed with a Hamming window was used, adding two extra seconds of real data at each side of each segment as padding.

1.4.5. Magnetic resonance imaging data

All T1WI used in this thesis was acquired using the same process and parameters. In more detail, all T1WI were acquired in a General Electric 1.5 Tesla system. A high-resolution antenna was employed together with a homogenization Phased array Uniformity Enhancement filter (Fast Spoiled Gradient Echo sequence, TR/TE/TI = 11.2/4.2/450 ms; flip angle 12°; 1 mm slice thickness; 256 × 256 voxels per slice and field of vision 25 cm). After the acquisition, the T1WI processing was performed using FreeSurfer 6.0 “recon-all” procedure as described in (Dale, Fischl, & Sereno, 1999; Fischl, Liu, & Dale, 2001; Fischl, Sereno, & Dale, 1999; Fischl, Sereno, Tootell, & Dale, 1999; F. Ségonne et al., 2004; Florent Ségonne, Pacheco, & Fischl, 2007). First, this procedure performs a motion correction, corrects intensity non-uniformity, and performs an intensity normalization. It then performs a segmentation of the different brain tissues and constructs a cortical surface mesh for each T1WI. It registers an inflated sphere version of this cortical mesh to a common surface-space. Finally, it uses an anatomical atlas (this atlas must also be an inflated version of the surface atlas and register to the common surface-space) to assign a neuroanatomical label to each native brain voxel. For this thesis, two anatomical atlases have been used: the automatic anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002) and the Schaefer atlas (Schaefer et al., 2018). As the last step, we obtained the cortical thickness using the FreeSurfer software.

For all the DWI in this thesis, the acquisition parameters were as follows: TE/TR 96.1/12,000 ms; the number of averages 3 for increasing the signal-to-noise ratio; 2.4 mm slice thickness, 128 × 128 voxels per slice, and 30.7 cm field of vision yielding an isotropic voxel of 2.4 mm; 1 image with no diffusion sensitization; and 25 DWI ($b = 900 \text{ s/mm}^2$). Data were recorded with a single-shot echo-planar imaging sequence. The DWI data was processed using the MRtrix3 software (J. D. Tournier et al., 2019). The DWI processing was compounded from the following sequential steps:

- 1.- A DWI improvement that consists of DWI denoising (Veraart et al., 2016), Gibbs ringing artifacts removal (Kellner, Dhital, & Reiser, 2015), eddy current and movement correction (Andersson & Sotiropoulos, 2016), and DWI bias field correction.

- 2.- Generating a tissue-type segmented image appropriate for anatomically constrained tractography (R. E. Smith, Tournier, Calamante, & Connelly, 2012).

3.- Estimating WM, GM, and CSF response functions for each subject (Dhollander, Raffelt, & Connelly, 2016). The final response function used for the whole sample is the average of all subjects' response functions.

4.- Single-Shell 3-Tissue CSD (SS3T-CSD) was performed to obtain WM-like fiber orientation distribution as well as GM-like and CSF-like compartments in all voxels (Dhollander & Connelly, 2016), using MRtrix3Tissue (<https://3Tissue.github.io>).

5.- Multi-tissue informed log-domain intensity normalization (Raffelt et al., 2017).

6.- Generation of the tractography (25 million streamlines, maximum tract length = 250, FA cutoff = 0.06, dynamical seeding) (J.-D. Tournier & F. Calamante, 2010) with anatomical constraints for tractography optimization (R. E. Smith, Tournier, Calamante, & Connelly, 2015).

In what follows, we present and discuss the three studies regarding FC, SC, and combined FC-SC as independent chapters 2, 3, and 4, respectively. To conclude, we present in chapter 5 the global conclusions summarizing the insight gained into the problem by the three different approaches.

Chapter 2

Functional connectivity in first-degree relatives of AD*

2.1. Introduction

There is still a debate regarding the initial factors triggering the cascade of neuropathophysiological events leading first to cognitive impairment and later to dementia along the continuum of sporadic (or late-onset) AD. As commented in the Introduction, the current dominant hypothesis suggests that soluble amyloid-beta ($A\beta$) protein or its aggregates in $A\beta$ plaques induce toxic effects in neuronal cells, leading to synaptic dysfunction, phosphorylation of tau protein, brain atrophy, and neuroinflammation (Bateman et al., 2012; Jack et al., 2013, 2010; Sperling et al., 2011; Villemagne et al., 2013). These toxic amyloid forms have been observed to cause the characteristic synaptic dysfunction present in AD (Cirrito et al., 2005; Selkoe, 2002). Stereological analyses of human brain tissues have revealed how, in the vicinity of the amyloid plaques, there is a reduction of GABAergic terminals (Garcia-Marin et al., 2009). This loss of inhibitory inputs induces the disruption of the excitation/inhibition balance (E/I balance), as demonstrated in animal models (Busche & Konnerth, 2016). The main feature of the E/I imbalance is represented as neuronal hyperactivity in the vicinity of $A\beta$ plaques during the first stages of AD (Busche & Konnerth, 2016), hypoactivity in the middle stages, and final collapse of the brain networks (Zott, Busche, Sperling, & Konnerth, 2018).

This E/I imbalance leads to hypersynchrony of the oscillatory activity produced by neural assemblies, affecting the function of different brain networks and, ultimately, causing cognitive impairment. This result was shown by Bajo et al. (2010) in patients with MCI in a study where a hypersynchronization of the oscillatory activity in the alpha band over the prefrontal cortex was the main difference between MCI patients and age-matched controls. A later study (David López-Sanz, Bruña, et al., 2017) showed that this anterior hypersynchronization in the alpha band was also present in subjects with SCD. Nakamura and colleagues (2018, 2017) associated such functional

* The results presented in this chapter have been published in (Ramírez-Toraño et al., 2021)

changes with the accumulation of the amyloid protein in frontal and parietal regions (Nakamura et al., 2018, 2017). Besides, the synchronicity between the anterior cingulate cortex (ACC) and the medial temporal lobe correlated with the level of phosphorylated tau in the cerebrospinal fluid (Canuet et al., 2015). Furthermore, in a previous study from our group, we found hypersynchronization in the alpha band between ACC and posterior brain regions in MCI to AD converters compared with non-converters (M. E. López et al., 2014).

In an attempt to unify the above results with those obtained in their longitudinal study, Pusil et al. (2019) proposed a model of progression from MCI to AD: the 'X' model, according to which *"the hypersynchronization would precede the conversion from MCI to Alzheimer's disease. Therefore, it could be considered a biomarker for the increased risk for the development of dementia"*.

Although the above-described profiles were mainly identified in the prodromal stages, pathophysiological features show decades before the main cognitive symptoms appear. Therefore, it is of great interest to assess whether the hypersynchronization phenomenon is already present in populations at risk. One population at increased risk are first-degree relatives of AD patients, at a two- to three-fold increased risk for developing dementia. This probability increases if relatives are carriers of the *APOE* $\epsilon 4$ allele (B. B. Bendlin et al., 2010). In previous studies, AD relatives with SCD showed alterations in the medial visual network (Hafkemeijer et al., 2013) and the posterior Default Mode Network (DMN) and medial temporal memory system (Verfaillie et al., 2018). However, young AD relatives without SCD have not been evaluated yet under the framework of the E/I unbalance hypothesis and the "X" model. The present study aims to strengthen and enhance the "hyperexcitability model," evaluating FC in young first-degree relatives of AD patients (without SCD) decades before the average age of clinical onset in sporadic Alzheimer's (Huff, Growdon, Corkin, & Rosen, 1987). Therefore, in the present study, we will examine the degree of FC, using magnetoencephalographic recordings, in a sample of healthy young adults at increased risk of developing AD: *APOE* $\epsilon 4$ carriers or individuals with a family history of AD (B. B. Bendlin et al., 2010; Martinez et al., 1998). In a hypothesis-guided approach, we will select brain areas known for early amyloid accumulation, namely the precuneus and the ACC (Aizenstein et al., 2008; Rowe et al., 2007). Such regions were also found to exhibit hypersynchrony in MCI to AD converters (M. E. López et al., 2014; Pusil et al., 2019). We will study FC between these areas and the rest of the brain in the alpha band, known to be the most affected in the pre-clinical stages of sporadic AD (Adler, Brassen, & Jajcevic, 2003; Babiloni et al., 2006; Jelic et al., 2000; David López-Sanz, Bruña, et al., 2017; M. E.

López et al., 2014; Nakamura et al., 2018, 2017). As this is a hypothesis-driven study where we want to evaluate our population, results previously found in other works, we have mimicked their approach, using the same source reconstruction algorithm and the same FC metric. Finally, we will check the possible correlation between the FC results and neuropsychological tests, and hippocampal volume measurements. This one is the first MEG study of FC in young adults with a family history of AD to the best of our knowledge. We hypothesize that AD patients' young relatives will show increased synchronization in the alpha band in the regions selected compared to non-relatives age- and education-matched controls.

2.2. Material and methods

Participants

In this first study, two additional constraints to those indicated in Section 1.4.1 were imposed on the population: the participant must be older than 50 and younger than 65 years old; the participant must have a valid MEG recording and T1-weighted image. The first constraint remarks the spirit of the project and the study, which detects early signs of impairment due to AD. The second constraint is quality control, vital for any scientific study. Hence, the population in this first study consisted of 92 first-degree relatives and 35 non-relatives. The characteristics of the sample are displayed in Table 3.

Table 3. Sample characteristics.

	First-degree relatives	Controls
N	92	35
Age	56,58 ± 4,11	57,20 ± 4,35
<i>APOE</i> ε4 carriers	34 (+) / 58 (-)	8 (+) / 27 (-)
Sex	36 M/ 56 F	16 M / 19 F
MMSE	29,23 ± 0,82	29,14 ± 0,84
Years of education	16,48 ± 4,53	17,48 ± 5,86
Left hippocampus volume	3812,02 ± 508,00	3819,19 ± 447,62
Right hippocampus volume	3935,48 ± 440,24	3959,31 ± 398,07

Values are presented as mean ± standard deviation. M = Male; F = Female; (+) = Carrier; (-) = Non-carrier.

Source reconstruction and analyses

In this study, the sources were labeled were to the AAL atlas (Tzourio-Mazoyer et al., 2002), resulting in 1210 cortical sources clustered in 80 Regions of Interest (ROIs). The beamformers used to obtain the source-space time series were calculated using the covariance matrix of the data

filtered in low alpha (8 to 10 Hz) and high alpha (10 to 12 Hz). Consequently, the FC was estimated for these two classical bands.

The FC was estimated using the PLV, ranging from 0 to 1, i.e., no connectivity to full connectivity. PLV shows high test-retest reliability (Colclough et al., 2016; Garcés, Martín-Buro, & Maestú, 2016), rendering it adequate to estimate FC in a meaningful way. The connectivity was calculated between the source locations belonging to the seed areas under study (namely, right and left precuneus and right and left anterior cingulate) and the rest of the cortical sources, resulting in a 1x1210 PLV vector for each area and band. The intra-area connectivity values were ignored. Figure 27 shows a visual overview of this process.

The 1210 cortical sources' power spectrum was calculated using averaged periodograms, a Hann window, and 0.25 Hz of spectral resolution. The relative power of low alpha and high alpha was calculated as the ratio between each band's power and the total broadband power between 2 and 45 Hz, resulting in a value ranging from 0 to 1. The mean power spectrum of all the source locations belonging to that area was calculated to obtain a specific power spectrum per area.

Figure 27. Estimation of the functional connectivity.

(A) The PLV value is obtained for each of the 1210 cortical sources. (B) Based on the AAL atlas, the sources belonging to the corresponding seed areas are selected. (C) The mean seed-with-all connectivity vectors are calculated and then averaged across subjects to perform the group comparison. (D) Finally, a CBPT with a t-test as the basic statistic is performed.

Statistical analyses

First, demographic characteristics were assessed. Age, MMSE, and years of education distributions were compared with a t-test, while sex and *APOE* $\epsilon 4$ carriage distributions were compared with a chi-square test.

FC's differences were addressed using a non-parametric cluster-based permutation test (CBPT) (Bullmore et al., 1999). This analysis reveals significant differences in cortical sources without the spatial constraint of atlas-defined areas. We used an independent sample t-test based on CBPT, with both the source-level and the cluster-level significance thresholds set to 0.05. The CBPT deals with the multiple comparison problem for the multiplicity of sources, but not with the multitude of frequency bands and areas. Therefore, we also applied a false discovery rate (FDR) correction (Benjamini & Hochberg, 1995) to the cluster significance level, taking into account the two frequency bands and the four seed areas under study.

PLV is known to be affected by source leakage; this is, the spatial smoothing introduced while solving the inverse problem (Schoffelen & Gross, 2009). If two nearby sources are spatially smoothed, the activity of each of them *leakages* into the other one, spuriously increasing the FC estimation. Two different factors can originate differences in FC due to leakage: first, the spatial smoothing in one group can be more extensive than in the other, directly increasing the leakage; second, the strength of the electrophysiological activity can be more extensive in one group than in the other, causing the spatial smoothing to reach farther distances before becoming negligible. The first effect can be estimated from the beamformer weights' correlation (Garcés et al., 2014) between the two reconstructed sources. The second can be calculated from the reconstructed signal's power (Muthukumaraswamy & Singh, 2011). To control these two proxies of source leakage, we checked all the results found in the cluster-based procedure using an ANOVA contrast, where the source power (in both the seed and the target) and the beamformer correlation weights, together with their interactions, were used as covariates. This approach removes these proxies' effects for source leakage, allowing for a more precise interpretation of the results.

We conducted a set of Pearson correlation tests to link significant FC differences with neuropsychological performances and cortical integrity. The mean PLV values of the significant clusters were compared separately with those of the neuropsychological tests and the left and right hippocampus volume. An FDR correction (Benjamini & Hochberg, 1995) was applied to each of the three correlation tests, taking into account the number of significant clusters and the number of comparisons.

Finally, the influence of being an *APOE* $\epsilon 4$ carrier was addressed. A two-way ANOVA test, with being relative and being an *APOE* $\epsilon 4$ carrier as factors, was performed in the significant clusters. Furthermore, we tested the influence of being an *APOE* $\epsilon 4$ carrier in the risk group using an independent samples t-test in the significant clusters.

Statistical analysis was performed using FieldTrip software (Oostenveld, Fries, Maris, & Schoffelen, 2011) and a set of in-house MATLAB scripts.

2.3. Results

Sample Characteristics

We did not find between-group differences in any of the characteristics controlled in this study. The results are displayed in Table 4. As well, no differences between groups were found in the neuropsychological test. Results are shown in Supplementary Table 1 (page 103).

Table 4. Statistical analyses on the sample characteristics.

	p-value	Effect size
N		
Age	0,454	0,149
<i>APOE</i> ε4 carriers	0,194	0,134
Sex	0,637	0,060
MMSE	0,592	0,103
Years of education	0,114	0,203
Left hippocampus volume	0,927	0,014
Right hippocampus volume	0,337	0,056

We performed a t-test for age, MMSE, and years of education, and hippocampal volume. In addition, we conducted a chi-square test for the percentage of *APOE* ε4 carriers and sex distribution. The effect size for the t-test was obtained using Cohen's D. The effect size of the chi-square test was obtained using Cramer's V. M = Male; F = Female; (+) = Carrier; (-) = Non-carrier.

Functional Connectivity

As an overall result, we found a hypersynchronization in the high alpha band among anterior and posterior areas and the dorsal and middle cingulate cortex in the group of first-degree relatives. Specifically, we found a hypersynchronization between the left ACC and a posterior cluster comprising both left and right posterior cingulate cortex, the left inferior occipital area, and a part of the left precuneus (Figure 28A). A hypersynchronization between the left precuneus and the left posterior and middle cingulate cortices, the posterior segment of the superior frontal gyrus, and the right precuneus was found (Figure 28B). Finally, we found a hypersynchronization between the right precuneus and left and right middle cingulate cortices, the right posterior cingulate cortex, and both left and right superior frontal gyri (Figure 28C). We found no significant results using the right ACC as seed. We found no significant results in the low alpha band. We present the results in Table 5.

For the sake of clarity, we only included significant effects. To get a better intuition of the results, we also pictured them in Figure 28.

Table 5. Significant functional connectivity values.

Seed areas	PLV (mean $\pm$ SD)		<i>p</i> -value	Effect size	Ratio
	Relatives	Controls			
Left anterior cingulate cortex	0,375 $\pm$ 0,016	0,359 $\pm$ 0,015	0,025	0,559	83,33%
Left precuneus	0,463 $\pm$ 0,032	0,436 $\pm$ 0,026	0,034	0,513	77,36%
Right precuneus	0,448 $\pm$ 0,034	0,420 $\pm$ 0,033	0,026	0,519	75,00%

PLV values are presented as mean $\pm$ standard deviation (SD). The *p*-values were obtained using a non-parametric cluster-based permutation test and with FDR correction. The effect size was obtained through Cohen's *D*, using each cluster's mean *t*-statistic for the calculation. The ratio expresses the percentage of sources that remain significant after the leakage correction. The ratio of leakage-corrected findings (last column) was calculated using an ANOVA model, including the correlation of beamformer weights and the source power as covariates. For the sake of clarity, only significant results are presented.

Correlation Analyses

No significant correlations were found between the FC clusters and the scores in any neuropsychological tests administered. Besides, there were no significant correlations between the FC clusters and the hippocampal volumes. The results are displayed in Supplementary Table 2 (page 104).

PLV leakage problem

Table 5 shows the ratio of links (i.e., members of the identified cluster), which remained significant, for each seed, after including the proxies for source leakage in the ANOVA model. In all the cases, this ratio was above 75%.

Figure 28. Functional connectivity results.

Functional connectivity results between the areas under study, namely (A) left anterior cingulate cortex, (B) left precuneus, and (C) right precuneus. On the left panel, we present the clusters with significant differences in functional connectivity. Green areas represent the seed area under study, and the red ones represent the clusters with significant differences in FC with the seed area. The red color map indicates a hypersynchronization in the relatives' group between the seed and the cluster. The red color map represents the t-statistic values of each source of the significant cluster obtained using an independent samples *t*-test. The FC's average between the seed area and the significant cluster is presented on the right panel. The plot shows each subject's PLV value (the crosses represent outlier values), the probability density estimates of the data, the sample median, and the quartiles' boundaries.

APOE $\epsilon 4$ Factor

The ANOVA results (with being a relative and an *APOE* $\epsilon 4$ carrier as factors) showed that being an *APOE* $\epsilon 4$ carrier does not affect the obtained clusters. However, being a relative is still significant in those clusters, even when controlling for the *APOE* genotype. In the same way, there is no

difference between *APOE* $\epsilon 4$ carriers and non-carriers within the relatives' group. The results are displayed in Table 6.

Table 6. Significance of being relative and being *APOE* $\epsilon 4$ carrier.

