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MARINA WEILER

AVALIAÇÃO POR RESSONÂNCIA MAGNÉTICA DAS CONECTIVIDADES FUNCIONAL E ESTRUTURAL DAS REDES NEUROFUNCIONAIS NA DEMÊNCIA DA DOENÇA DE ALZHEIMER LEVE E COMPROMETIMENTO COGNITIVO LEVE AMNÉSTICO.

Evaluation by magnetic resonance imaging of functional and structural connectivities of neurofunctional networks in mild Alzheimer's disease dementia and amnestic mild cognitive impairment subjects.

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ORIENTADOR: Marcio Luiz Figueredo Balthazar

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RESUMO

A demência por doença de Alzheimer (DA) é uma doença neurodegenerativa na qual ocorrem alterações cognitivas, neuropsiquiátricas e funcionais. Avanços recentes no estudo da neuroimagem na DA mostraram que as alterações presentes nesse grupo de pacientes não se restringem apenas às estruturas anatômicas particulares (tema abordado no ARTIGO 2), mas estendem-se também às redes neurofuncionais, as quais podem gerar problemas de memória e função executiva, entre outros. Devido às alterações estruturais apresentadas por atrofia, ao depósito de placas senis e emaranhados neurofibrilares, bem como à redução no metabolismo de glicose presente nas suas regiões, a *Default Mode Network* (DMN) tornou-se a rede neurofuncional de maior interesse no campo da DA. O principal objetivo desta Tese foi, dessa maneira, avaliar a conectividade de redes neurofuncionais na DA - enfatizando sobretudo na DMN, e suas relações com a cognição. Além disso, estudamos também outros aspectos anatômicos na DA - como alterações de substância branca e cinzenta no cérebro inteiro.

No ARTIGO 1 mostramos que as alterações causadas pela doença afetam também áreas subcorticais como o tálamo e o corpo caloso, o que se relaciona com o déficit cognitivo dos pacientes. Em outro estudo com carácter mais exploratório (ARTIGO 6), mostramos que à medida em que a doença progrediu, as alterações na substância branca ocorreram de maneira mais extensa do que o esperado, levando em consideração as alterações estruturais encontradas na substância cinzenta. Assim, nossos dados sugerem que danos na substância branca possam ocorrer de maneira independente ao dano cortical. O ARTIGO 8, nesse contexto, traz um apanhado de resultados moleculares e de imagem que reforçam a hipótese de uma degeneração de redes neurofuncionais específicas em doenças neurodegenerativas, onde a propagação de proteínas alteradas ocorre ao longo dos tratos de substância branca (no caso da DA, em especial e primariamente nos tratos da DMN).

Com isso em mente, no ARTIGO 4 tivemos como objetivo isolar apenas os tratos da DMN para avaliar o quão íntegro estruturalmente eles se apresentam na doença. De fato, observamos que pacientes com DA apresentam alterações

microestruturais nos tratos da DMN, que contribuem para o déficit na performance cognitiva desses pacientes. No ARTIGO 5, investigamos não apenas a conectividade funcional das regiões da DMN, como também a média das amplitudes de baixa frequência (ALFF) do sinal dependente de oxigenação do sangue dessas regiões. Encontramos que sujeitos com Comprometimento Cognitivo Leve amnéstico (CCLa, sujeitos sob risco de desenvolverem DA) por exemplo, possuem ALFF reduzido em regiões específicas da DMN, porém sem apresentar desconexão funcional entre elas. Pacientes com DA, entretanto, possuem não apenas ALFF reduzido em algumas regiões, mas também desconexão funcional entre elas. As amplitudes de ambos os grupos, entretanto, não possuem relação com o déficit cognitivo apresentado pelos pacientes; ao contrário da conectividade funcional dessas regiões.

No ARTIGO 3, mostramos que outras redes neurofuncionais, como a de Linguagem e a de Controle Executivo também estão alteradas na DA. Em termos de correlatos neuropsicológicos apenas a conectividade da DMN mostrou relação com a performance em testes de memória episódica. No ARTIGO 7, por vez, tivemos como objetivo explorar outras hipóteses envolvendo a função da DMN. Nele, abordamos a questão da alteração do *self* nos pacientes com DA, e sugerimos uma relação com a atividade intrínseca do cérebro e o sentimento de auto-continuidade no tempo.

Palavras-chave: Doença de Alzheimer, Comprometimento Cognitivo Leve amnéstico, redes neurofuncionais, Default Mode Network, conectividade funcional, conectividade estrutural.

ABSTRACT

Alzheimer's (AD) is a neurodegenerative disease that presents with cognitive, neuropsychiatric and functional alterations. Recent studies in the neuroimaging field of AD have shown that the alterations observed in these patients are not limited to specific anatomic structures (as shown in Chapter 2) but also compromise neurofunctional networks, which can lead to memory and executive function impairment, among others. Due to the structural alterations such as atrophy, burden of amyloid beta and hyperphosphorilated tau, and metabolism reduction presented in its regions, the Default Mode Network (DMN) has become the most studied network in the AD field. Thus, the main objective of this thesis was to evaluate the functional and structural connectivities of the neurofunctional networks in AD – emphasizing the DMN, and the relationship with cognition. Besides that, we have also studied some other anatomical aspects in AD, such as alterations in white and grey matter.

In Chapter 1, we have shown that the alterations caused by the disease also affect subcortical areas such as the thalamus and the corpus callosum, which correlates to the cognitive deficit of the patients. In another exploratory study (Chapter 6), we observed that as the disease progressed, the alterations in white matter occurred were more extensive than expected, considering the structural alterations of the grey matter. Our results suggest that damage in white matter can occur independently of grey matter damage. In this context, Chapter 8 brings molecular and imaging results that reinforce the hypothesis that neurodegenerative diseases affect specific neurofunctional networks, and the propagation of altered proteins occur through white matter tracts (along DMN tracts).

Keeping this in mind, in Chapter 4 we had as a main objective to isolate only the DMN tracts, in order to evaluate its structural connectivity in AD. Indeed, we observed that structural microalterations are present in DMN tracts of AD patients, contributing to their cognitive deficits. In Chapter 5, we not only investigated the functional connectivity of DMN regions, but also the amplitude of low frequency fluctuations (ALFF) of the blood oxygenation level signal of these regions. We found that amnestic mild cognitive impairment subjects (aMCI –

subjects at risk for developing AD) for instance, have decreased ALFF in specific regions of the DMN, though not presenting alterations in functional connectivity. AD patients, however, present both reduced ALFF and connectivity in the DMN regions. Interestingly, ALFF values did not correlate with the cognitive impairment of the patients; but connectivity values did.

In Chapter 3, we have shown that other networks such as the Language and Executive Functions are also altered in AD. The functional connectivity of the DMN, in turn, correlated with episodic memory function. In Chapter 7, our main objective was to explore some other hypothesis involving the DMN function. Here, we mentioned the alterations in the self presented in AD patients and suggest a relationship with the brain intrinsic activity and the feeling of self continuity across time.

Key-words: Alzheimer's disease, amnestic mild cognitive impairment subjects, neurofunctional networks, Default Mode Network, functional connectivity, structural connectivity.

LISTA DE ABREVIATURAS

ALFF – *amplitude of low frequency fluctuations,* média das amplitudes de baixa frequência do sinal BOLD

BOLD - *blood oxygenation level dependent*, dependente do nível de oxigenação do sangue

CCL - comprometimento cognitivo leve

CCLa - comprometimento cognitivo leve amnéstico

DA - doença de Alzheimer

DMN – *default mode network*, rede de modo padrão

DTI - *diffusion tensor imaging*, imagem por tensor de difusão

ENF - emaranhados neurofibrilares

FA – *fractional anisotropy*, anisotropia fracionada

FDG-PET - ^{18}F-flúor-deoxi-2-glicose *positron emission tomography*, tomografia por emissão de positrons

MD – *mean diffusivity*, difusividade média

PET - *positron emission tomography*, tomografia por emissão de pósitrons

RM - ressonância magnética

RMf - RM funcional

βA - beta amiloide

SUMÁRIO

INTRODUÇÃO

A Doença de Alzheimer

A demência da doença de Alzheimer (DA) é uma doença neurodegenerativa com surgimento em geral após a sétima década, na qual ocorrem alterações cognitivas, neuropsiquiátricas e funcionais. É a principal causa de demência na população idosa (Ferri *et al.*, 2005), responsável por cerca de 70% dentre todas as demências (Qiu, Kivipelto e von Strauss, 2009) e sua prevalência está aumentando progressivamente devido, sobretudo, ao envelhecimento da população. O número de pessoas com DA está previsto para dobrar a cada 20 anos (Wimo, Jonsson e Winblad, 2006), atingindo 42 milhões em 2020 e 81 milhões em 2040 (Ferri *et al.*, 2005). A cada ano, cerca de 5 milhões de pessoas são diagnosticadas (Brookmeyer *et al.*, 2007), e as chances de isso ocorrer dobram a cada 5 anos após os 65 (Qiu, Kivipelto e von Strauss, 2009). Na cidade de São Paulo, especificamente, a prevalência da DA chega a 4,1% da população acima de 60 anos (Bottino *et al.*, 2008).