			ANOVA			t-test
			Relatives	<i>APOE</i> $\epsilon 4$ carrier	Interaction	<i>APOE</i> $\epsilon 4$ carrier
Left anterior cingulate cortex	p-values		< 0,001	0,361	0,749	0,195
	Effect size		0,138	0,005	< 0,001	0,281
Left precuneus	p-values		< 0,001	0,678	0,890	0,780
	Effect size		0,096	0,001	< 0,001	0,060
Right precuneus	p-values		< 0,001	0,610	0,707	0,353
	Effect size		0,090	0,001	0,001	0,201

The mean PLV value of each significant cluster is compared. P-values obtained from the two-way ANOVA test and the independent samples t-test are presented. The effect size was obtained using Cohen's D. The area names are defined by the AAL atlas.

2.4. Discussion

The present study demonstrates that young and cognitively healthy adults at increased risk of developing sporadic AD increased the synchronization of their anterior-posterior functional networks in the higher alpha band. This profile of hypersynchronization was found mainly between the left ACC and left and right precuneus with the rest of the brain.

The group of first-degree relatives of AD patients did not differ in neuropsychological performance or hippocampal volume from the control group of age and educational level matched participants. Therefore, group differences cannot be attributed to cognitive or morphological factors. The study of two risk factors combined, being an *APOE* $\epsilon 4$ carrier and being a first-degree relative, showed that being an *APOE* $\epsilon 4$ carrier does not affect the hypersynchronization pattern. These results suggest that decades before the typical age at onset of cognitive impairment, a brain network dysfunction could be identified.

Studies in human tissue and animal models of AD showed, in the vicinity of amyloid plaques, a reduction of GABAergic inhibition of hyperactive neurons and enhanced GABAergic inhibition of silent neurons (Busche & Konnerth, 2016; Garcia-Marin et al., 2009). This E/I imbalance produces hyperactivity leading to increased synchronization. Furthermore, the presence of A β oligomers impairs the oscillatory activity between widely distributed cortical areas. In this sense, the increased

synchrony of the oscillatory activity found in the current study could result from an early amyloid deposition leading to an E/I unbalance.

The seed areas selected for this study (ACC and precuneus) are the earliest and most affected AD regions. They are also critical nodes of the DMN, which has been proven to accumulate A β in its key nodes (Aizenstein et al., 2008; Rowe et al., 2007). Increased alpha band synchrony in both local (Nakamura et al., 2018) and long-range networks (Nakamura et al., 2017) has been associated with an increased A β burden in the frontal lobe and posterior regions, respectively. Therefore, our results suggest that the accumulation of A β in certain brain regions alters brain networks within this specific frequency band. Also, the particular brain regions under study are areas where previous works showed hypersynchronization in AD (Koelewijn et al., 2017, 2019; Ranasinghe et al., 2014), MCI (Bajo et al., 2012; Canuet et al., 2015; María Eugénía López et al., 2014; Nakamura et al., 2017) and SCD populations (Hafkemeijer et al., 2013; David López-Sanz, Bruña, et al., 2017).

Taking all these works into account, our lab proposed the “X” model (Pusil et al., 2019), which predicts the pattern of change in brain synchrony dynamics from MCI to dementia. In the same direction, the sample in this study presented a profile of hypersynchronization in the alpha band in the same brain networks described by the “X” model, indicating an early network disruption without brain atrophy or cognitive impairment. The subjects included in this study were all in the range of 50 to 65 years old and well adapted to their social or work activities, not reporting cognitive complaints. Therefore, this neurophysiological pattern could be classified as a subclinical sign of network disruption. Besides, this hypersynchronization could be reflecting a personal neurophysiological trait that increases the risk for dementia, and it might be incorporated into other factors such as diet, physical exercise, cognitive reserve, or genetics. The sample used in this study is currently enrolled in a longitudinal study to evaluate a possible clinical progression over time; therefore, some of these hypotheses could be better evaluated during the follow-up period.

It is also interesting to notice that being an *APOE* ϵ 4 carrier seems not to affect such an early stage of the continuum. Our first guess was that the effect could have been diluted due to the low sample of control *APOE* ϵ 4 non-carriers (8 subjects). For this reason, we investigated the impact of being an *APOE* ϵ 4 carrier within the group of first-degree relatives, where the number of participants was similar in both groups. This comparison did not provide statistically significant results, indicating a more substantial effect of being a first-degree relative than being a carrier of the *APOE* ϵ 4.

To conclude, neuronal hyperactivation by itself can cause cell damage due to calcium toxicity. Therefore, increased brain synchrony early in time could be one of the factors contributing to cell

death, brain atrophy, and cognitive impairment (de Haan, van Straaten, Gouw, & Stam, 2017). It is then essential to further evaluate whether an early intervention over brain hypersynchronization can delay the disease's process. FC obtained from non-invasive neurophysiological recordings may become an important biomarker to characterize the AD continuum, even in preclinical stages.

Limitations

Unfortunately, A β deposition biomarkers could not be obtained for this study. Therefore, it should be a matter of future research to evaluate the effect of A β in FC in this same population of first-degree relatives of AD patients. Nevertheless, subjects in the present study are significantly younger than the average onset age of sporadic AD (Huff et al., 1987). Therefore, it is reasonable to suspect that the presence of A β would probably be in an oligomeric form, which is harder to detect (Yamin & Teplow, 2017).

LCMV source reconstruction is known to have problems when reconstructing highly correlated brain activities, as is the case of bilateral auditory stimulation (Dalal, Sekihara, & Nagarajan, 2006; Gascoyne, Furlong, Hillebrand, Worthen, & Witton, 2016; Popescu, Popescu, Chan, Blunt, & Lewine, 2008). However, when dealing with resting-state data, there is no reason to expect highly correlated brain activities among distant brain areas, rendering this issue unlikely to affect us. While there are alternative approaches, such as MCMV beamforming (Moiseev, Gaspar, Schneider, & Herdman, 2011; Nunes et al., 2020), that directly addressed the signal leakage, this method is only valid for scenarios where no more than ten sources are estimated (Nunes et al., 2020). This constraint makes this approach unsuitable for the type of analysis presented in this work. However, it would be interesting to address this issue in a more direct manner using different analysis under a similar FC framework.

Also, the FC estimation through PLV might be affected by source leakage (Popescu et al., 2008), thus being spuriously overestimated. This study took into account two different proxies for source leakage, namely the correlation of the beamformer weights and the reconstructed activity's power. More than 75% of the results remained significant. While they do not guarantee that our findings are free from the effects of source leakage, these results indicate that they are unlikely to be driven by them. Although the methodology in this work has been successfully employed in the past (Garcés et al., 2014; David López-Sanz, Garcés, et al., 2017; María Eugenia López et al., 2014; Nakamura et al., 2017; Pusil et al., 2019), it is important to bear these methodological caveats in mind to obtain a complete intuition of the results.

Chapter 3

Structural connectivity in first-degree relatives of AD

3.1. Introduction

In recent years, the brain organization's concept has shifted to a complex system where different subnetworks support human cognitive functions. In this framework, neurodegenerative diseases would not solely affect the brain at a locally molecular level but the normal functions and interactions among these subnetworks. The evolution of impairment and the subnetworks involved would be unique for each neurodegenerative disease (Seeley et al., 2009). In the case of sporadic AD, the network-level malfunction would start in highly connected posterior regions, progressing to hyperconnectivity with ventral and anterior areas (Jones et al., 2016). This model, denominated cascading network failure, accords with the AD pathophysiology observed with other biomarkers.

DWI is a specific MRI technique that measures the movement of water particles in the brain (Le Bihan & Breton, 1985). The DWI information and water movement properties in the brain's different tissues allow extracting more complex information about the brain's structural integrity. DTI technique characterizes the magnitude, degree of anisotropy, and orientation of water diffusion and, thus, tests the brain's structural integrity. DTI technique has been widely used in the study of AD. The observation most commonly reported in advanced AD stages and MCI is a decrease in fractional anisotropy and an increase in mean diffusivity, especially in the hippocampal cingulum and in the posterior, temporal and parietal areas of white matter (Bozzali et al., 2002; Douaud et al., 2011; Mayo et al., 2019; Nir et al., 2013). Fewer studies might be found with cognitively healthy populations at risk of developing AD, but the reports show an initial impairment in posterior and temporal areas (Barbara B. Bendlin et al., 2010; Gold et al., 2010; C. D. Smith et al., 2010).

Nevertheless, since no considerable impairments are expected in the cognitively healthy population at risk, the classical straightforward comparison techniques might obscure subtle connectivity patterns. Amico et al. (2017) developed an independent component approach (*connICA*) that untangles different brain connectivity patterns present in the population under study to address this matter. This approach estimates independent connectivity patterns (or *traits*)

present in the whole population without stratifying subjects or classifying them. Instead, the presence of traits on each subject is evaluated *a posteriori* to assess possible associations with cognition or behavior. This framework has been used to identify connectivity traits related to levels of consciousness (Amico et al., 2017), to mild cognitive impairment and Alzheimer's disease (Contreras et al., 2017), structural-functional connectivity traits that support cognitive tasks (Amico & Goñi, 2018) and, more recently, family history of alcoholism (Amico et al., 2020).

This study estimates independent structural components under the framework of *connICA* using classical DTI measures and different cortical parcellation resolutions. We then test the association of the obtained traits with a collection of demographic, genetic, neuropsychological, and neurophysiological factors that have been proven to be related to AD and structural connectivity. Specially, we seek structural connectivity traits associated with the family history of AD.

3.2. Material and methods

Participants

In this study, only one additional constraint to those indicated in Section 1.4.1 was imposed on the population: the participant must have a valid T1-weighted and DWI image. Hence, the final sample finally consisted of 123 first-degree relatives and 61 non-relatives. The characteristics of the sample are displayed in Table 7.

Table 7. Demographic characteristics of the population.

	First-degree relative	Controls
N	123	61
Age	57,88 ± 6,85	62,72 ± 9,31
APOE ε4 carriers	46 (+) / 77 (-)	13 (+) / 48 (-)
Sex	42 M / 81 F	25 M / 36 F
Years of education	15,50 ± 3,84	16,92 ± 3,85
MoCA	26,12 ± 2,78	26,00 ± 2,54
TPA	1,44 ± 1,19	1,55 ± 1,16
Average cortical thickness	2,37 ± 0,07	2,35 ± 0,08
Hippocampi volume	3820,99 ± 377,23	3751,36 ± 445,17

Values are presented as mean ± standard deviation. TPA values are normalized by actigraphy wear time. The cortical thickness is expressed in *mm*. The hippocampus volume is the sum of left and right hippocampi volumes normalized by the estimated total intracranial volume. M = Male; F = Female; (+) = Carrier; (-) = Non-carrier; TPA = total physical activity.

Structural connectomes

This study used the cortex parcellation scheme proposed by (Schaefer et al., 2018). This parcellation scheme divides the cortex into seven functional networks with ten different spatial granularity levels (from 100 parcels up to 1000 parcels). For completeness of those atlases, subcortical regions were added as obtained using the *FIRST* command provided by FSL software (Patenaude, 2007). An example of a whole-brain structural connectome organized into hemispheres and resting-state networks is shown in Supplementary Figure 1 (page 99).

After the DWI processing, we obtained a set of structural connectomes corresponding to each of the ten Schaefer parcellations for each tractography. In particular, for each parcellation, structural connectomes were estimated based on the five measures presented in Section 1.3.2.2, namely, NoS, FA, ADiff, MDiff, and RDiff. Overall, this process results in 50 structural connectomes for each subject (5 structural measures and ten parcellations).

Independent Component Analysis of structural connectomes

The workflow to obtain independent components from structural connectomes is based on the *connICA* methodology used for functional connectomes (Amico et al., 2017). For a single parcellation and structural measure, each SC was transformed into a column vector keeping only the SC's upper triangular part. The SC vectors of the whole population were concatenated into a single matrix. To avoid possible noise sources, we performed a principal component analysis (PCA) and reconstructed the whole-population matrix using the number of components needed to explain 95% of the variance. Note that the number of PCA components needed is different for each measure and parcellation. Over this reconstructed matrix, we performed an ICA by running the *FastICA* algorithm (Hyvärinen, 1999). The number of independent components (ICs) was set to 20. For each IC, we obtained two output vectors: the first output vector will be referred to as SC-trait, and it represents the IC itself; the second output vector will be referred to as weights, and it quantifies the importance or the presence of this SC-trait in each subject. A scheme of this framework is shown in Figure 29. This process was repeated for all combinations of structural measures and parcellation schemes.

The non-deterministic nature of the *FastICA* algorithm represents a problem for the consistency of the solutions obtained. To address this issue, we ran the *FastICA* algorithm 500 times and imposed two constraints to keep only the “robust” SC-traits: first, it has to be present, at least, in 75% of the runs; second, an SC-trait from one run “is present” in another run if it has a correlation of 0.7 or higher with any of the SC-traits of that second run.

Figure 29. Scheme of the *connICA* framework applied to structural connectomes.

The original structural connectomes are vectorized and assembled to create a structural connectivity matrix for the whole population. The matrix is preprocessed using PCA, and an ICA is performed. The output consists of 20 SC-traits, presents in the whole population, and their associated weights, quantifying each subject's SC-trait presence.

Statistical analyses

The first statistical analysis compares each SC-trait presence in the relatives and control groups through a t-test. A t-test was performed using the weights of each of the robust SC-traits obtained. An FDR correction (Benjamini & Hochberg, 1995) was applied considering the number of robust SC-traits found, the number of structural measures, and the parcellation resolution to address the multiple comparison problem. The significance level for the FDR correction was set to $q < 0.05$.

The second statistical analysis consists of an incremental multiple linear regression (MLR) model using the weights of the robust SC-traits aforementioned and nine predictors. The selected predictors are a collection of demographic, genetic, neuropsychological, and neurophysiological factors that have been proven to be related to AD and SC. The last predictor added is a family history of AD to evaluate the isolated contribution of familial history when accounting for all other eight predictors. Predictors are presented in Table 7.

To avoid possible biases driven by our cohort's unbalanced stratification into family history (N=123) and controls (N=61), the MLR model was estimated using sampling without replacement. For each bootstrap iteration, 61 first-degree relatives are selected randomly without replacement. Only iterations where the 61 first-degree relatives selected show no significant differences with the control group in any remaining eight predictors are kept for further evaluation. We avoid any group bias on the predictors that might interact or confound with being first-degree relatives or not with this constraint. This procedure is repeated until obtaining 100 unbiased samplings (runs) of the family history group. For each sampling, the statistics of the corresponding MLR model are saved. Results are summarized by averaging the statistics across runs, namely: predicted values, standard residuals, predictability of the model (R^2), regression coefficients of each predictor (β), and t-statistic of each predictor. The p-value associated with each predictor is calculated using the averaged t-statistic. To address the multiple comparison problem, an FDR correction (Benjamini & Hochberg, 1995) was applied considering the number of robust SC-traits found, the number of predictors, the number of structural measures, and the parcellation resolution. The significance level for the FDR correction was set to $q < 0.05$.

Finally, to investigate the neuropsychological implications of the robust SC-traits, correlation tests between the SC-traits weights and the neuropsychological tests were performed.

3.3. Results

This section presents the SC patterns found in a young, cognitively healthy population using the *connICA* technique. This technique reveals independent components of structural connectivity, SC-traits, present in the whole population under study without any prior stratification. First, we study the presence of the robust SC-traits in the relatives and non-relatives groups. Finally, we study the association of these SC-traits with demographic, neuropsychological, and neurophysiological variables of interest utilizing a multiple linear regression.

Robust SC-traits

An SC-trait is defined as robust when it is present, at least, in 75% of the 500 runs as defined in (Amico et al., 2017). We explored the presence of robust SC-traits across structural measures and Schaefer parcellations. Results are shown in Table 8. Note that no further investigation on the traits or their possible association with demographic or cognition was performed for the cases where no robust SC-traits were found.

Table 8. The number of robust SC-traits found for each structural measure and atlas resolution.

Structural measures	Atlas resolution				
	100	200	300	400	500
NoS	3	9	8	5	8
FA	0	5	11	7	6
ADiff	0	15	12	11	11
MDiff	-	-	-	-	-
RDiff	-	-	-	-	-

Structural measures	Atlas resolution				
	600	700	800	900	1000
NoS	8	7	6	2	8
FA	9	10	10	10	10
ADiff	12	10	9	7	9
MDiff	-	-	-	-	-
RDiff	-	-	-	-	-

The dash symbol ('-') indicates the cases where it was impossible to obtain 20 independent components.

Presence of the SC-traits

One SC-trait survived the FDR correction when compared to the weights of the relatives and non-relatives groups. The SC-trait associated with a family history of AD is obtained with the FA measure and the 800 area parcellation. This SC-trait shows a pattern of altered interhemispheric connectivity, with a large negative cluster in the interhemispheric temporal-occipital connections. Figure 30 shows the significant SC-trait in its matrix form and the SC-trait presence in each subject (i.e., the weights obtained by *connICA*).

Figure 30. Family history of AD SC-trait.

On the left, the matrix represents the connectivity patterns of the SC-trait. The positive values (yellow) represent increased FA connectivity in the AD relatives group, and the negative values (blue) represent a decreased FA connectivity in the AD relatives group. On the right, the weights of this SC-trait for each subject, i.e., the presence of this SC-trait in each subject's connectivity pattern. The violin plots present the distribution of the weight of each group with the quartiles and the median. The p-value is the result of a two-tailed t-test. LH = Left hemisphere; RH = Right hemisphere.

Multiple linear regression results

The SC-trait associated with a family history of AD is further analyzed. The SC-trait was considered for associations with different characteristics of the subjects. To do so, we used demographic, genetic, neuropsychological, and neurophysiological factors as predictors in a multilinear regression model to predict the subjects' weights of the SC-trait.

The predictability of this MLR model using the nine predictors above was $R^2 = 0.2121$. We assessed each predictor's relative contribution to the model's general predictability, adding the predictors sequentially to the MLR model. The most relevant predictors for the MLR model were a family history of AD (relative contribution of 0.1084), sex (0.0341), and age (0.0303). The only predictor significantly associated with this SC-trait is a family history of AD ($t(112) = 3.8713, p = 0.0002$) with a regression coefficient $\beta = 0.0080$. The positive sign of the regression coefficient indicates that first-degree relatives have a greater presence of this SC-trait. The information of the MLR model is visually summarized in Figure 31. To see the complete information of the MLR model, refer to Supplementary Table 3 (page 105).

Figure 31. Multiple linear regression model.

The t-statistic is associated with each of the nine predictors used to define the top panel model. On the mid panel, the model's quality, i.e., each subject's real weight versus the weight predicted by the model and the error associated with each prediction. On the bottom panel, the contribution of each predictor to the overall predictability of the model.

Anatomical areas of the family history of AD SC-trait

To understand the SC-trait implications, we condensed the SC-trait into the seven functional networks defined by (Schaefer et al., 2018). First, the significant SC-trait is split into two sub-traits, reflecting enhanced and diminished integrity, respectively. As shown in Figure 30 and Figure 31, the

presence of this SC-trait is significantly higher in first-degree relatives. Hence, the enhanced integrity represents a pattern of increased integrity in the first-degree relative group, while the diminished integrity group presents decreased integrity in the first-degree relatives. Hence, from now on, we will refer to those sub-traits as family history (FH) enhanced and diminished integrity, respectively. We retained the giant component of each sub-trait's connectivity pattern, thereby discarding spurious edges involving one or a few brain areas (Goñi et al., 2013). Then, we estimated the presence of each resting-state network (RSN) as the sum of the weights of the within and between RSN edges divided by their respective total number of edges. The denominator takes into account the RSNs size so that results are comparable within and between RSNs. We hypothesize that FH subjects will not show an integrity gain in connectivity with respect to the control group. Instead, we expect them to possibly have a loss of structural integrity (Barbara B. Bendlin et al., 2010; Gold et al., 2010; C. D. Smith et al., 2010). Following this hypothesis, we use the highest value of RSNs presence in FH enhanced sub-trait (0.6076) as a threshold to detect FH diminished RSNs in the FH diminished sub-trait.

The three over-represented within and between RSNs in the FH diminished sub-trait are: visual-visual, visual-limbic, and visual-subcortical. Results are shown in Figure 32A. To get an anatomical visualization of the results, we extracted the streamlines from the original tractography for each over-represented result (Figure 32B).

Figure 32. Anatomical visualization of the family history of AD SC-trait.

The family history of AD SC-trait is split into one positive and one negative matrix, excluding spurious connections. The most active networks are estimated as the sum of the connectivity patterns of the areas belonging to that network divided by the numbers of areas. The most active networks were found in the negative matrix, and they were the visual-visual network (blue), the visual-limbic network (purple), and the visual-subcortical network (green). The tracts of these networks are visually presented over an MRI for clarity purposes.

This process revealed that the most active functional networks were found in the negative matrix, i.e., areas where the first-degree relatives present a reduced SC pattern, involving the visual-

visual network, the visual limbic network, and the visual-subcortical network as defined by (Schaefer et al., 2018). Anatomically, we observed a decreased connectivity pattern between posterior regions, between posterior and superior temporal regions (including the insula), and between posterior regions and the amygdala.

Neuropsychological tests correlations

None of the neuropsychological tests presented a significant correlation with the SC-trait weights.

3.4. Discussion

This chapter investigated the presence of disrupted structural connectivity patterns associated with AD's family history. The population under study is about one decade younger than the average onset of sporadic AD and presents no differences in AD's relevant markers, namely: *APOE* $\epsilon 4$ carriage, demographic characteristics, neuropsychological performance, hippocampi volume, and cortical thickness. The SC-traits were obtained for five different DTI measures and ten different brain parcellation resolutions using the *connICA* technique. The SC-traits obtained with this method are present in the whole population without any stratification of the subjects and without knowing if they have any relation with the variables of interest. The posterior MLR analysis revealed an SC-trait obtained from FA associated with the family history of AD. The SC-trait presents a reduced FA pattern in first-degree relatives in the tracts connecting posterior and temporal areas.

Therefore, this study's essence is the exploratory analysis of SC in a young, healthy population to reveal possible early effects of sporadic AD. With this purpose, we have selected the DTI measures most used in the literature and a brain parcellation scheme that allows us to obtain different levels of anatomical resolution of the same brain functional networks (Schaefer et al., 2018). Furthermore, the expected changes in the first-degree relatives might be so subtle that straightforward connectivity matrix comparisons may lose early AD signs in the young population at risk. The *connICA* technique extracts independent structural connectivity patterns (SC-traits) present in the population without any stratification or a priori assumption about the population. Later, we analyzed the association of the SC-traits to different variables of interest. This framework has been previously used in different experiments such as levels of consciousness (Amico et al., 2017), mild cognitive impairment and AD (Contreras et al., 2017), structural-functional connectivity traits that support cognitive tasks (Amico & Goñi, 2018), and family history of alcoholism (Amico et al., 2020).

Table 8 shows the diverse number of SC-traits obtained for each parcellation resolution and DTI measure, indicating our framework's benefits for an exploratory study.

The multiple linear regression models the relationship between the SC-traits and the demographic, genetic, neuropsychological, and neurophysiological factors relevant to sporadic AD. Among all the SC-traits, one showed a significant association with the family history of AD. Furthermore, AD's family history is the only significant predictor for this SC-trait, addressing current discussions in AD literature. The fact that the two groups do not differ in hippocampal volume or cortical thickness and the fact that these two predictors are not associated with this SC-trait support the idea that white matter alterations are not caused by Wallerian degeneration secondary to grey matter atrophy (Caballero et al., 2018; Gold et al., 2010; Zhuang et al., 2012). Neither being an *APOE* $\epsilon 4$ carrier is associated with this SC-trait, a surprising outcome previously reported in the literature (Barbara B. Bendlin et al., 2010; Chalmers, Wilcock, & Lovet, 2005). This same outcome was found in this population in a previous study of functional connectivity using MEG (Ramírez-Toraño et al., 2021). The null contribution of *APOE* $\epsilon 4$ to this SC-trait does not imply that *APOE* $\epsilon 4$ does not affect AD's progression. We can only infer the presence of an abnormal connectivity pattern related to the risk factor of family history of AD. *APOE* $\epsilon 4$ has been associated with amyloid plaques' deposition, which could be a different pathway of AD pathology than the disrupted white matter integrity. These two pathways could be interacting at some point in the pathology but seem relatively independent at this stage of the AD continuum.

The AD SC-trait family history shows decreased connectivity between posterior areas, between posterior areas and temporal areas, and between posterior areas and the amygdala. Decreased FA has been reported in preclinical AD, amnesic MCI, and clinically diagnosed AD dementia patients (Barbara B. Bendlin et al., 2010; Chua, Wen, Slavin, & Sachdev, 2008; Kantarci et al., 2017; Liu et al., 2011; L. Wang et al., 2009). Recently, (Jones et al., 2016) proposed a model for AD progression defined as a cascading network failure. According to this model: *“The failure begins in the posterior default mode network, which then shifts processing burden to other systems containing prominent connectivity hubs.”* The *“systems containing prominent connectivity hubs”* would be the temporal and frontal areas. The family history of AD SC-trait presented in this study might be supporting evidence of this model. The AD SC-trait family history resembles this model definition, presenting posterior-posterior and posterior-temporal abnormal connectivity patterns in the population at increased AD risk. These results might demonstrate an early structural impairment

before the presence of any clinical or neurophysiological alteration. The association of this finding with the disease's typical pathophysiology is hard to establish with the current data.

Nevertheless, the early disruption of the white matter integrity could be due to an initial effect of the tau-pathology associated with AD. The tau protein's hyperphosphorylation affects the axonal microtubules' structure and, consequently, the axon structure. This effect could be lastly seen in the reduction of the tract's integrity by DTI. This relationship has already been demonstrated in AD patients with posterior cortical atrophy (Sintini et al., 2019). This hypothesis needs to be tested by mixing SC and tau-PET scans in subjects with an AD family history.

The main limitation of the study design is the lack of A β and tau biomarkers. Nevertheless, this study's population age is significantly younger than the average onset age of sporadic AD (Huff et al., 1987). Therefore, it is reasonable to suspect that the presence of A β would probably be in an oligomeric form, a form that is harder to detect than the plaques (Yamin & Teplow, 2017). The main limitation of the methodological design is the instability or non-deterministic nature of the independent component analysis. As commented in the Method section of this chapter, we addressed this limitation by enforcing the use of “robust” components as defined by (Amico et al., 2017).

There are two main lines of future work for this study. As shown in Chapter 2, we found exciting results in the same population regarding MEG FC. Thus, the next study will try to model the association (if any) between the FC and SC patterns found in the population. In parallel, the sample used in this study is currently enrolled in a longitudinal study to evaluate a possible clinical progression over time; therefore, some of the hypotheses presented could be better evaluated during the follow-up period. Finally, the inclusion of additional neuroimaging techniques as tau-PET could help interpret the current results.

Chapter 4

On the association between MEG functional connectivity and structural connectivity

4.1. Introduction

In this third, final chapter, we will explore the possible relationship between FC and SC changes discovered in chapters 2 and 3, respectively. In sporadic AD, the cascading network failure model characterizes the disease's progression starting in highly connected posterior regions progressing to hyperconnectivity with ventral and anterior areas (Jones et al., 2016). Such impairment concerns physical anatomical connections (SC) between networks and their interactions (FC).

The FC impairment due to AD has been widely characterized and reported in the literature. One of the most consistent results is related to the FC disruption in the alpha band, although this disruption's characteristics depend on the AD continuum stages. As a general, simplified description, the FC of people who eventually develop AD will increase along with AD progression until a drastic decrease of FC in the final stages of AD (compared to age-matched controls with no symptoms). More in detail, hypersynchronization in the posterior areas has been reported in preclinical stages of AD, such as first-degree relatives of patients with AD ((Ramírez-Toraño et al., 2021), see Chapter 2) and people with SCD (David López-Sanz, Bruña, et al., 2017). This hypersynchronization is reported in the MCI stage in more areas of the brain (Bajo et al., 2010; David López-Sanz, Bruña, et al., 2017; María Eugenia López et al., 2014). A recent longitudinal study captured the transition from hyper to hyposynchronization in the MCI stage: people with MCI who later developed AD showed an initial hypersynchronization and a final hyposynchronization compared to people with MCI whose diagnosis remained stable at the end of the longitudinal study. As already mentioned, Pusil et al. (2019) define this phenomenon as the 'X model'. Finally, a network disruption characterized as a hyposynchronization pattern is found in AD patients (Damoiseaux, Prater, Miller, & Greicius, 2012; C. J. Stam et al., 2006).

Regarding SC, NoS and FA have been widely used to characterize the AD continuum. In comparison with FC, structural changes in AD's preclinical stages are more subtle, resulting in different reported results (Selnes et al., 2012; Y. Wang et al., 2012). In the MCI stage, more consistent results can be found in the literature. MCI patients present a loss of fibers in temporal, parietal, and frontal areas (Filippi et al., 2020; Tucholka et al., 2018), decreased FA in the posterior cingulate (Chua et al., 2009), and white matter tracts related to the corpus callosum (Liu et al., 2011; L. Wang et al., 2009). This tendency to fibers loss and decreased FA is worsened with the AD progression, and it is widespread across the brain (Liu et al., 2011; Qin, Guo, McClure, & Mu, 2020).

The relationship between FC and SC has been studied for years, mostly using fMRI to define the FC (Buckner et al., 2009; Greicius, Supekar, Menon, & Dougherty, 2009; C. J. Honey et al., 2009; Van Den Heuvel, Mandl, Kahn, & Hulshoff Pol, 2009). In addition, some studies have used EEG/MEG to define the FC, although they are not many as the ones using fMRI. Chu et al. (2015), using EEG and probabilistic tractography, observed that direct and indirect anatomical connections shape FC. This relationship is more critical in high-frequency bands, which require the existence of direct structural connections. Other studies have found a correlation between SC and FC using fMRI and MEG (Garcés, Pereda, et al., 2016). The same authors had previously used MEG-FC and SC to characterize pathological processes in MCI (Garcés et al., 2014) and showed that MCI presents a disruption in the alpha band and lower white matter integrity as measure by FA both results per the literature. Furthermore, these results presented a direct correlation in the MCI group between SC and FC changes.

The study described henceforth had two main objectives. The first one was to characterize the functional and structural connectivity in a young and cognitively healthy population using three variants of PLV and two SC measures obtained from probabilistic tractography. The second one consisted of examining whether the FC-SC relationship is affected or modulated by AD's family history.

4.2. Material and methods

Participants

In this study, two additional constraints to those indicated in Section 1.4.1 were imposed on the population: the participant must be older than 50 and younger than 65 years old; the participant must have a valid MEG recording, T1-weighted image, and DWI. Hence, the population in this third

study consisted of 83 first-degree relatives and 34 non-relatives. The characteristics of the sample are shown in Table 9.

Table 9. Sample characteristics.

	Relatives	Controls
N	83	34
Age	56,39 ± 4,11	57,03 ± 4,30
APOE ε4 carriers	32 (+) / 51 (-)	7 (+) / 27 (-)
Sex	32 M / 51 F	16 M / 18 F
MMSE	29,20 ± 0,82	29,12 ± 0,84
Years of education	16,04 ± 3,46	16,97 ± 3,84
Left hippocampus volume	3828,66 ± 720,73	3814,16 ± 453,35
Right hippocampus volume	3905,66 ± 699,03	3949,60 ± 399,82

Values are presented as mean ± standard deviation. M = Male; F = Female; (+) = Carrier; (-) = Non-carrier.

Maximal information coefficient

The maximal information coefficient (MIC) is a measure of dependence for a two-variable relationship. MIC belongs to a larger class of maximal information-based nonparametric exploration (MINE) statistics for identifying and classifying relationships (D. N. Reshef et al., 2011). As a simple concept, MIC is based on the idea that if there is a relationship between two variables, this relationship can be encapsulated dividing the scatterplot of the two variables using a specific grid.

Let define two random variables, X and Y , and a set of n observation pairs $(x_1, y_1) \dots (x_n, y_n)$ that can be represented in a scatterplot. Over this scatterplot, a set of grids $G_i(k, l)$ is defined, where k and l are the number of rows and columns of the grid, respectively. Furthermore, different grids are defined as modifying the grid cells' width and height for the same number of rows and columns. For each grid, the mutual information induced on the grid cells is estimated, where the probability of a box is proportional to the number of points falling inside the cell. Then, the mutual information is normalized, taking into account the number of rows and columns of the grid. This normalization allows obtaining values between 0 and 1, and it ensures a fair comparison between grids. With all these values, the characteristic matrix $M(X, Y) = (m_{k,l})$ is defined, where $m_{k,l}$ is the highest normalized mutual information achieved by any of the k -by- l grids. Finally, MIC is the maximum value in M . Formally, this measure is defined as:

$$\begin{aligned}
MIC &= \sup M(X, Y), \text{ where} \\
M(X, Y) &= (m_{ij}) \in \mathbb{R}^{k \times l} \\
m_{i,j} &= \max\left\{\frac{I_G}{\log(\min\{i, j\})}\right\}
\end{aligned} \tag{14}$$

There has been a lengthy discussion on the literature on MIC properties. Specifically, whether it possesses *equitability*, i.e., its value is lower if the association between the two variables decreases no matter how the relationship's nature may be. As a result of such discussion, the same authors later defined a new index associated with MIC: the total information coefficient (TIC) (D. N. Reshef, Reshef, Sabeti, & Mitzenmacher, 2018; Y. A. Reshef, Reshef, Finucane, Sabeti, & Mitzenmacher, 2016). TIC is defined as the sum of the entries of $M(X, Y)$. Formally:

$$TIC = \sum_{\substack{i \leq k \\ j \leq l}} m_{i,j} \tag{15}$$

Estimating the supreme of a matrix involves taking the maximum over a set of estimation. Thus, this procedure may lead to an undesirable positive bias in the decision of statistical independence. This bias is provoked by disregarding all the useful matrix information apart from its maximum. If a more stable property is used, such as the sum of the entries in the characteristic matrix, it may reduce the bias and improve the statistical power. Therefore, TIC is useful for testing for the presence or absence of a relationship rather than quantifying the relationship strength (Albanese, Riccadonna, Donati, & Franceschi, 2018).

To get a better intuition, Figure 33 presents the process of MIC and TIC estimation.

Figure 33. MIC estimation.

For a bivariate signal, grids of different resolutions are used until a maximal resolution of k rows and l columns. For each cell of a grid, the mutual information is estimated proportionally to the number of points falling inside the cell. Then, the mutual information of a specific grid resolution is normalized, considering the grid's number of cells. Once all the values are normalized, the characteristic matrix is constructed, selecting the grids with the maximum normalized mutual information for each resolution. Finally, MIC and TIC values are obtained from the characteristic matrix. Modified from (D. N. Reshef et al., 2011).

An essential property of MIC/TIC is that they can estimate the relationship between two variables that may be of a different type, as the two indices use the probability distribution of the variables rather than their values. They make them ideal candidates to explore the structure-function relationship in the brain from SC and FC matrices. However, to the best of our knowledge, this is the first study undertaking such analysis.

Functional and structural connectivity associations

The idea is to obtain, for each subject, a pair of vectors representing the FC and SC to estimate the MIC and TIC measures. To focus the study, we have selected parameters and measures of interest based on the results of the previous studies. Regarding functional connectivity, we selected the three measures presented in Section 1.3.1.5 (PLV, $\text{PLV}_{\text{unbiased}}$, and ciPLV) in the two frequency bands of interest (low and high alpha). Regarding SC, the two measures of interest selected were

the NoS and FA. All matrixes range from 0 to 1 except the NoS matrixes. The NoS matrix was normalized using the maximum value across all subjects to compare measures within the same range. Thus, we obtained eight symmetrical matrixes per subject (two structural ones and three functional ones in two frequency bands) of 90 x 90, whose values range from 0 to 1.

Depending on the information extracted from each matrix, different relationships can be obtained. First, the matrixes were preprocessed to compare whole-brain FC and SC in a young and cognitively healthy population. Second, the matrixes were preprocessed to compare FC and SC between the areas of interest revealed by the FC study (precuneus and ACC) and the rest of the brain.

Accordingly, the first preprocessing workflow consists of:

- Since the matrixes are symmetrical, we only considered their upper triangular part.
- The diagonals of the matrixes were also discarded to remove the areas' intra-connectivity,
- MEG functional connectivity is not reliable in subcortical sources when estimating whole-brain FC (Attal & Schwartz, 2013; Krishnaswamy et al., 2017). Therefore, the entries related to the amygdala, caudate nucleus, putamen, pallidum, and thalamus were discarded from both FC and SC matrixes.
- Finally, we address the problem of areas without SC between them. There are entries with value zero in the SC matrix, i.e., areas not physically connected but maybe functionally connected. This situation should be considered abnormal when analyzing the FC-SC relation using dependence measures such as MIC or TIC. Accordingly, entries with value zero in the SC matrix were discarded from further analysis.

The pre-processing workflow explained above transforms each matrix of 90x90 into a vector of 1x1389 entries ranging from 0 to 1. The vectors were averaged across subjects resulting in eight vectors (three functional for each of the two frequency bands and two structural) representing the whole sample. A TIC value (TIC_{whole_head}) was obtained for each of the twelve combinations of functional and structural relations using a default maximal-allowed grid resolution of 9 cells. This resolution guarantees good performance in terms of statistical power against independence in most situations (D. N. Reshef et al., 2018). This process is visually presented in Figure 34.

Figure 34. MIC and TIC estimation of the whole-head connectivity.

In this case, both matrixes are vectorized, keeping the upper triangular of each one. From each vector, we removed the subcortical areas as well as those without structural connections. Finally, we estimated the MIC and TIC between the FC and the SC vectors. For clarity purposes, the matrixes presented have a significantly smaller size than the study's actual ones.