Atualmente, segundo o *National Institute on Aging - Alzheimer's Association* (McKhann *et al.*, 2011), o diagnóstico da demência da DA é feito primariamente com base nas definições clínicas da demência e exclusão de outros fatores que possam ser causa da mesma. Um dos principais objetivos da pesquisa contemporânea na DA é encontrar biomarcadores que possam facilitar o diagnóstico, principalmente nas fases iniciais da doença ou até mesmo antes que haja o desenvolvimento de demência. Isso se deve, sobretudo, não apenas ao melhor prognóstico com o tratamento farmacológico e não-farmacológico, mas também pela perspectiva do desenvolvimento de novas drogas que possam mudar a história natural destas patologias. A neuroimagem, nesse contexto – particularmente a ressonância magnética (RM) – serve tanto como um importante ‘apoio’ para o diagnóstico final da doença (Teipel *et al.*, 2008) como também constitui uma importante ferramenta na busca por esses biomarcadores.

Durante as últimas décadas, o entendimento de que a DA se caracteriza quando já há a ocorrência clínica da demência fez com que um novo conceito fosse empregado, Comprometimento Cognitivo Leve (CCL) (Petersen

et al., 1999). CCL é um termo clínico aplicado a pacientes com uma ou mais alterações cognitivas objetivas, sem que haja prejuízo significativo das atividades de vida diária (ou seja, sem que sejam preenchidos critérios para diagnóstico de demência) (Albert *et al.*, 2011). Hoje em dia, cada vez mais aceita-se o fato de que sujeitos com CCL, principalmente o subtipo amnéstico (CCLa), possuem chances aumentadas de virem a desenvolver a demência da DA.

As Redes Neurofuncionais

A organização cerebral das funções mentais ocorre como um sistema complexo de redes interconectadas - cada uma contribuindo especificamente para o funcionamento do sistema como um todo (Luria, 1970). A degeneração de redes neurofuncionais específicas pode, por sua vez, gerar problemas cognitivos e/ou psiquiátricos. De fato, avanços recentes no estudo da neuroimagem na DA mostraram que as alterações presentes nesse grupo de pacientes não se restringem apenas às estruturas anatômicas particulares (como atrofia de áreas do lobo temporal medial), mas estendem-se também às redes neurofuncionais, as quais podem gerar problemas de memória, habilidades visuoespaciais, linguagem, funções executivas, entre outros (Bokde, Ewers e Hampel, 2009).

As redes neurofuncionais podem ser descritas como grupos de estruturas corticais e/ou subcorticais espacialmente distintas, mas funcionalmente conectadas (Power *et al.*, 2011). Essas redes podem ser identificadas pelo estudo do padrão de sincronia do sinal BOLD (*blood oxygenation level dependent*, dependente do nível de oxigenação do sangue), mesmo na ausência de um paradigma experimental ou estímulo externo – método conhecido como RM funcional (RMf) de repouso (Biswal *et al.*, 1995). Interessantemente, essas redes neurofuncionais obtidas durante o repouso recapitulam e refletem a topografia que seria obtida durante determinadas tarefas cognitivas (Smith *et al.*, 2009), e dentre elas destacam-se a rede de Modo Padrão (*Default Mode Network,* DMN), a rede de Linguagem (*Language Network*), a rede de Atenção Dorsal (*Dorsal Attention Network*), a rede de Controle Executivo (*Executive Control Network*), a rede de Relevância Emocional (*Salience Network*), a rede Visuoespacial (*Visuospatial Network*), a rede Sensorio-motora (*Sensorimotor Network*), entre outras.

Conectividades Estrutural e Funcional

As funções mentais, conforme acima descrito, podem ser caracterizadas pelo padrão de conectividade e atividade de diferentes regiões cerebrais. A maneira como avaliamos o quão íntegro estão essas conexões se baseia em duas principais abordagens: a conectividade estrutural e a conectividade funcional.

O termo conectividade estrutural refere-se à estruturação física que liga diferentes áreas cerebrais - os tratos de substância branca. Usando técnicas avançadas de ressonância magnética como o DTI (*diffusion tensor imaging* - imagem por tensor de difusão), podemos analisar a conectividade anatômica das redes neurofuncionais. Esse método quantifica a difusão da água de maneira não invasiva (Poupon *et al.*, 2000; Madden *et al.*, 2004; Sotak, 2004) e baseia-se na teoria de que a água encontrada nos tecidos apresenta propriedades de difusão anisotrópicas (ou seja, a difusão não é igual em todas as direções) (Teipel *et al.*, 2007). Quando um feixe de axônios apresenta valores alterados para alguma das métricas extraídas pelo método de DTI, assume-se que esse trato esteja com algum tipo de alteração na sua microestrutura. Dentre as principais métricas obtidas com o DTI, destacam-se a anisotropia fracionada e a difusividade média.