For the second preprocessing, we selected PLV as the functional measure. The reasoning is explained in Section 4.3. Using again the original matrixes, the second preprocessing workflow consists of:

- For a specific area, its row corresponding is extracted, obtaining a vector of 1 x 90.
- The intra-connectivity entry is discarded.
- The amygdala, caudate nucleus, putamen, pallidum, and thalamus were discarded from any matrix.
- Finally, entries with value zero in the SC matrix were discarded.

After this pre-processing step, 16 vectors of different lengths were obtained (the possible combinations of two structural measures and two frequency bands for each of the four anatomical areas of interest). The vectors' length depends on the number of zeroes in the SC row for each area. The vectors were averaged across subjects representing the whole sample. A TIC and a MIC value (TIC_{area} and MIC_{area} respectively) was obtained for each of the combinations of structural measures, frequency bands, and anatomical areas of interest using a default maximal-allowed grid resolution of 9 cells. This resolution guarantees good performance in terms of statistical power against independence in most situations (D. N. Reshef et al., 2018). This process is visually presented in Figure 35.

Figure 35. Selected areas.

In this case, both matrixes are vectorized, keeping the row of interest corresponding to a specific cortical area under study. From each vector, the subcortical areas and the areas without structural connections are removed. Finally, with the pair FC-SC, the MIC and TIC values are estimated. As in the former Figure, the actual matrixes were larger than the ones depicted here.

Statistical analyses

Age, MMSE, and years of education distributions were compared with a t-test, while sex and *APOE* ε4 carriage distributions were compared with a chi-square test.

We tested the dependence between whole-brain FC and SC brain using a permutation-based strategy. This first step allows checking if the TIC measure can capture any FC-SC association in a young, cognitively healthy population. The process is explained for a pair of functional and structural connectivity vectors. Once the original TIC value for the whole head (TIC_{whole_head}) is stored, an empirical null distribution needs to be estimated to obtain the associated p -value. The null distribution was estimated using 200.000 iterations, as suggested in (Albanese et al., 2018). In every iteration, the structural vector is randomly permuted. A new TIC value (TIC_{iperm}) is estimated using the original FC vector and the *permuted* SC vector. Finally, the associated p -value of this pair of FC-SC vectors is the fraction of TIC_{iperm} that exceeds TIC_{whole_head} . Formally:

$$p\text{-value} = \frac{1 + \#\{TIC_{iperm} > TIC_{whole_head}\}}{1 + \text{number of permutations}} \quad (16)$$

Secondly, we tested if the whole-brain FC-SC relationship (if any) yields for the areas of interest (precuneus and ACC). We used here the same permutation-based strategy: the null

distribution was estimated again using 200.000 iterations. In this case, the value used was TIC_{area} for each of the possible combinations.

Finally, we tested if the FC-SC relationship in each of the two areas is different in first-degree relatives and controls. First, we tested if there were differences in the structural data that may bias the results. The difference of NoS and FA between the selected areas and the rest of the brain was tested using a t-test. For that purpose, each vector obtained in the second preprocessing workflow was averaged to obtain a mean connectivity measure of that area with the rest of the brain, and then, these values were compared using a t-test. Second, the association between functional and structural connectivity was tested using the MIC_{area} . The difference between MIC_{area} values between first-degree relatives and controls were tested through a t-test.

Finally, we applied an FDR correction (Benjamini & Hochberg, 1995) to all tests to consider the number of SC and FC measures, frequency bands, and areas of interest depending on each case of study to address the multiple comparison problem. The significance level for the FDR correction was set to $q < 0.05$.

4.3. Results

As an overall result, we did find an FC-SC relationship in a young, cognitively healthy population. This relationship holds both for whole-brain and seed-based connectivity. Finally, we did not find any difference between first-degree relatives and controls in the FC-SC relationship.

Sample characteristics

We did not find between-group differences in any of the characteristics controlled in this study. The results are displayed in Table 10.

Table 10. Statistical analyses on the sample characteristics.

	p-value	Effect size
N		
Age	0,449	0,155
<i>APOE</i> ϵ 4 carriers	0,061	0,173
Sex	0,396	0,079
MMSE	0,607	0,105
Years of education	0,202	0,261
Left hippocampus volume	0,869	0,034
Right hippocampus volume	0,564	0,118

We performed a t-test for age, MMSE, and years of education, and hippocampal volume. In addition, we conducted a chi-square test for the percentage of *APOE* ϵ 4 carriers and sex distribution. The effect size for the t-test was obtained using Cohen's D. The effect size of the chi-square test was obtained using Cramer's V. M = Male; F = Female; (+) = Carrier; (-) = Non-carrier.

Functional and structural connectivity dependence

All combinations of functional and structural connectivity matrixes present significant dependence. When visually inspected, NoS presents a significant direct correlation with all the FC measures. On the other hand, FA presents a significant inverse correlation with all the FC measures. The results are visually presented in Figure 36.

Figure 36. Whole brain FC-SC relationship

The figure presents the associations between functional and structural connectivity for all FC measures, SC measures, and frequency bands. All combinations presented significant dependence. The results showed a

direct correlation between NoS and the FC measures. The results showed an inverse correlation between FA and the FC measures.

The results showed that PLV and $PLV_{unbiased}$ captured the same information, as indicated by TIC_{whole_head} (Figure 36). Another remarkable result is the effect of ciPLV in the overall association between FC and SC. As we can see, the direct and inverse correlation of NoS and FA with the FC disappears when the ciPLV measure is used. This effect is due to the nature of ciPLV and the anatomical characteristics of the human brain. Essentially, ciPLV is defined to be immune to volume conduction and source leakage effects. These two problems affect mainly those sources that are closer within the brain. Thus, when ciPLV is used, such sources show lower values than with the PLV measure, just by definition of ciPLV. In addition, the brain's anatomical organization presents more structural connections between close areas than between distant areas. Thus, the definition of ciPLV “punishes” the FC of close areas and “punishes” areas that are more connected. This effect is shown in Figure 37.

Figure 37. Difference between PLV and ciPLV.

This figure shows the effect of ciPLV on both FC and SC. The ciPLV aims to remove spurious values due to the volume conduction and source leakage. Unfortunately, these problems are more associated with close brain sources that, at the same time, present more structural connections due to the anatomical properties of the brain. Thus, ciPLV “punishes” the FC and SC simultaneously, which is an undesirable outcome.

Considering these results, for the rest of the statistical analyses, we only used PLV.

Seed-based FC-SC relationship in the areas of interest

The results showed that the FC-SC relationship between the areas of interest and the rest of the brain aligns with the whole brain analysis results: NoS and FA present a significant direct and inverse correlation with PLV, respectively. This tendency holds for all frequency bands and all areas

of interest. It is noteworthy that these areas are not significantly affected by the natural bias of PLV. For the sake of clarity, only the results of one area are presented in Figure 38. The rest of the areas presented a similar behavior, and their results are presented in Supplementary Figure 2 (page 100).

Figure 38. Functional and structural dependence involving the right anterior cingulate cortex.

The figure presents the FC-SC relationships for all combinations of PLV, SC measures, and frequency bands. All combinations presented significant dependence. As it happened for the whole-brain FC-SC associations, the results showed a direct correlation between NoS and PLV and an inverse correlation between FA and PLV in all frequency bands.

Effect of family history of AD in the FC-SC associations

When comparing the SC of first-degree relatives and controls, there is no difference in NoS and FA that can bias the results. The average NoS between the right ACC and the rest of the brain differs in both groups. However, such a difference did hold after the FDR correction. The p-values and effect size of each test are presented in Table 11. The NoS and FA values for each group are presented in Supplementary Table 4 (page 106).

Table 11. Comparison of SC measures between groups.

Areas	SC measure	p-values	Effect size
Left ACC	NoS	0.626	0,010
	FA	0.405	0,170
Right ACC	NoS	0.008	0,546
	FA	0.237	0,242
Left precuneus	NoS	0.699	0,079
	FA	0,505	0,136
Right precuneus	NoS	0,248	0,236
	FA	0,507	0,135

Statistical results of the SC measures in both groups. The effect size for the t-test was obtained using Cohen's D.

The results of the t-test showed no differences in the MIC_{area} values between first-degree relatives and the control group. The p-values and effect size of each test are presented in Table 12. The values of MIC_{area} for each group are presented in Supplementary Table 5 (page 107).

Table 12. Significance of the FC-SC dependence between groups.

Area	Frequency band	t-test	p-values	Effect size
Left ACC	Low alpha	MIC_{NoS}	0,295	0,214
		MIC_{FA}	0,453	0,153
	High alpha	MIC_{NoS}	0,148	0,297
		MIC_{FA}	0,105	0,333
Right ACC	Low alpha	MIC_{NoS}	0,535	0,127
		MIC_{FA}	0,722	0,073
	High alpha	MIC_{NoS}	0,170	0,281
		MIC_{FA}	0,299	0,213
Left precuneus	Low alpha	MIC_{NoS}	0,498	0,139
		MIC_{FA}	0,422	0,164
	High alpha	MIC_{NoS}	0,228	0,247
		MIC_{FA}	0,533	0,128
Right precuneus	Low alpha	MIC_{NoS}	0,807	0,050
		MIC_{FA}	0,643	0,095
	High alpha	MIC_{NoS}	0,969	0,008
		MIC_{FA}	0,991	0,002

Statistical results of the FC-SC dependence in the areas of interest in both groups. The effect size for the t-test was obtained using Cohen's D.

4.4. Discussion

This study aimed to characterize the FC-SC relationship in a young and cognitively healthy population. In a second step, we tested if this relationship differs due to AD's family history, the risk factor for AD analyzed in this thesis. The FC was estimated using MEG and the PLV/icPLV measures, the SC was estimated using DTI, and the NoS and FA measures, and the degree of dependence was estimated using the MIC and TIC measures of the MINE statistics. The results showed a significant linear relationship between SC and FC measures when analyzing the whole-brain connectivity. Specifically, NoS presented a direct correlation with PLV while FA presented an inverse correlation with FA. These relationships yield significant analyzing the connectivity between the areas of interest (precuneus and ACC) and the rest of the brain. Finally, the level of dependence (or level of association) between FC and SC did not differ between first-degree relatives and controls.

To limit this exploratory study's scope, we used the information (or results) of the previous works presented in this thesis. To characterize FC, PLV, and two related measures, ciPLV and $PLV_{unbiased}$, have been used. The PLV measure has proved to allow detecting subtle changes in the population at risk of developing dementia (Section 2.3). The same frequency bands and the areas of interest of this study have been used, namely low and high alpha, precuneus, and ACC. The first test showed that PLV and $PLV_{unbiased}$ captures the same information, resulting in the same level of association. On the other hand, the nature of ciPLV and the human brain's anatomical characteristics makes ciPLV a bad estimator of FC for this particular study (Figure 37). For these reasons, FC was estimated using PLV in this study. Regarding SC, the significant measure FA observed in Section 3.3 has been used in this study. To obtain a complete view or information about SC, we decided to include NoS, although it presented no significance in the previous study. We argue that these two SC measures are complementary, giving a more comprehensive view of the SC “quality”: it is equally important to estimate the number of connections between two areas (NoS) than the integrity of these connections (FA). Finally, we did not have any *a priori* hypothesis about the nature of the relationship between FC and SC (linear, exponential, ...). Therefore, we reviewed the literature searching for dependence measures to detect a broader range of relationships beyond the linear one. MINE statistics have been proved to detect different kinds of associations (D. N. Reshef et al., 2011) and used in different scientific fields, not only neuroscience.

The first tests showed a linear relationship between FC and SC across the whole brain areas (Figure 36). These relationships are also present between the areas of interest and the rest of the brain (Figure 38 and Supplementary Figure 2). In the case of NoS, there is a direct correlation with

FC, a result extensively reported in the literature (Garcés et al., 2014; C. J. Honey et al., 2009; Skudlarski et al., 2008). Although the vast majority of studies reported that the underlying SC supports FC, the studies also point out that the underlying structure cannot fully explain all FC.

Since high values of FA, i.e., great axonal integrity, have been commonly associated with a healthy state, the inverse correlation of FA and PLV may cause a first confusing impression: an increased FA should improve the FC between areas. Therefore, the FC should increase. Taking into account the results of the first study of this thesis and results from the literature, an increased FC is not a desirable feature *per se*, but it can express neurodegeneration in diseases such as AD (D. López-Sanz et al., 2016; María Eugenia López et al., 2014; Pusil et al., 2019). In contrast to NoS, not many studies have tried to estimate the FC-SC association using FA as a structural measure (Straathof, Sinke, Dijkhuizen, & Otte, 2019). Furthermore, the correlation values reported are not very consistent, and many studies are difficult to compare to the one presented in this thesis. Hirsiger et al. (2016) reported no relation between cingulum's SC and FC, and Garcés et al. (2014) neither found any FC-SC correlation in areas of the DMN using an SC measure related to FA. Collin, Sporns, Mandl, & Van Den Heuvel (2014) defined three types of anatomical connections (rich-club connections, feeder connections, and local connections) and reported three different patterns of associations between FC-SC: they observed a direct correlation between FA and the FC measure in the rich-club connections and an inverse correlation between FA and the FC measure in the feeder and local connections.

Finally, no differences were found between first-degree relatives and the control group in the FC-SC associations. This result might have several explanations. As the comparison of NoS and FA between groups showed, there is no differences in SC, although the SC study (Section 3.3) showed differences in connectivity patterns. This could indicate that the impairment may be so subtle that we are not able to measure it. Even more, there may be no impairment yet considering the age and cognitive health of the population under study. This explanation does not seem like a valid or at least a complete one since the results of the two previous studies and those found in the literature showed differences in FC associated with AD's family history. The differences in FC and SC might be subtle enough that the differences are faded in the process by combining FC-SC. We have also compared the connectivity of the areas of interest with the rest in the brain, which is not the more precise approach. The first study showed differences between those areas and some clusters of cortical sources. The differences might have been shaded with all the connections with the rest of the brain.

As we have briefly discussed in this section, there is a contrast between the quasi-static nature of SC and the highly dynamic nature of FC. The decisions made to estimate the FC (time scale, frequency band, measure...) might have also affected this study's results. Regarding all the possible time scales that one can use to estimate FC, several studies showed that different time scales result in different FC-SC associations (Christopher J. Honey, Kötter, Breakspear, & Sporns, 2007; Messé, Hütt, König, & Hilgetag, 2015). Similarly, FC in different frequency bands is modulated by the distance between the brain areas (Chu et al., 2015; Wu et al., 2008), and the brain functional efficiency might arise from a trade-off between short- and long-distance structural connections (Sepulcre et al., 2010; Vértes et al., 2012). Finally, it is widely reported and accepted that the functional brain organization shifts depending on the person's state, for example, if the person is resting with no external output or performing any type of task (Fox et al., 2005). We have analyzed resting-state networks, while the first signs of impairments might be present in specific tasks and specific functional subnetworks. In summary, there might be an alteration caused by the family history of AD in one of the multiple FC-SC associations, but it may be that we did not evaluate the 'appropriate' FC-SC association.

This research opens several lines for future work. First, to use a personalized atlas that includes interest areas and the cluster of cortical sources that presented significant differences in the first study. This new atlas will allow obtaining more accurate results in the exploratory study. Second, to include in the study indirect structural connections. Although it is not a straightforward process, we will need to handle the indirect structural connections that affect FC, as seen in the literature. Third, the different FC-SC associations resulting from the selection of time scales, frequency bands, or cognitive tasks need further evaluation. Since FC has already shown significant results in the first study using that time scale and frequency band, it is of particular interest to evaluate FC-SC during a cognitive task. Fortunately, in addition to a resting-state MEG recording, the participants underwent a memory task during a MEG recording. The association of SC and the FC obtained during a memory task will be further evaluated. Finally, the participants are currently enrolled in a longitudinal study to evaluate a possible clinical progression over time; therefore, some of the FC-SC associations' impairment might be better evaluated during the follow-up period.

Chapter 5

General discussion

In this thesis, we included an individual discussion for each study. This chapter is devoted to sorting out and jointly discussing the different results obtained in the three studies to draw the thesis's general conclusions. Eventually, this chapter should allow any reader to grasp this thesis's spirit without going through each experiment.

AD is a neurodegenerative process that progressively impairs cognitive functions such as memory, language, and behavior, resulting in dementia in the final stage. AD has been defined as a continuum (Figure 4) that might start decades before the first neuropsychological symptoms are noticed (Morris, 2005). The different stages of the continuum have been defined based on the evolution of neuropsychological symptoms and the two neurophysiological hallmarks of AD: A β accumulation and tau hyperphosphorylation. For decades, several AD progression models have been proposed using the collected evidence of these hallmarks during the disease's evolution. Here, we present two models vital to understand and give perspective to the thesis's results: the cascading network failure model and the E/I imbalance.

The cascading network failure model (Jones et al., 2016) defines the brain as a complex system where different subnetworks support human cognitive functions. Under this view, neurodegenerative diseases would affect the brain at a local, molecular level, and these pathological changes would modify, at the system level, the interactions among these subnetworks. In the preclinical phase, regions with tau deposition start suffering a processing overload due to the degenerative tau process. These brain regions shift their preprocessing load to the posterior default mode network (DMN), a core-processing hub. The posterior DMN becomes overloaded and begins to shift its processing load to other connectivity hubs. This increased load leads to noisier synaptic activity and alterations in APP processing that eventually provokes the amyloid plaque formation. The inability to shift additional processing load, the uncompensated stress in the original system now leads to a rapid acceleration of the previously indolent tau-associated neurodegenerative process (Figure 9). In this loop, local degeneration will provoke functional connectivity impairments that lead to more severe local degeneration.

The E/I imbalance animal model (Busche & Konnerth, 2016) addresses the effect of A β presence in neurons' surroundings and how it escalates to the network level. During the preclinical stages, amyloid plaques and also soluble A β oligomers alter local neuronal circuits' function by disrupting the balance of synaptic excitation and inhibition. This E/I imbalance leads to hyperactivity in cortical and hippocampal neurons, a breakdown of slow-wave oscillations, and, eventually, network hypersynchrony.

Once we have laid out this theoretical framework, we can discuss our results. The thesis's main objective was the characterization of AD in its preclinical stages using neuroimaging techniques. A total of 262 young and cognitively healthy people volunteered for the study. Each participant underwent an extensive battery of medical tests, including neuropsychological tests, MRI scanners, MEG sessions, blood extraction and genotype tests, nutritional tests, and ophthalmological tests. Only a part of the vast information collected from the participants has been used in this thesis. Other colleagues in the lab currently carry out their research using the rest of the information, and much work remains to be done. Following this thesis's main objective, these 262 participants were classified as first-degree relatives of AD (population at higher risk of developing AD) and a control group. With these two groups, we tried to characterize AD's preclinical stages, focusing on three vital features of the brain: the functional connectivity, the structural connectivity, and the relationship between them. These three features resulted in three different studies presented in this thesis.

The first study was designed with a straightforward *a priori* hypothesis: there is a difference in FC between first-degree relatives of AD patients and the control group. Taking into account the age and cognitive status of the participants, we imposed two additional constraints: we evaluated the FC of areas known for early amyloid accumulation, namely precuneus and ACC (Aizenstein et al., 2008; Rowe et al., 2007), and we evaluated it in the alpha band, known to be the most affected frequency band in the pre-clinical stages of sporadic AD (Adler et al., 2003; Babiloni et al., 2006; Jelic et al., 2000).

The hypothesis is based on previous results from the lab and the literature. It is well-known that AD starts decades before the onset of clinical symptoms, and different neurophysiological changes have been observed using different neuroimaging techniques. In FC's case, a consistent and evolving effect along the AD continuum is the hypersynchronization of specific brain areas in the first stages of AD and a final hyposynchronization in the advanced AD, presumably due to the brain network's impairment. This evolution has been observed in SCD (David López-Sanz, Bruña, et al.,

2017), MCI (Bajo et al., 2012; Canuet et al., 2015; María Eugénía López et al., 2014; Nakamura et al., 2017) and MCI-to-AD converters (Pusil et al., 2019).

As an overall result, we found a hypersynchronization in the high alpha band in the first-degree relatives among anterior and posterior areas and the dorsal and middle cingulate cortex. Specifically, we found hypersynchronization between the left ACC and a posterior cluster comprising both left and right posterior cingulate cortex, the left inferior occipital area, and a part of the left precuneus (Figure 28A). A hypersynchronization between the left precuneus and the left posterior and middle cingulate cortices, the posterior segment of the superior frontal gyrus, and the right precuneus was found (Figure 28B). Finally, we found a hypersynchronization between the right precuneus and left and right middle cingulate cortices, the right posterior cingulate cortex, and both left and right superior frontal gyri (Figure 28C). We found no significant results using the right ACC as seed. We found no significant results in the low alpha band. Surprisingly, *APOE* ϵ 4 carriage seems to not affect functional connectivity at such an early stage of the continuum. Our results support this AD model's progression, suggesting that the hypersynchronization phenomenon is present at earlier stages of the disease in the population at risk. Therefore, increased brain synchrony early in time could be one of the factors contributing to cell death, brain atrophy, and cognitive impairment (de Haan et al., 2017). The FC obtained from non-invasive neurophysiological recordings may become an important biomarker to characterize the AD continuum, even in preclinical stages.

The second study, based on SC, has a laxer hypothesis: there is a difference in SC between the first-degree relatives and the control group. In contrast to the FC, we did not know if there would be changes in SC and if those changes (if any) could be measure or detectable at such an early stage of AD. Thus, we designed a study that could detect changes without prior constraints. First, we used an anatomical atlas with ten different resolution levels (from sparse to dense). Second, we used the framework of *connICA* (Amico et al., 2017) to detect subtle structural connectivity patterns (SC-traits). The presence of the SC-traits obtained in both groups was later compared to evaluate differences related to AD's family history.

One SC-trait, obtained with the FA measure and the 800 areas parcellation, was associated with AD's family history (Figure 30). This SC-trait showed a reduced interhemispheric connectivity pattern in the first-degree relatives, especially in the interhemispheric temporal-occipital connections. The SC-trait associated with a family history of AD was further analyzed using multiple linear regression. The SC-trait was considered for associations with demographic, genetic, neuropsychological, and neurophysiological factors as predictors in a multilinear regression model

to predict the weights of the SC-trait subjects. The most relevant predictors for the MLR model were family history of AD, sex, and age, being the family history of AD, the only factor significantly associated with this SC-trait (Figure 31).

These results have three critical interpretations. First, the AD SC-trait family history might be supporting evidence of the cascading network failure model. The family history of AD SC-trait resembles this model definition, presenting posterior-posterior and posterior-temporal abnormal connectivity patterns, as measured by FA, in the population at increased AD risk. Decreased FA has been reported in preclinical AD, amnesic MCI, and clinically diagnosed AD dementia patients (Barbara B. Bendlin et al., 2010; Chua et al., 2008; Kantarci et al., 2017; Liu et al., 2011; L. Wang et al., 2009). These results might demonstrate an early structural impairment caused by an initial effect of the tau-pathology associated with AD. Second, the fact that the two groups do not differ in hippocampal volume or cortical thickness and that these two predictors are not associated with this SC-trait support the idea that white matter alterations are not caused by Wallerian degeneration secondary to grey matter atrophy (Caballero et al., 2018; Gold et al., 2010; Zhuang et al., 2012). Thus, white matter alterations and grey matter impairments parallel the same neurodegenerative process. Third, as in the first study, being an *APOE* $\epsilon 4$ carrier is not associated with this SC-trait, a surprising outcome previously reported in the literature (Barbara B. Bendlin et al., 2010; Chalmers et al., 2005). The null contribution of *APOE* $\epsilon 4$ to this SC-trait does not imply that *APOE* $\epsilon 4$ does not affect AD's progression. Instead, we can only infer an abnormal connectivity pattern related to the risk factor of AD's family history. *APOE* $\epsilon 4$ has been associated with amyloid plaques' deposition, which could be a different AD pathology pathway than the disrupted white matter integrity. These two pathways could be interacting at some point in the pathology but seem relatively independent at this AD continuum stage.

The third study focused on the FC-SC relationship. This final study was purely data-driven, i.e., with no *a priori* hypothesis but an intuition: if there is an association between FC and SC, as reported in the literature, this association may be different between the first-degree relatives and the control group. The FC was estimated using MEG and the PLV-related measures, the SC was estimated using DTI and the NoS and FA measures, and the degree of dependence was estimated using the MIC and TIC measures of the MINE statistics. Firstly, the relationship between FC and SC was evaluated for the whole population, i.e., with no stratification into risk and control groups. Secondly, we tested whether the FC-SC relationship (if any) across the whole brain yields for the areas of interest

(precuneus and ACC). Finally, we tested if the relationship between functional and structural connectivity in interest areas is different in first-degree relatives and controls.

The results showed a significant linear relationship between SC and FC measures when analyzing whole-brain connectivity. Specifically, NoS presented a direct correlation with PLV while FA presented an inverse correlation with FA (Figure 36). These relationships yield significant analyzing the connectivity between the areas of interest, precuneus, ACC, and the rest of the brain (Figure 38 and Supplementary Figure 2). Finally, the level of dependence (or level of association) between FC and SC did not differ between first-degree relatives and controls.

The direct correlation of NoS with FC has been extensively reported in the literature (Garcés et al., 2014; C. J. Honey et al., 2009; Skudlarski et al., 2008). Although the vast majority of studies reported that the underlying NoS support FC, it is essential to remark that the underlying structure cannot explain all the FC. For example, areas with no direct SC may present FC due to a “relay” area interconnecting them (C. J. Honey et al., 2009). In addition, the different, even opposite nature of FC (dynamic) and SC (static) supports this idea. While SC remains stable during a single MRI session, different aspects of FC can be estimated. For example, different time scales and different frequency bands for FC estimation reflect different brain interactions' properties. Furthermore, the FC highly depends on the person's state during the fMRI session, presenting very different FC profiles during resting-state or any task performance. All these phenomena provide essential insights into the complex brain FC-SC relationship.

In contrast, not many studies have estimated the FC-SC association using FA as a structural measure (Straathof et al., 2019), and those that have done it reported inconsistent results (Collin et al., 2014; Garcés et al., 2014; Hirsiger et al., 2016). Since high values of FA, i.e., great axonal integrity, have been commonly associated with a healthy state, the inverse correlation of FA and PLV may cause a first confusing impression: an increased FA should improve the FC between areas. Therefore, the FC should be increased. However, as we have discussed earlier, an increased FC is not a desirable feature *per se*, but it can express neurodegeneration in diseases such as AD. It is possible that an increased FA, i.e., good white matter integrity, allows tuning the FC between areas to the optimal value, neither hyper- nor hyposynchronized.

Finally, no differences were found between first-degree relatives and the control group in the FC-SC associations. It is important to remark that although no differences were found, it does not imply that no processes affect the FC-SC relationship. The impairment may be so subtle that we are not able to measure it. This not seems contradictory to the results of the two previous studies, but

the differences in both FC and SC might be subtle enough that the differences are faded in the process when combining FC-SC. Another limitation is that we have compared the connectivity of interest areas with the rest of the brain. This is not precisely the same framework for the first study, where the areas of interest were compared across cortical sources and not whole areas as defined by the AAL atlas. The differences found in the first study were between the areas of interest and clusters of cortical sources.

Finally, it is of great interest to discuss the dynamic nature of FC to consider all its implications in the results of the third study and to gain insights into the FC-SC relationship. As we have briefly discussed, there is a contrast between the quasi-static nature of SC and the highly dynamic nature of FC. This dynamic nature forces any researcher to select different parameters or characteristics for the evaluation of FC, for example, the time window to obtain the FC or the frequency band of interest, or if the interest of the research is the resting-state brain or specific cognitive performance. Regarding all the possible time scales that one can use to estimate FC, several studies showed that different time scales result in different FC-SC associations (Christopher J. Honey et al., 2007; Messé et al., 2015). Similarly, FC in different frequency bands is modulated by the distance between the brain areas (Chu et al., 2015; Wu et al., 2008), and the brain functional efficiency might arise from a trade-off between short- and long-distance structural connections (Sepulcre et al., 2010; Vértes et al., 2012). Finally, it is widely reported and accepted that the functional brain organization shifts depending on the person's state, for example, if the person is resting with no external output or performing any type of task (Fox et al., 2005). In summary, there might be an alteration caused by AD's family history in one of the multiple FC-SC associations, but it may be that such alteration is only apparent during specific tasks.

We selected the regions, frequency bands, and time scale that showed significant results in the first study. At the same time, this decision was made based on the progression models of AD. To this day, we have no reason or argument to explore other frequency bands or other regions, i.e., if we evaluate the FC in another frequency band, it would be a purely exploratory and data-driven study without any *a priori* hypothesis. Accordingly, we decided to study resting-state networks, but it might be interesting to study task-related networks. Brain functional networks are mostly related to memory since memory is one of the first and more impaired cognitive domains in AD. Fortunately, in addition to a resting-state MEG recording, the participants underwent a memory task during a MEG recording. It will be of great interest to evaluate the association between static SC and the functional networks related to memory.

Considering all the results presented in this thesis, AD's neurodegenerative process could start decades before the onset of clinical symptoms. Indeed, they support the use of FC as an early biomarker of the disease and its evolution along the AD continuum as proposed in our Laboratory. Furthermore, they also support, to some extent, the cascading network failure model previously described.

CONCLUSIONS

As a final summary, we present the main conclusions of this thesis:

- Regarding the FC, first-degree relatives presented a hypersynchronization in the high alpha band in the first-degree relatives among anterior and posterior areas and the dorsal and middle cingulate cortex. These areas are known for their early amyloid accumulation and for exhibiting hypersynchrony along with the AD progression.

- This increased synchrony of the oscillatory activity could result from amyloid's early presence in the soluble form, leading to an E/I unbalance.
- The hypersynchronization in posterior areas resembles the AD progression model previously proposed by the Laboratory of Cognitive and Computational Neuroscience: The synchrony of posterior areas with different brain areas gradually increases until the network collapses a hyposynchronization can be found in AD converters.
- This early impairment seems associated with AD's family history, and the APOE $\epsilon 4$ carriage does not modulate it.
- FC obtained from non-invasive neurophysiological recordings may be considered an important biomarker to characterize the AD continuum, even in preclinical stages.

- First-degree relatives showed a connectome integrity diminishment between interhemispheric posterior areas, between posterior and temporal areas, and between posterior areas and the amygdala. Considering nine different risk factors for AD, this SC-trait was associated exclusively with the family history of AD.

- The early disruption of the white matter integrity could be due to an initial effect of the tau-pathology associated with AD.
- The SC-trait associated with a family history of AD supports the cascading network failure model, presenting posterior-posterior and posterior-temporal abnormal connectivity patterns.
- The absence of hippocampal atrophy or cortical thinning supports the idea that white matter alterations are not caused by Wallerian degeneration secondary to grey matter atrophy.

- There is a dependence between FC and SC. In particular, NoS presented a direct correlation with PLV while FA presented an inverse correlation with PLV.

- This dependence did not differ between first-degree relatives and the control group.

- The results presented in this thesis suggest that AD starts decades before the symptoms onset. Furthermore, the first signs could be present in earlier stages than the SCD stage.

- Family history of AD, conceptualized as known and unknown susceptibility genes and environmental circumstances, should be considered a risk factor of developing AD.

Supplementary material

Supplementary Figures

Supplementary Figure 1. Schaefer parcellation and matrix form.

Organization of the SC matrix. The terminology used for the seven functional networks is as in (Schaefer et al., 2018).

Supplementary Figure 2. Functional and structural dependence involves the rest of the interest areas, namely the left anterior cingulate cortex

Left anterior cingulate cortex

Left precuneus

Right precuneus

The figures present the associations between functional and structural connectivity for all combinations of PLV, SC measures, and frequency bands. All combinations presented significant dependence. As it happened when estimating the FC-SC association across the whole brain, the results showed a direct correlation between NoS and PLV and an inverse correlation between FA and PLV in all frequency bands.

Supplementary Tables

Supplementary Table 1. Neuropsychological performance and hippocampus volumes

	Relatives	Control	<i>p-values</i>
Direct Digits span	6,11 ± 1,29	6,06 ± 1,25	0,337
Inverse Digits span	4,43 ± 1,36	4,62 ± 1,13	0,726
Direct Digits total	9,89 ± 2,21	10,06 ± 2,3	0,492
Inverse Digits total	6,7 ± 2,34	6,91 ± 2,12	0,303
Logic Memory immediate units	39,21 ± 11,99	40,09 ± 11,31	0,176
Logic Memory delayed units	25,29 ± 9,26	25,71 ± 8,29	0,857
Logic Memory immediate gist	14,73 ± 3,39	14,22 ± 3,32	0,481
Logic Memory delayed gist	9,98 ± 2,28	9,81 ± 2,1	0,517
F-A-S	43,08 ± 10,08	39,19 ± 11,04	0,510
Left hippocampus volume	3812,02 ± 508,00	3819,19 ± 447,62	0,927
Right hippocampus volume	3935,48 ± 440,24	3959,31 ± 398,07	0,337

Values are presented as mean ± standard deviation. A t-test was conducted for all comparisons. The p-values presented are not corrected, and they are not significant when an FDR correction is applied.

Supplementary Table 2. Correlation values.

	High alpha					
	Anterior cingulate left		Precuneus left		Precuneus right	
	rho	p	rho	p	rho	p
Direct Digits span	0,018	0,846	0,131	0,159	0,107	0,252
Inverse Digits span	0,110	0,239	0,075	0,421	0,058	0,534
Direct Digits total	<0,001	0,997	0,007	0,943	0,009	0,925
Inverse Digits total	0,031	0,742	0,032	0,735	0,033	0,721
Logic Memory immediate units	-0,003	0,976	0,086	0,358	0,080	0,390
Logic Memory delayed units	-0,014	0,885	0,047	0,615	0,038	0,684
Logic Memory immediate gist	0,047	0,618	0,065	0,487	0,081	0,388
Logic Memory delayed gist	-0,050	0,592	0,076	0,415	0,069	0,460
F-A-S	0,051	0,582	0,170	0,067	0,202	0,029
Left hippocampus volume	-0,033	0,725	-0,041	0,660	-0,056	0,549
Right hippocampus volume	0,059	0,529	-0,109	0,244	-0,049	0,602

Correlation estimate (ρ and significance) between the FC's significant values and the neuropsychological values and the hippocampus volumes. The p-values presented are not corrected, and they are not significant when an FDR correction is applied.

Supplementary Table 3. Multiple linear regression model statistics with the nine predictors.

MLR model statistics			
	Regression coefficients	t-statistic	p-value
Family history of AD	0,008	3,871	<0,001
Age	-0,001	-0,947	0,346
<i>APOE</i> ε4 carriers	-0,002	-0,819	0,415
Sex	0,004	1,653	0,101
Years of education	0,001	0,222	0,825
MoCA	0,001	0,140	0,889
TPA	0,001	1,519	0,132
Average cortical thickness	-0,014	-0,928	0,356
Hippocampus volume	0,001	0,920	0,360

The regression coefficients, t-statistics, and p-values of the nine predictors as obtained by the MLR model.

Supplementary Table 4. SC measures values for each group in different areas of interest.

Areas	SC measure	Relatives	Controls
Left ACC	NoS	0,455 ± 0,001	0,458 ± 0,001
	FA	0,460 ± 0,001	0,457 ± 0,000
Right ACC	NoS	0,427 ± 0,001	0,444 ± 0,001
	FA	0,456 ± 0,001	0,451 ± 0,000
Left precuneus	NoS	0,495 ± 0,004	0,499 ± 0,003
	FA	0,441 ± 0,000	0,438 ± 0,000
Right precuneus	NoS	0,424 ± 0,002	0,435 ± 0,002
	FA	0,453 ± 0,000	0,451 ± 0,000

Values are presented as mean ± standard deviation.

Supplementary Table 5. MIC values for each FC-SC association.

Areas	Frequency band	<i>MIC_{NoS}</i>	
		Relatives	Controls
Left ACC	Low alpha	2,876 ± 0,149	2,966 ± 0,250
	High alpha	2,975 ± 0,232	3,112 ± 0,170
Right ACC	Low alpha	2,846 ± 0,270	2,780 ± 0,273
	High alpha	2,825 ± 0,304	2,983 ± 0,346
Left precuneus	Low alpha	2,339 ± 0,340	2,419 ± 0,305
	High alpha	2,268 ± 0,359	2,408 ± 0,215
Right precuneus	Low alpha	2,047 ± 0,233	2,070 ± 0,174
	High alpha	2,017 ± 0,235	2,021 ± 0,206
Areas	Frequency band	<i>MIC_{FA}</i>	
		Relatives	Controls
Left ACC	Low alpha	1,556 ± 0,138	1,615 ± 0,183
	High alpha	1,501 ± 0,134	1,636 ± 0,240
Right ACC	Low alpha	1,431 ± 0,103	1,453 ± 0,063
	High alpha	1,391 ± 0,084	1,454 ± 0,102
Left precuneus	Low alpha	2,117 ± 0,144	2,053 ± 0,176
	High alpha	2,072 ± 0,143	2,120 ± 0,143
Right precuneus	Low alpha	1,629 ± 0,096	1,657 ± 0,073
	High alpha	1,685 ± 0,093	1,685 ± 0,084

Values are presented as mean ± standard deviation.

Bibliography

- 2020 Alzheimer's disease facts and figures. (2020). *Alzheimer's and Dementia*. <https://doi.org/10.1002/alz.12068>
- Adler, G., Brassen, S., & Jajcevic, A. (2003). EEG coherence in Alzheimer's dementia. *Journal of Neural Transmission*, 110(9), 1051–1058. <https://doi.org/10.1007/s00702-003-0024-8>
- Aizenstein, H. J., Nebes, R. D., Saxton, J. A., Price, J. C., Mathis, C. A., Tsopelas, N. D., ... Klunk, W. E. (2008). Frequent amyloid deposition without significant cognitive impairment among the elderly. *Archives of Neurology*, 65(11), 1509–1517. <https://doi.org/10.1001/archneur.65.11.1509>
- Alamian, G., Hincapié, A. S., Combrisson, E., Thiery, T., Martel, V., Althukov, D., & Jerbi, K. (2017). Alterations of intrinsic brain connectivity patterns in depression and bipolar disorders: A critical assessment of magnetoencephalography-based evidence. *Frontiers in Psychiatry*. <https://doi.org/10.3389/fpsy.2017.00041>
- Albanese, D., Riccadonna, S., Donati, C., & Franceschi, P. (2018). A practical tool for maximal information coefficient analysis. *GigaScience*. <https://doi.org/10.1093/gigascience/giy032>
- Amico, E., Dzemidzic, M., Oberlin, B. G., Carron, C. R., Harezlak, J., Goñi, J., & Kareken, D. A. (2020). The disengaging brain: Dynamic transitions from cognitive engagement and alcoholism risk. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2020.116515>
- Amico, E., & Goñi, J. (2018). Mapping hybrid functional-structural connectivity traits in the human connectome. *Network Neuroscience*, 2(3), 306–322. https://doi.org/10.1162/netn_a_00049
- Amico, E., Marinazzo, D., Di Perri, C., Heine, L., Annen, J., Martial, C., ... Goñi, J. (2017). Mapping the functional connectome traits of levels of consciousness. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2017.01.020>
- Andersson, J. L. R., & Sotiropoulos, S. N. (2016). An integrated approach to correction for off-resonance effects and subject movement in diffusion MR imaging. *NeuroImage*, 125, 1063–1078. <https://doi.org/10.1016/j.neuroimage.2015.10.019>
- Arbib, M. A., Erdi, P., & Szentagothai, J. (1997). *Neural Organization: Structure, Function, and Dynamics*. A Bradford Book.
- Attal, Y., & Schwartz, D. (2013). Assessment of Subcortical Source Localization Using Deep Brain Activity Imaging Model with Minimum Norm Operators: A MEG Study. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0059856>
- Auburtin, E. S. A. (1861). Reprise de la discussion sur la forme et le volume du cerveau. *Bulletins de La Société d'Anthropologie de Paris*, 2, 209–220.
- Aydore, S., Pantazis, D., & Leahy, R. M. (2013). A note on the phase locking value and its properties. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2013.02.008>
- Babiloni, C., Ferri, R., Binetti, G., Cassarino, A., Forno, G. D., Ercolani, M., ... Rossini, P. M. (2006). Fronto-parietal coupling of brain rhythms in mild cognitive impairment: A multicentric EEG

study. *Brain Research Bulletin*, 69(1), 63–73.
<https://doi.org/10.1016/j.brainresbull.2005.10.013>

- Bajo, R., Castellanos, N. P., Cuesta, P., Aurteneixe, S., Garcia-Prieto, J., Gil-Gregorio, P., ... Maestu, F. (2012). Differential Patterns of Connectivity in Progressive Mild Cognitive Impairment. *Brain Connectivity*, 2(1), 21–24. <https://doi.org/10.1089/brain.2011.0069>
- Bajo, R., Maestú, F., Nevado, A., Sancho, M., Gutiérrez, R., Campo, P., ... Del-Pozo, F. (2010). Functional connectivity in mild cognitive impairment during a memory task: Implications for the disconnection hypothesis. *Journal of Alzheimer's Disease*, 22(1), 183–193. <https://doi.org/10.3233/JAD-2010-100177>
- Bateman, R. J., Xiong, C., Benzinger, T. L. S., Fagan, A. M., Goate, A., Fox, N. C., ... Morris, J. C. (2012). Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. *New England Journal of Medicine*, 367(9), 795–804. <https://doi.org/10.1056/NEJMoa1202753>
- Bendlin, B. B., Carlsson, C. M., Gleason, C. E., Johnson, S. C., Sodhi, A., Gallagher, C. L., ... Asthana, S. (2010, February). Midlife predictors of Alzheimer's disease. *Maturitas*. <https://doi.org/10.1016/j.maturitas.2009.12.014>
- Bendlin, Barbara B., Ries, M. L., Canu, E., Sodhi, A., Lazar, M., Alexander, A. L., ... Johnson, S. C. (2010). White matter is altered with parental family history of Alzheimer's disease. *Alzheimer's and Dementia*. <https://doi.org/10.1016/j.jalz.2009.11.003>
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)*. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
- Bozzali, M., Falini, A., Franceschi, M., Cercignani, M., Zuffi, M., Scotti, G., ... Filippi, M. (2002). White matter damage in Alzheimer's disease assessed in vivo using diffusion tensor magnetic resonance imaging. *Journal of Neurology Neurosurgery and Psychiatry*. <https://doi.org/10.1136/jnnp.72.6.742>
- Braak, H., & Braak, E. (1991). Neuropathological staging of Alzheimer-related changes. *Acta Neuropathologica*. <https://doi.org/10.1007/BF00308809>
- Bressler, S. L. (1999). The Dynamic Manifestation of Cognitive Structures in the Cerebral Cortex. In *Understanding Representation in the Cognitive Sciences*. https://doi.org/10.1007/978-0-585-29605-0_13
- Bressler, S. L., & Tognoli, E. (2006). Operational principles of neurocognitive networks. *International Journal of Psychophysiology*, 60(2), 139–148. <https://doi.org/10.1016/j.ijpsycho.2005.12.008>
- Broca, P. (1861). Remarques sur le siège de la faculté du langage articulé, suivies d'une observation d'aphémie (perte de la parole). *Bulletins de La Société Anatomique de Paris*.
- Bruña, R. (2019). *Simultaneous MEG/EEG recordings for the study of source domain brain connectivity in neurodegenerative diseases*. Universidad Politécnica de Madrid. <https://doi.org/10.20868/UPM.thesis.54514>
- Bruña, R., Maestú, F., & Pereda, E. (2018). Phase locking value revisited: Teaching new tricks to an old dog. *Journal of Neural Engineering*. <https://doi.org/10.1088/1741-2552/aacfe4>
- Buckner, R. L., Sepulcre, J., Talukdar, T., Krienen, F. M., Liu, H., Hedden, T., ... Johnson, K. A. (2009).

Cortical hubs revealed by intrinsic functional connectivity: Mapping, assessment of stability, and relation to Alzheimer's disease. *Journal of Neuroscience*. <https://doi.org/10.1523/JNEUROSCI.5062-08.2009>

Bullmore, E. T., Suckling, J., Overmeyer, S., Rabe-Hesketh, S., Taylor, E., & Brammer, M. J. (1999). Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural mr images of the brain. *IEEE Transactions on Medical Imaging*, 18(1), 32–42. <https://doi.org/10.1109/42.750253>

Busche, M. A., & Konnerth, A. (2016). Impairments of neural circuit function in Alzheimer's disease. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371(1700), 20150429. <https://doi.org/10.1098/rstb.2015.0429>

Buzsáki, G. (2009). *Rhythms of the Brain*. *Rhythms of the Brain*. <https://doi.org/10.1093/acprof:oso/9780195301069.001.0001>

Buzsáki, G., Anastassiou, C. A., & Koch, C. (2012). The origin of extracellular fields and currents-EEG, ECoG, LFP and spikes. *Nature Reviews Neuroscience*. <https://doi.org/10.1038/nrn3241>

Caballero, M. Á. A., Suárez-Calvet, M., Duering, M., Franzmeier, N., Benzinger, T., Fagan, A. M., ... Ewers, M. (2018). White matter diffusion alterations precede symptom onset in autosomal dominant Alzheimer's disease. *Brain*. <https://doi.org/10.1093/brain/awy229>

Cajal, S. R. y. (1899). Comparative study of the sensory areas of the human cortex. *Journal of Chemical Information and Modeling*.

Canuet, L., Pusil, S., López, M. E., Bajo, R., Pineda-Pardo, J. Á., Cuesta, P., ... Maestú, F. (2015). Network disruption and cerebrospinal fluid amyloid-beta and phospho-tau levels in mild cognitive impairment. *Journal of Neuroscience*. <https://doi.org/10.1523/JNEUROSCI.0704-15.2015>

Chalmers, K., Wilcock, G., & Lovet, S. (2005). Contributors to white matter damage in the frontal lobe in Alzheimer's disease. *Neuropathology and Applied Neurobiology*. <https://doi.org/10.1111/j.1365-2990.2005.00678.x>

Cherubini, A., Péran, P., Spoletini, I., Di Paola, M., Di Iulio, F., Hagberg, G. E., ... Spalletta, G. (2010). Combined volumetry and DTI in subcortical structures of mild cognitive impairment and Alzheimer's disease patients. *Journal of Alzheimer's Disease*. <https://doi.org/10.3233/JAD-2010-091186>

Chu, C. J., Tanaka, N., Diaz, J., Edlow, B. L., Wu, O., Hämäläinen, M., ... Kramer, M. A. (2015). EEG functional connectivity is partially predicted by underlying white matter connectivity. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2014.12.033>

Chua, T. C., Wen, W., Chen, X., Kochan, N., Slavin, M. J., Trollor, J. N., ... Sachdev, P. S. (2009). Diffusion tensor imaging of the posterior cingulate is a useful biomarker of mild cognitive impairment. *American Journal of Geriatric Psychiatry*. <https://doi.org/10.1097/JGP.0b013e3181a76e0b>

Chua, T. C., Wen, W., Slavin, M. J., & Sachdev, P. S. (2008). Diffusion tensor imaging in mild cognitive impairment and Alzheimer's disease: A review. *Current Opinion in Neurology*. <https://doi.org/10.1097/WCO.0b013e3282f4594b>

- Cirrito, J. R., Yamada, K. A., Finn, M. B., Sloviter, R. S., Bales, K. R., May, P. C., ... Holtzman, D. M. (2005). Synaptic activity regulates interstitial fluid amyloid- β levels in vivo. *Neuron*. <https://doi.org/10.1016/j.neuron.2005.10.028>
- Cline, E. N., Bicca, M. A., Viola, K. L., & Klein, W. L. (2018). The Amyloid- β Oligomer Hypothesis: Beginning of the Third Decade. *Journal of Alzheimer's Disease*. <https://doi.org/10.3233/JAD-179941>
- Cohen, D. (1968). Magnetoencephalography: Evidence of magnetic fields produced by alpha-rhythm currents. *Science*, 161(3843), 784–786. <https://doi.org/10.1126/science.161.3843.784>
- Cohen, D. (1972). Magnetoencephalography: Detection of the brain's electrical activity with a superconducting magnetometer. *Science*. <https://doi.org/10.1126/science.175.4022.664>
- Colclough, G. L., Woolrich, M. W., Tewarie, P. K., Brookes, M. J., Quinn, A. J., & Smith, S. M. (2016). How reliable are MEG resting-state connectivity metrics? *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2016.05.070>
- Collin, G., Sporns, O., Mandl, R. C. W., & Van Den Heuvel, M. P. (2014). Structural and functional aspects relating to cost and benefit of rich club organization in the human cerebral cortex. *Cerebral Cortex*. <https://doi.org/10.1093/cercor/bht064>
- Contreras, J. A., Goñi, J., Risacher, S. L., Amico, E., Yoder, K., Dziedzic, M., ... Saykin, A. J. (2017). Cognitive complaints in older adults at risk for Alzheimer's disease are associated with altered resting-state networks. *Alzheimer's and Dementia: Diagnosis, Assessment and Disease Monitoring*. <https://doi.org/10.1016/j.dadm.2016.12.004>
- Coupé, P., Manjón, J. V., Lanuza, E., & Catheline, G. (2019). Lifespan Changes of the Human Brain In Alzheimer's Disease. *Scientific Reports*. <https://doi.org/10.1038/s41598-019-39809-8>
- Cuesta, P., Garcés, P., Castellanos, N. P., López, M. E., Aurteneixe, S., Bajo, R., ... Maestú, F. (2015). Influence of the APOE ϵ 4 allele and mild cognitive impairment diagnosis in the disruption of the MEG resting state functional connectivity in sources space. *Journal of Alzheimer's Disease*, 44(2), 493–505. <https://doi.org/10.3233/JAD-141872>
- Dalal, S. S., Sekihara, K., & Nagarajan, S. S. (2006). Modified Beamformers for Coherent Source Region Suppression. *IEEE Transactions on Biomedical Engineering*, 53(7), 1357–1363. <https://doi.org/10.1109/TBME.2006.873752>
- Dale, A. M., Fischl, B., & Sereno, M. I. (1999). Cortical surface-based analysis: I. Segmentation and surface reconstruction. *NeuroImage*. <https://doi.org/10.1006/nimg.1998.0395>
- Damadian, R. (1971). Tumor detection by nuclear magnetic resonance. *Science*. <https://doi.org/10.1126/science.171.3976.1151>
- Damasio, H., & Damasio, A. (1989). *Lesion analysis in neuropsychology*. New York: Oxford University Press.
- Damoiseaux, J. S., Prater, K. E., Miller, B. L., & Greicius, M. D. (2012). Functional connectivity tracks clinical deterioration in Alzheimer's disease. *Neurobiology of Aging*. <https://doi.org/10.1016/j.neurobiolaging.2011.06.024>
- de Haan, W., van Straaten, E. C. W., Gouw, A. A., & Stam, C. J. (2017). Altering neuronal excitability to preserve network connectivity in a computational model of Alzheimer's disease. *PLoS*

- Del Gratta, C., Pizzella, V., Tecchio, F., & Romani, G. L. (2001). Magnetoencephalography-a noninvasive brain imaging method with 1 ms time resolution. *Reports on Progress in Physics*. <https://doi.org/10.1088/0034-4885/64/12/204>
- Dhollander, T., & Connelly, A. (2016). A novel iterative approach to reap the benefits of multi-tissue CSD from just single-shell ($b=0$) diffusion MRI data. In *24th International Society of Magnetic Resonance in Medicine*. Singapore.
- Dhollander, T., Raffelt, D., & Connelly, A. (2016). Unsupervised 3-tissue response function estimation from single-shell or multi-shell diffusion MR data without a co-registered T1 image. *ISMRM Workshop on Breaking the Barriers of Diffusion MRI*.
- Donix, M., Small, G. W., & Bookheimer, S. Y. (2012). Family history and APOE-4 genetic risk in Alzheimer's disease. *Neuropsychology Review*. <https://doi.org/10.1007/s11065-012-9193-2>
- Douaud, G., Jbabdi, S., Behrens, T. E. J., Menke, R. A., Gass, A., Monsch, A. U., ... Smith, S. (2011). DTI measures in crossing-fibre areas: Increased diffusion anisotropy reveals early white matter alteration in MCI and mild Alzheimer's disease. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2010.12.008>
- Engels, M. M. A., van der Flier, W. M., Stam, C. J., Hillebrand, A., Scheltens, P., & van Straaten, E. C. W. (2017). Alzheimer's disease: The state of the art in resting-state magnetoencephalography. *Clinical Neurophysiology*. <https://doi.org/10.1016/j.clinph.2017.05.012>
- Fernández, A., Maestú, F., Amo, C., Gil, P., Fehr, T., Wienbruch, C., ... Ortiz, T. (2002). Focal temporoparietal slow activity in Alzheimer's disease revealed by magnetoencephalography. *Biological Psychiatry*. [https://doi.org/10.1016/S0006-3223\(02\)01366-5](https://doi.org/10.1016/S0006-3223(02)01366-5)
- Filippi, M., Basaia, S., Canu, E., Imperiale, F., Magnani, G., Falautano, M., ... Agosta, F. (2020). Changes in functional and structural brain connectome along the Alzheimer's disease continuum. *Molecular Psychiatry*. <https://doi.org/10.1038/s41380-018-0067-8>
- Finger, H., Bönstrup, M., Cheng, B., Messé, A., Hilgetag, C., Thomalla, G., ... König, P. (2016). Modeling of Large-Scale Functional Brain Networks Based on Structural Connectivity from DTI: Comparison with EEG Derived Phase Coupling Networks and Evaluation of Alternative Methods along the Modeling Path. *PLoS Computational Biology*, 12(8), e1005025. <https://doi.org/10.1371/journal.pcbi.1005025>
- Fischl, B., Liu, A., & Dale, A. M. (2001). Automated manifold surgery: Constructing geometrically accurate and topologically correct models of the human cerebral cortex. *IEEE Transactions on Medical Imaging*. <https://doi.org/10.1109/42.906426>
- Fischl, B., Sereno, M. I., & Dale, A. M. (1999). Cortical surface-based analysis: II. Inflation, flattening, and a surface-based coordinate system. *NeuroImage*. <https://doi.org/10.1006/nimg.1998.0396>
- Fischl, B., Sereno, M. I., Tootell, R. B. H., & Dale, A. M. (1999). High-resolution intersubject averaging and a coordinate system for the cortical surface. *Human Brain Mapping*. [https://doi.org/10.1002/\(SICI\)1097-0193\(1999\)8:4<272::AID-HBM10>3.0.CO;2-4](https://doi.org/10.1002/(SICI)1097-0193(1999)8:4<272::AID-HBM10>3.0.CO;2-4)
- Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for

grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*. [https://doi.org/10.1016/0022-3956\(75\)90026-6](https://doi.org/10.1016/0022-3956(75)90026-6)

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., & Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences of the United States of America*, 102(27), 9673–9678. <https://doi.org/10.1073/pnas.0504136102>

Friston, K. J., Frith, C. D., Liddle, P. F., & Frackowiak, R. S. J. (1993). Functional connectivity: The principal-component analysis of large (PET) data sets. *Journal of Cerebral Blood Flow and Metabolism*. <https://doi.org/10.1038/jcbfm.1993.4>

Fuller, S. C. (1907). A STUDY OF THE NEUROFIBRILS IN DEMENTIA PARALYTICA, DEMENTIA SENILIS, CHRONIC ALCOHOLISM, CEREBRAL LUES AND MICROCEPHALIC IDIOCY. *American Journal of Psychiatry*. <https://doi.org/10.1176/ajp.63.4.415>

Garcés, P., Ángel Pineda-Pardo, J., Canuet, L., Aurtenetxe, S., López, M. E., Marcos, A., ... Maestú, F. (2014). The Default Mode Network is functionally and structurally disrupted in amnesic mild cognitive impairment-A bimodal MEG-DTI study. *NeuroImage: Clinical*. <https://doi.org/10.1016/j.nicl.2014.09.004>

Garcés, P., López-Sanz, D., Maestú, F., & Pereda, E. (2017). Choice of Magnetometers and Gradiometers after Signal Space Separation. *Sensors*, 17(12), 2926. <https://doi.org/10.3390/s17122926>

Garcés, P., Martín-Buro, M. C., & Maestú, F. (2016). Quantifying the Test-Retest Reliability of Magnetoencephalography Resting-State Functional Connectivity. *Brain Connectivity*, 6(6), 448–460. <https://doi.org/10.1089/brain.2015.0416>

Garcés, P., Pereda, E., Hernández-Tamames, J. A., Del-Pozo, F., Maestú, F., & Ángel Pineda-Pardo, J. (2016). Multimodal description of whole brain connectivity: A comparison of resting state MEG, fMRI, and DWI. *Human Brain Mapping*. <https://doi.org/10.1002/hbm.22995>

Garcia-Marin, V., Blazquez-Llorca, L., Rodriguez, J. R., Boluda, S., Muntane, G., Ferrer, I., & DeFelipe, J. (2009). Diminished perisomatic GABAergic terminals on cortical neurons adjacent to amyloid plaques. *Frontiers in Neuroanatomy*, 3(NOV). <https://doi.org/10.3389/neuro.05.028.2009>

Gascoyne, L., Furlong, P. L., Hillebrand, A., Worthen, S. F., & Witton, C. (2016). Localising the auditory N1m with event-related beamformers: localisation accuracy following bilateral and unilateral stimulation. *Scientific Reports*, 6, 31052. <https://doi.org/10.1038/srep31052>

Gold, B. T., Powell, D. K., Andersen, A. H., & Smith, C. D. (2010). Alterations in multiple measures of white matter integrity in normal women at high risk for Alzheimer's disease. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2010.05.036>

Goñi, J., Avena-Koenigsberger, A., Velez de Mendizabal, N., van den Heuvel, M. P., Betzel, R. F., & Sporns, O. (2013). Exploring the Morphospace of Communication Efficiency in Complex Networks. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0058070>

Greicius, M. D., Supekar, K., Menon, V., & Dougherty, R. F. (2009). Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cerebral Cortex*. <https://doi.org/10.1093/cercor/bhn059>

- Hafkemeijer, A., Altmann-Schneider, I., Oleksik, A. M., Van De Wiel, L., Middelkoop, H. A. M., Van Buchem, M. A., ... Rombouts, S. A. R. B. (2013). Increased functional connectivity and brain atrophy in elderly with subjective memory complaints. *Brain Connectivity*. <https://doi.org/10.1089/brain.2013.0144>
- Hardy, J. A., & Higgins, G. A. (1992). Alzheimer's disease: The amyloid cascade hypothesis. *Science*. <https://doi.org/10.1126/science.1566067>
- Head, H. (1920). Aphasia and kindred disorders of speech. *Brain*. <https://doi.org/10.1093/brain/43.2.87>
- Hincapié, A. S., Kujala, J., Mattout, J., Pascarella, A., Daligault, S., Delpuech, C., ... Jerbi, K. (2017). The impact of MEG source reconstruction method on source-space connectivity estimation: A comparison between minimum-norm solution and beamforming. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2017.04.038>
- Hirsiger, S., Koppelmans, V., Mérellat, S., Liem, F., Erdeniz, B., Seidler, R. D., & Jäncke, L. (2016). Structural and functional connectivity in healthy aging: Associations for cognition and motor behavior. *Human Brain Mapping*. <https://doi.org/10.1002/hbm.23067>
- Honey, C. J., Sporns, O., Cammoun, L., Gigandet, X., Thiran, J. P., Meuli, R., & Hagmann, P. (2009). Predicting human resting-state functional connectivity from structural connectivity. *Proceedings of the National Academy of Sciences of the United States of America*. <https://doi.org/10.1073/pnas.0811168106>
- Honey, Christopher J., Kötter, R., Breakspear, M., & Sporns, O. (2007). Network structure of cerebral cortex shapes functional connectivity on multiple time scales. *Proceedings of the National Academy of Sciences of the United States of America*. <https://doi.org/10.1073/pnas.0701519104>
- Huff, F. J., Growdon, J. H., Corkin, S., & Rosen, T. J. (1987). Age at Onset and Rate of Progression of Alzheimer's Disease. *Journal of the American Geriatrics Society*, 35(1), 27–30. <https://doi.org/10.1111/j.1532-5415.1987.tb01315.x>
- Hyvärinen, A. (1999). Fast and robust fixed-point algorithms for independent component analysis. *IEEE Transactions on Neural Networks*. <https://doi.org/10.1109/72.761722>
- Jack, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., ... Silverberg, N. (2018). NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's and Dementia*. <https://doi.org/10.1016/j.jalz.2018.02.018>
- Jack, C. R., Knopman, D. S., Jagust, W. J., Petersen, R. C., Weiner, M. W., Aisen, P. S., ... Trojanowski, J. Q. (2013, February). Tracking pathophysiological processes in Alzheimer's disease: An updated hypothetical model of dynamic biomarkers. *The Lancet Neurology*. [https://doi.org/10.1016/S1474-4422\(12\)70291-0](https://doi.org/10.1016/S1474-4422(12)70291-0)
- Jack, C. R., Knopman, D. S., Jagust, W. J., Shaw, L. M., Aisen, P. S., Weiner, M. W., ... Trojanowski, J. Q. (2010). Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *The Lancet Neurology*. Lancet Publishing Group. [https://doi.org/10.1016/S1474-4422\(09\)70299-6](https://doi.org/10.1016/S1474-4422(09)70299-6)
- Jaklevic, R. C., Lambe, J., Silver, A. H., & Mercereau, J. E. (1964). Quantum interference effects in Josephson tunneling. *Physical Review Letters*. <https://doi.org/10.1103/PhysRevLett.12.159>

- Jelic, V., Johansson, S. E., Almkvist, O., Shigeta, M., Julin, P., Nordberg, A., ... Wahlund, L. O. (2000). Quantitative electroencephalography in mild cognitive impairment: Longitudinal changes and possible prediction of Alzheimer's disease. *Neurobiology of Aging*, 21(4), 533–540. [https://doi.org/10.1016/S0197-4580\(00\)00153-6](https://doi.org/10.1016/S0197-4580(00)00153-6)
- Jerskey, B. A. (2018). Rey Complex Figure Test. In *Encyclopedia of Clinical Neuropsychology*. https://doi.org/10.1007/978-3-319-57111-9_1399
- Jessen, F., Amariglio, R. E., Van Boxtel, M., Breteler, M., Ceccaldi, M., Chételat, G., ... Wagner, M. (2014). A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. *Alzheimer's and Dementia*, 10(6), 844–852. <https://doi.org/10.1016/j.jalz.2014.01.001>
- Johnson, K. A., Schultz, A., Betensky, R. A., Becker, J. A., Sepulcre, J., Rentz, D., ... Sperling, R. (2016). Tau positron emission tomographic imaging in aging and early Alzheimer disease. *Annals of Neurology*. <https://doi.org/10.1002/ana.24546>
- Jones, D. T., Graff-Radford, J., Lowe, V. J., Wiste, H. J., Gunter, J. L., Senjem, M. L., ... Jack, C. R. (2017). Tau, amyloid, and cascading network failure across the Alzheimer's disease spectrum. *Cortex*. <https://doi.org/10.1016/j.cortex.2017.09.018>
- Jones, D. T., Knopman, D. S., Gunter, J. L., Graff-Radford, J., Vemuri, P., Boeve, B. F., ... Jack, C. R. (2016). Cascading network failure across the Alzheimer's disease spectrum. *Brain*. <https://doi.org/10.1093/brain/awv338>
- Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. H., ... Müller-Hill, B. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature*. <https://doi.org/10.1038/325733a0>
- Kantarci, K., Murray, M. E., Schwarz, C. G., Reid, R. I., Przybelski, S. A., Lesnick, T., ... Dickson, D. W. (2017). White-matter integrity on DTI and the pathologic staging of Alzheimer's disease. *Neurobiology of Aging*. <https://doi.org/10.1016/j.neurobiolaging.2017.04.024>
- Kellner, E., Dhital, B., & Reiser, M. (2015). Gibbs-Ringing Artifact Removal Based on Local Subvoxel-shifts. *Magnetic Resonance in Medicine*, 76(5), 1574–1581. Retrieved from <http://arxiv.org/abs/1501.07758>
- Klimesch, W. (1996). Memory processes, brain oscillations and EEG synchronization. *International Journal of Psychophysiology*. [https://doi.org/10.1016/S0167-8760\(96\)00057-8](https://doi.org/10.1016/S0167-8760(96)00057-8)
- Koelewijn, L., Bompas, A., Tales, A., Brookes, M. J., Muthukumaraswamy, S. D., Bayer, A., & Singh, K. D. (2017). Alzheimer's disease disrupts alpha and beta-band resting-state oscillatory network connectivity. *Clinical Neurophysiology*, 128(11), 2347–2357. <https://doi.org/10.1016/j.clinph.2017.04.018>
- Koelewijn, L., Lancaster, T. M., Linden, D., Dima, D. C., Routley, B. C., Magazzini, L., ... Singh, K. (2019). Oscillatory hyperactivity and hyperconnectivity in young APOE-ε4 carriers and hypoconnectivity in Alzheimer's disease. *eLife*, 8. <https://doi.org/10.7554/eLife.36011>
- Koo, E. H., & Squazzo, S. L. (1994). Evidence that production and release of amyloid β-protein involves the endocytic pathway. *Journal of Biological Chemistry*.
- Krishnaswamy, P., Obregon-Henao, G., Ahveninen, J., Khan, S., Babadi, B., Iglesias, J. E., ... Purdon,

- P. L. (2017). Sparsity enables estimation of both subcortical and cortical activity from MEG and EEG. *Proceedings of the National Academy of Sciences of the United States of America*. <https://doi.org/10.1073/pnas.1705414114>
- Lachaux, J.-P., Rodriguez, E., Martinerie, J., & Varela, F. J. (1999). Measuring phase synchrony in brain signals. *Human Brain Mapping*, 8(4), 194–208. [https://doi.org/10.1002/\(SICI\)1097-0193\(1999\)8:4<194::AID-HBM4>3.0.CO;2-C](https://doi.org/10.1002/(SICI)1097-0193(1999)8:4<194::AID-HBM4>3.0.CO;2-C)
- Lau, M., Yam, D., & Burneo, J. G. (2008). A systematic review on MEG and its use in the presurgical evaluation of localization-related epilepsy. *Epilepsy Research*. <https://doi.org/10.1016/j.eplepsyres.2008.01.004>
- Lauterbur, P. C. (1973). Image formation by induced local interactions: Examples employing nuclear magnetic resonance. *Nature*. <https://doi.org/10.1038/242190a0>
- Lauterbur, Paul C. (1974). Magnetic Resonance Zeugmatography. *Pure and Applied Chemistry*. <https://doi.org/10.1351/pac197440010149>
- Le Bihan, D., & Breton, E. (1985). Imagerie de diffusion in-vivo par résonance magnétique nucléaire. *Comptes-Rendus de l'Académie Des Sciences*.
- Ledberg, A., Bressler, S. L., Ding, M., Coppola, R., & Nakamura, R. (2007). Large-scale visuomotor integration in the cerebral cortex. *Cerebral Cortex*. <https://doi.org/10.1093/cercor/bhj123>
- Liu, Y., Spulber, G., Lehtimäki, K. K., Könönen, M., Hallikainen, I., Gröhn, H., ... Soininen, H. (2011). Diffusion tensor imaging and Tract-Based Spatial Statistics in Alzheimer's disease and mild cognitive impairment. *Neurobiology of Aging*. <https://doi.org/10.1016/j.neurobiolaging.2009.10.006>
- López-Sanz, D., Brunã, R., Garcés, P., Camara, C., Serrano, N., Rodríguez-Rojo, I. C., ... Maestú, F. (2016). Alpha band disruption in the AD-continuum starts in the Subjective Cognitive Decline stage: A MEG study. *Scientific Reports*, 6(November), 1–11. <https://doi.org/10.1038/srep37685>
- López-Sanz, David, Bruña, R., Garcés, P., Martín-Buro, M. C., Walter, S., Delgado, M. L., ... Maestú, F. (2017). Functional connectivity disruption in subjective cognitive decline and mild cognitive impairment: A common pattern of alterations. *Frontiers in Aging Neuroscience*. <https://doi.org/10.3389/fnagi.2017.00109>
- López-Sanz, David, Garcés, P., Álvarez, B., Delgado-Losada, M. L., López-Higes, R., & Maestú, F. (2017). Network Disruption in the Preclinical Stages of Alzheimer's Disease: From Subjective Cognitive Decline to Mild Cognitive Impairment. *International Journal of Neural Systems*, 27(08), 1750041. <https://doi.org/10.1142/S0129065717500411>
- López, M. E., Cuesta, P., Garcés, P., Castellanos, P. N., Aurtinetxe, S., Bajo, R., ... Fernandez, A. (2014). MEG spectral analysis in subtypes of mild cognitive impairment. *Age (Dordrecht, Netherlands)*, 36(3), 9624. <https://doi.org/10.1007/s11357-014-9624-5>
- López, María Eugénia, Bruña, R., Aurtinetxe, S., Pineda-Pardo, J. Á., Marcos, A., Arrazola, J., ... Maestú, F. (2014). Alpha-band hypersynchronization in progressive mild cognitive impairment: A magnetoencephalography study. *Journal of Neuroscience*, 34(44), 14551–14559. <https://doi.org/10.1523/JNEUROSCI.0964-14.2014>
- Lubke, J., Markram, H., Frotscher, M., & Sakmann, B. (1996). Frequency and dendritic distribution

of autapses established by layer 5 pyramidal neurons in the developing rat neocortex: Comparison with synaptic innervation of adjacent neurons of the same class. *Annals of Anatomy*. [https://doi.org/10.1016/S0940-9602\(96\)80077-X](https://doi.org/10.1016/S0940-9602(96)80077-X)

- Lúriya, A. R. (1973). *The working brain: An introduction to neuropsychology*. London: Penguin Books.
- Maestú, F., Fernández, A., Simos, P. G., Gil-Gregorio, P., Amo, C., Rodriguez, R., ... Ortiz, T. (2001). Spatio-temporal patterns of brain magnetic activity during a memory task in Alzheimer's disease. *NeuroReport*. <https://doi.org/10.1097/00001756-200112210-00013>
- Mansfield, P., & Grannell, P. K. (1975). "Diffraction" and microscopy in solids and liquids by NMR. *Physical Review B*. <https://doi.org/10.1103/PhysRevB.12.3618>
- Marco, E. J., Hinkley, L. B. N., Hill, S. S., & Nagarajan, S. S. (2011). Sensory processing in autism: A review of neurophysiologic findings. *Pediatric Research*. <https://doi.org/10.1203/PDR.0b013e3182130c54>
- Martinez, M., Champion, D., Brice, A., Hannequin, D., Dubois, B., Didierjean, O., ... Clerget-Darpoux, F. (1998). Apolipoprotein E $\epsilon 4$ allele and familial aggregation of Alzheimer disease. *Archives of Neurology*, 55(6), 810–816. <https://doi.org/10.1001/archneur.55.6.810>
- Marty, M. A., Pepin, R., June, A., & Segal, D. L. (2010). Geriatric Depression Scale. In *Principles and Practice of Geriatric Psychiatry: Third Edition*. <https://doi.org/10.1002/9780470669600.ch29>
- Mayo, C. D., Garcia-Barrera, M. A., Mazerolle, E. L., Ritchie, L. J., Fisk, J. D., & Gawryluk, J. R. (2019). Relationship between DTI metrics and cognitive function in Alzheimer's disease. *Frontiers in Aging Neuroscience*. <https://doi.org/10.3389/fnagi.2018.00436>
- Mazziotta, J. C., Toga, A. W., Evans, A., Fox, P., & Lancaster, J. (1995). A probabilistic atlas of the human brain: theory and rationale for its development. The International Consortium for Brain Mapping (ICBM). *NeuroImage*.
- McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of alzheimer's disease: Report of the NINCDS-ADRDA work group* under the auspices of department of health and human services task force on alzheimer's disease. *Neurology*, 34(7), 939–944. <https://doi.org/10.1212/wnl.34.7.939>
- Messé, A., Hütt, M. T., König, P., & Hilgetag, C. C. (2015). A closer look at the apparent correlation of structural and functional connectivity in excitable neural networks. *Scientific Reports*. <https://doi.org/10.1038/srep07870>
- Mitzdorf, U. (1988). Evoked Potentials and Their Physiological Causes: An Access to Delocalized Cortical Activity. https://doi.org/10.1007/978-3-642-71531-0_8
- Moiseev, A., Gaspar, J. M., Schneider, J. A., & Herdman, A. T. (2011). Application of multi-source minimum variance beamformers for reconstruction of correlated neural activity. *NeuroImage*, 58(2), 481–496. <https://doi.org/10.1016/j.neuroimage.2011.05.081>
- Morris, J. C. (2005). Early-stage and preclinical Alzheimer disease. In *Alzheimer Disease and Associated Disorders*. <https://doi.org/10.1097/01.wad.0000184005.22611.cc>
- Murakami, S., & Okada, Y. (2006). Contributions of principal neocortical neurons to magnetoencephalography and electroencephalography signals. *Journal of Physiology*. <https://doi.org/10.1113/jphysiol.2006.105379>

- Muthukumaraswamy, S. D., & Singh, K. D. (2011, November). A cautionary note on the interpretation of phase-locking estimates with concurrent changes in power. *Clinical Neurophysiology*. <https://doi.org/10.1016/j.clinph.2011.04.003>
- Nakamura, A., Cuesta, P., Fernández, A., Arahata, Y., Iwata, K., Kuratsubo, I., ... Kato, T. (2018). Electromagnetic signatures of the preclinical and prodromal stages of Alzheimer's disease. *Brain*, 141(5), 1470–1485. <https://doi.org/10.1093/brain/awy044>
- Nakamura, A., Cuesta, P., Kato, T., Arahata, Y., Iwata, K., Yamagishi, M., ... Ito, K. (2017). Early functional network alterations in asymptomatic elders at risk for Alzheimer's disease. *Scientific Reports*, 7(1). <https://doi.org/10.1038/s41598-017-06876-8>
- Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., ... Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: A brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society*. <https://doi.org/10.1111/j.1532-5415.2005.53221.x>
- Nelson, E., Wasson, J., Kirk, J., Keller, A., Clark, D., Dietrich, A., ... Zubkoff, M. (1987). Assessment of function in routine clinical practice: Description of the coop chart method and preliminary findings. *Journal of Chronic Diseases*. [https://doi.org/10.1016/S0021-9681\(87\)80033-4](https://doi.org/10.1016/S0021-9681(87)80033-4)
- Nir, T. M., Jahanshad, N., Villalon-Reina, J. E., Toga, A. W., Jack, C. R., Weiner, M. W., & Thompson, P. M. (2013). Effectiveness of regional DTI measures in distinguishing Alzheimer's disease, MCI, and normal aging. *NeuroImage: Clinical*. <https://doi.org/10.1016/j.nicl.2013.07.006>
- Nolte, G. (2003). The magnetic lead field theorem in the quasi-static approximation and its use for magnetoencephalography forward calculation in realistic volume conductors. *Physics in Medicine and Biology*, 48(22), 3637–3652. <https://doi.org/10.1088/0031-9155/48/22/002>
- Nolte, G., Bai, O., Wheaton, L., Mari, Z., Vorbach, S., & Hallett, M. (2004). Identifying true brain interaction from EEG data using the imaginary part of coherency. *Clinical Neurophysiology*, 115(10), 2292–2307. <https://doi.org/10.1016/j.clinph.2004.04.029>
- Nunes, A. S., Moiseev, A., Kozhemiako, N., Cheung, T., Ribary, U., & Doesburg, S. M. (2020). Multiple constrained minimum variance beamformer (MCMV) performance in connectivity analyses. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2019.116386>
- Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J. M. (2011). FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Computational Intelligence and Neuroscience*, 2011. <https://doi.org/10.1155/2011/156869>
- Patenaude, B. (2007). Bayesian Statistical Models of Shape and Appearance for Subcortical Brain Segmentation. *Department of Clinical Neurology*.
- Penny, W., Friston, K., Ashburner, J., Kiebel, S., & Nichols, T. (2007). *Statistical Parametric Mapping: The Analysis of Functional Brain Images*. *Statistical Parametric Mapping: The Analysis of Functional Brain Images*. Elsevier Ltd. <https://doi.org/10.1016/B978-0-12-372560-8.X5000-1>
- Pereda, E., Quiroga, R. Q., & Bhattacharya, J. (2005). Nonlinear multivariate analysis of neurophysiological signals. *Progress in Neurobiology*. <https://doi.org/10.1016/j.pneurobio.2005.10.003>
- Petroni, F., Panzeri, S., Hilgetag, C. C., Kötter, R., & Young, M. P. (2001). Simultaneity of responses

in a hierarchical visual network. *NeuroReport*. <https://doi.org/10.1097/00001756-200108280-00032>

- Pikovsky, A., Rosenblum, M., Kurths, J., & Hilborn, R. C. (2002). Synchronization: A Universal Concept in Nonlinear Science. *American Journal of Physics*. <https://doi.org/10.1119/1.1475332>
- Popescu, M., Popescu, E.-A., Chan, T., Blunt, S. D., & Lewine, J. D. (2008). Spatio–Temporal Reconstruction of Bilateral Auditory Steady-State Responses Using MEG Beamformers. *IEEE Transactions on Biomedical Engineering*, 55(3), 1092–1102. <https://doi.org/10.1109/TBME.2007.906504>
- Puce, A., & Hämäläinen, M. S. (2017). A review of issues related to data acquisition and analysis in EEG/MEG studies. *Brain Sciences*. <https://doi.org/10.3390/brainsci7060058>
- Pusil, S., López, M. E., Cuesta, P., Bruña, R., Pereda, E., & Maestú, F. (2019). Hypersynchronization in mild cognitive impairment: The ‘X’ model. *Brain*. <https://doi.org/10.1093/brain/awz320>
- Qin, L., Guo, Z., McClure, M. A., & Mu, Q. (2020). White matter changes from mild cognitive impairment to Alzheimer’s disease: a meta-analysis. *Acta Neurologica Belgica*. <https://doi.org/10.1007/s13760-020-01322-5>
- Rabi, I. I., Zacharias, J. R., Millman, S., & Kusch, P. (1938). A new method of measuring nuclear magnetic moment. *Physical Review*. <https://doi.org/10.1103/PhysRev.53.318>
- Raffelt, D., Dhollander, T., Tournier, J. D., Tabbara, R., Smith, R. E., Pierre, E., & Connelly, A. (2017). Bias Field Correction and Intensity Normalisation for Quantitative Analysis of Apparent Fibre Density. In *25th International Society of Magnetic Resonance in Medicine*. Honolulu.
- Ramírez-Toraño, F., Bruña, R., de Frutos-Lucas, J., Rodríguez-Rojo, I. C., Marcos de Pedro, S., Delgado-Losada, M. L., ... Maestú, F. (2021). Functional Connectivity Hypersynchronization in Relatives of Alzheimer’s Disease Patients: An Early E/I Balance Dysfunction? *Cerebral Cortex*, 31(2), 1201–1210. <https://doi.org/10.1093/cercor/bhaa286>
- Ranasinghe, K. G., Hinkley, L. B., Beagle, A. J., Mizuiri, D., Dowling, A. F., Honma, S. M., ... Vossel, K. A. (2014). Regional functional connectivity predicts distinct cognitive impairments in Alzheimer’s disease spectrum. *NeuroImage: Clinical*. <https://doi.org/10.1016/j.nicl.2014.07.006>
- Reshef, D. N., Reshef, Y. A., Finucane, H. K., Grossman, S. R., McVean, G., Turnbaugh, P. J., ... Sabeti, P. C. (2011). Detecting novel associations in large data sets. *Science*. <https://doi.org/10.1126/science.1205438>
- Reshef, D. N., Reshef, Y. A., Sabeti, P. C., & Mitzenmacher, M. (2018). An empirical study of the maximal and total information coefficients and leading measures of dependence. *Annals of Applied Statistics*. <https://doi.org/10.1214/17-AOAS1093>
- Reshef, Y. A., Reshef, D. N., Finucane, H. K., Sabeti, P. C., & Mitzenmacher, M. (2016). Measuring dependence powerfully and equitably. *Journal of Machine Learning Research*.
- Rosenblum, M., Pikovsky, A., Kurths, J., Schäfer, C., & Tass, P. A. (2001). *Phase synchronization: From theory to data analysis*. *Handbook of Biological Physics* (Vol. 4). North-Holland. [https://doi.org/10.1016/S1383-8121\(01\)80012-9](https://doi.org/10.1016/S1383-8121(01)80012-9)

- Rowe, C. C., Ng, S., Ackermann, U., Gong, S. J., Pike, K., Savage, G., ... Villemagne, V. L. (2007). Imaging β -amyloid burden in aging and dementia. *Neurology*, 68(20), 1718–1725. <https://doi.org/10.1212/01.wnl.0000261919.22630.ea>
- Scahill, R. I., Schott, J. M., Stevens, J. M., Rossor, M. N., & Fox, N. C. (2002). Mapping the evolution of regional atrophy in Alzheimer's disease: Unbiased analysis of fluid-registered serial MRI. *Proceedings of the National Academy of Sciences of the United States of America*. <https://doi.org/10.1073/pnas.052587399>
- Schaefer, A., Kong, R., Gordon, E. M., Laumann, T. O., Zuo, X.-N., Holmes, A. J., ... Yeo, B. T. T. (2018). Local-Global Parcellation of the Human Cerebral Cortex from Intrinsic Functional Connectivity MRI. *Cerebral Cortex*. <https://doi.org/10.1093/cercor/bhx179>
- Schoffelen, J. M., & Gross, J. (2009, June). Source connectivity analysis with MEG and EEG. *Human Brain Mapping*. <https://doi.org/10.1002/hbm.20745>
- Seeley, W. W., Crawford, R. K., Zhou, J., Miller, B. L., & Greicius, M. D. (2009). Neurodegenerative Diseases Target Large-Scale Human Brain Networks. *Neuron*. <https://doi.org/10.1016/j.neuron.2009.03.024>
- Ségonne, F., Dale, A. M., Busa, E., Glessner, M., Salat, D., Hahn, H. K., & Fischl, B. (2004). A hybrid approach to the skull stripping problem in MRI. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2004.03.032>
- Ségonne, Florent, Pacheco, J., & Fischl, B. (2007). Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Transactions on Medical Imaging*. <https://doi.org/10.1109/TMI.2006.887364>
- Selkoe, D. J. (2002). Alzheimer's disease is a synaptic failure. *Science*. <https://doi.org/10.1126/science.1074069>
- Selnes, P., Fjell, A. M., Gjerstad, L., Bjornerud, A., Wallin, A., Due-Tønnessen, P., ... Fladby, T. (2012). White matter imaging changes in subjective and mild cognitive impairment. *Alzheimer's and Dementia*. <https://doi.org/10.1016/j.jalz.2011.07.001>
- Sepulcre, J., Liu, H., Talukdar, T., Martincorena, I., Thomas Yeo, B. T., & Buckner, R. L. (2010). The organization of local and distant functional connectivity in the human brain. *PLoS Computational Biology*. <https://doi.org/10.1371/journal.pcbi.1000808>
- Serrano-Pozo, A., Frosch, M. P., Masliah, E., & Hyman, B. T. (2011). Neuropathological alterations in Alzheimer disease. *Cold Spring Harbor Perspectives in Medicine*. <https://doi.org/10.1101/cshperspect.a006189>
- Serrano, N., López-Sanz, D., Bruña, R., Garcés, P., Rodríguez-Rojo, I. C., Marcos, A., ... Maestú, F. (2020). Spatiotemporal Oscillatory Patterns during Working Memory Maintenance in Mild Cognitive Impairment and Subjective Cognitive Decline. *International Journal of Neural Systems*, 30(1). <https://doi.org/10.1142/S0129065719500199>
- Shahani, N., & Brandt, R. (2002). Functions and malfunctions of the tau proteins. *Cellular and Molecular Life Sciences*. <https://doi.org/10.1007/PL00012495>
- Shoham, S., & Nagarajan, S. (2004). THE THEORY OF CENTRAL NERVOUS SYSTEM RECORDING (pp. 448–471). https://doi.org/10.1142/9789812561763_0014

- Siekmeier, P. J., & Stufflebeam, S. M. (2010, June). Patterns of spontaneous magnetoencephalographic activity in patients with schizophrenia. *Journal of Clinical Neurophysiology*. NIH Public Access. <https://doi.org/10.1097/WNP.0b013e3181e0b20a>
- Sintini, I., Schwarz, C. G., Martin, P. R., Graff-Radford, J., Machulda, M. M., Senjem, M. L., ... Whitwell, J. L. (2019). Regional multimodal relationships between tau, hypometabolism, atrophy, and fractional anisotropy in atypical Alzheimer's disease. *Human Brain Mapping*. <https://doi.org/10.1002/hbm.24473>
- Skudlarski, P., Jagannathan, K., Calhoun, V. D., Hampson, M., Skudlarska, B. A., & Pearlson, G. (2008). Measuring brain connectivity: Diffusion tensor imaging validates resting state temporal correlations. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2008.07.063>
- Smith, C. D., Chebrolu, H., Andersen, A. H., Powell, D. A., Lovell, M. A., Xiong, S., & Gold, B. T. (2010). White matter diffusion alterations in normal women at risk of Alzheimer's disease. *Neurobiology of Aging*. <https://doi.org/10.1016/j.neurobiolaging.2008.08.006>
- Smith, R. E., Tournier, J. D., Calamante, F., & Connelly, A. (2012). Anatomically-constrained tractography: Improved diffusion MRI streamlines tractography through effective use of anatomical information. *NeuroImage*, 62(3), 1924–1938. <https://doi.org/10.1016/j.neuroimage.2012.06.005>
- Smith, R. E., Tournier, J. D., Calamante, F., & Connelly, A. (2015). SIFT2: Enabling dense quantitative assessment of brain white matter connectivity using streamlines tractography. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2015.06.092>
- Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., ... Phelps, C. H. (2011). Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's and Dementia*, 7(3), 280–292. <https://doi.org/10.1016/j.jalz.2011.03.003>
- Spreen, O., & Benton, A. L. (1977). Neurosensory Center Comprehensive Examination for Aphasia: Manual of instructions (NCCEA). *Victoria, BC: University of Victoria*.
- Stam, C. J., Jones, B. F., Manshanden, I., van Cappellen van Walsum, A. M., Montez, T., Verbunt, J. P. A., ... Scheltens, P. (2006). Magnetoencephalographic evaluation of resting-state functional connectivity in Alzheimer's disease. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2006.05.033>
- Stam, Cornelis J., Nolte, G., & Daffertshofer, A. (2007). Phase lag index: Assessment of functional connectivity from multi channel EEG and MEG with diminished bias from common sources. *Human Brain Mapping*. <https://doi.org/10.1002/hbm.20346>
- Stejskal, E. O., & Tanner, J. E. (1965). Spin diffusion measurements: Spin echoes in the presence of a time-dependent field gradient. *The Journal of Chemical Physics*. <https://doi.org/10.1063/1.1695690>
- Stenroos, M., Hunold, A., & Haueisen, J. (2014). Comparison of three-shell and simplified volume conductor models in magnetoencephalography. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2014.01.006>
- Straathof, M., Sinke, M. R. T., Dijkhuizen, R. M., & Otte, W. M. (2019). A systematic review on the

quantitative relationship between structural and functional network connectivity strength in mammalian brains. *Journal of Cerebral Blood Flow and Metabolism*. <https://doi.org/10.1177/0271678X18809547>

Taulu, S., & Simola, J. (2006). Spatiotemporal signal space separation method for rejecting nearby interference in MEG measurements. *Physics in Medicine and Biology*, 51(7), 1759–1768. <https://doi.org/10.1088/0031-9155/51/7/008>

Teipel, S. J., Meindl, T., Wagner, M., Stieltjes, B., Reuter, S., Hauenstein, K. H., ... Hampel, H. (2010). Longitudinal changes in fiber tract integrity in healthy aging and mild cognitive impairment: A DTI follow-up study. *Journal of Alzheimer's Disease*. <https://doi.org/10.3233/JAD-2010-100234>

Thal, D. R., Rüb, U., Orantes, M., & Braak, H. (2002). Phases of A β -deposition in the human brain and its relevance for the development of AD. *Neurology*. <https://doi.org/10.1212/WNL.58.12.1791>

Tournier, J.-D., & , F. Calamante, and a. C. (2010). Improved probabilistic streamlines tractography by 2 nd order integration over fibre orientation distributions. *Isrmr*.

Tournier, J. D., Smith, R., Raffelt, D., Tabbara, R., Dhollander, T., Pietsch, M., ... Connelly, A. (2019, November 15). MRtrix3: A fast, flexible and open software framework for medical image processing and visualisation. *NeuroImage*. Academic Press Inc. <https://doi.org/10.1016/j.neuroimage.2019.116137>

Tucholka, A., Grau-Rivera, O., Falcon, C., Rami, L., Sánchez-Valle, R., Lladó, A., ... Molinuevo, J. L. (2018). Structural connectivity alterations along the Alzheimer's disease continuum: Reproducibility across two independent samples and correlation with cerebrospinal fluid amyloid- β and tau. *Journal of Alzheimer's Disease*. <https://doi.org/10.3233/JAD-170553>

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., ... Joliot, M. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage*. <https://doi.org/10.1006/nimg.2001.0978>

Van Den Heuvel, M. P., Mandl, R. C. W., Kahn, R. S., & Hulshoff Pol, H. E. (2009). Functionally linked resting-state networks reflect the underlying structural connectivity architecture of the human brain. *Human Brain Mapping*. <https://doi.org/10.1002/hbm.20737>

Van Veen, B. D., Van Drongelen, W., Yuchtman, M., & Suzuki, A. (1997). Localization of Brain Electrical Activity via Linearly Constrained Minimum Variance Spatial Filtering. *IEEE TRANSACTIONS ON BIOMEDICAL ENGINEERING*, 44(9).

Veraart, J., Novikov, D. S., Christiaens, D., Ades-aron, B., Sijbers, J., & Fieremans, E. (2016). Denoising of diffusion MRI using random matrix theory. *NeuroImage*, 142, 394–406. <https://doi.org/10.1016/j.neuroimage.2016.08.016>

Verfaillie, S. C. J., Pichet Binette, A., Vachon-Pressseau, E., Tabrizi, S., Savard, M., Bellec, P., ... Yu, E. (2018). Subjective Cognitive Decline Is Associated With Altered Default Mode Network Connectivity in Individuals With a Family History of Alzheimer's Disease. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, 3(5), 463–472. <https://doi.org/10.1016/j.bpsc.2017.11.012>

Vértes, P. E., Alexander-Bloch, A. F., Gogtay, N., Giedd, J. N., Rapoport, J. L., & Bullmore, E. T. (2012). Simple models of human brain functional networks. *Proceedings of the National Academy of*

- Villemagne, V. L., Burnham, S., Bourgeat, P., Brown, B., Ellis, K. A., Salvado, O., ... Masters, C. L. (2013). Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: A prospective cohort study. *The Lancet Neurology*, 12(4), 357–367. [https://doi.org/10.1016/S1474-4422\(13\)70044-9](https://doi.org/10.1016/S1474-4422(13)70044-9)
- Wang, L., Goldstein, F. C., Veledar, E., Levey, A. I., Lah, J. J., Meltzer, C. C., ... Mao, H. (2009). Alterations in cortical thickness and white matter integrity in mild cognitive impairment measured by whole-brain cortical thickness mapping and diffusion tensor imaging. *American Journal of Neuroradiology*. <https://doi.org/10.3174/ajnr.A1484>
- Wang, Y., West, J. D., Flashman, L. A., Wishart, H. A., Santulli, R. B., Rabin, L. A., ... Saykin, A. J. (2012). Selective changes in white matter integrity in MCI and older adults with cognitive complaints. *Biochimica et Biophysica Acta - Molecular Basis of Disease*. <https://doi.org/10.1016/j.bbadis.2011.08.002>
- Wechsler, D. (1997). WMS-III. Wechsler Memory Scale, 3rd Edn. *Psychological Corporation*. San Antonio, TX.
- Wernicke, C. (1874). *Der Aphasische Symptomencomplex. Ein psychologische studie auf anatomischer Basis*. Cohn & Weigert.
- Whitwell, J. L., Josephs, K. A., Murray, M. E., Kantarci, K., Przybelski, S. A., Weigand, S. D., ... Jack, C. R. (2008). MRI correlates of neurofibrillary tangle pathology at autopsy: A voxel-based morphometry study. *Neurology*. <https://doi.org/10.1212/01.wnl.0000324924.91351.7d>
- Wu, C. W., Gu, H., Lu, H., Stein, E. A., Chen, J. H., & Yang, Y. (2008). Frequency specificity of functional connectivity in brain networks. *NeuroImage*. <https://doi.org/10.1016/j.neuroimage.2008.05.035>
- Yamin, G., & Teplow, D. B. (2017). Pittsburgh Compound-B (PiB) binds amyloid β -protein protofibrils. *Journal of Neurochemistry*, 140(2), 210–215. <https://doi.org/10.1111/jnc.13887>
- Zhuang, L., Sachdev, P. S., Trollor, J. N., Kochan, N. A., Reppermund, S., Brodaty, H., & Wen, W. (2012). Microstructural white matter changes in cognitively normal individuals at risk of amnesic MCI. *Neurology*. <https://doi.org/10.1212/WNL.0b013e3182661f4d>
- Zott, B., Busche, M. A., Sperling, R. A., & Konnerth, A. (2018). What Happens with the Circuit in Alzheimer's Disease in Mice and Humans? *Annual Review of Neuroscience*, 41(1), 277–297. <https://doi.org/10.1146/annurev-neuro-080317-061725>