Quando falamos de conectividade funcional, entretanto, nos referimos a áreas cerebrais que estão sincronizadas entre si no desempenho de uma função, tarefa ou mesmo na manutenção de um estado mental (Reid e Evans, 2013). Essas áreas não possuem, necessariamente, conexão física entre elas (ou seja, presença de conectividade funcional não implica necessariamente em conectividade estrutural direta) (Greicius *et al.*, 2009). No caso da análise por RMf de repouso, esse sincronismo baseia-se na variação do sinal BOLD que, por vez, é medido pela razão entre a oxiemoglobina e a desoxiemoglobina – que possuem propriedades diamagnética e paramagnética, respectivamente (Seiyama *et al.*, 2004). Conforme ocorre um aumento do fluxo sanguíneo em determinada área cerebral (e esse evento é interpretado como uma manifestação indireta da ativação neural), há uma variação na concentração relativa da oxi e desoxiemoglobina, alterando a resposta magnética da região e aumentando o sinal da ressonância magnética (Toronov *et al.*, 2003). Assume-

se assim que as regiões cerebrais que possuem um padrão síncrono de variação temporal do sinal BOLD estão funcionalmente conectadas.

Estudos envolvendo técnicas de DTI e RMf têm crescido de maneira exponencial nos últimos anos. Através deles, obtivemos avanços significativos não apenas no entendimento do funcionamento das redes neurofuncionais, mas também na maneira como essas redes estão alteradas em doenças neurológicas ou psiquiátricas. Na DA, especificamente, inúmeros estudos têm apontado que há desconexão estrutural e funcional, principalmente na DMN, conforme descritos abaixo.

Default Mode Network **(A Rede de Modo Padrão)**

A descoberta da DMN ocorreu na década de 1990, de forma casual. A maioria dos estudos na época, em geral por imageamento por emissão de pósitrons (^{18}F-flúor-deoxi-2-glicose *Positron emission tomography*, ou FDG-PET), se concentrava na resposta da mudança do metabolismo cerebral de glicose após o indivíduo ser submetido a determinado estímulo (motor, cognitivo, emocional) durante o exame. Entretanto, foi constatado que algumas regiões cerebrais também estavam metabolicamente mais ativas quando o indivíduo era deixado em repouso mental (ou seja, sem estar engajado em nenhuma tarefa específica proposta pelo examinador) (Kety e Schmidt, 1948). Assumiu-se assim, que esse padrão de ativação neural que ocorre durante momentos mentais ‘passivos’, consistia no nível basal de atividade cerebral - ou modo padrão (Raichle *et al.*, 2001). Interessantemente, observou-se que não apenas o padrão dessa ativação era altamente organizado, mas também responsável pelo gasto de uma quantidade substancial de energia do metabolismo cerebral (Shulman, Hyder e Rothman, 2003).

Durante a execução de tarefas mentais, entretanto, viu-se que essas mesmas regiões apresentavam um padrão inverso de ativação em relação às áreas recrutadas no processamento cognitivo da tarefa proposta (Anticevic *et al.*, 2012). Ou seja, as regiões da DMN sempre apresentavam atividade metabólica diminuída em relação às demais. Assumiu-se, assim, que a DMN se ‘desativava’ quando um indivíduo se engajava em tarefas que envolviam atenção ao mundo exterior, passando assim a ser chamada de rede negativa de tarefa (*task-negative network*) (Lin *et al.*, 2011). Alguns anos mais tarde, no começo dos anos

2000, estudos envolvendo RMf de repouso também conseguiram isolar a DMN, devido ao fato de que suas regiões (assim como as de qualquer outra rede neurofuncional), permanecem conectadas mesmo na ausência de uma tarefa externa (Greicius *et al.*, 2003). Esses estudos foram particularmente informativos porque, semelhantemente aos exames de FDG-PET, também mostravam as regiões do cérebro funcionalmente conectadas, mesmo na ausência de um paradigma cognitivo.

A DMN na DA

Em nível molecular, a DA é caracterizada principalmente pelo acúmulo de placas senis extracelulares e insolúveis de beta amiloide (βA), e emaranhados neurofibrilares (ENFs) intracitoplasmáticos de proteína tau hiperfosforilada (Selkoe, 2001). Interessantemente, porém talvez não coincidentemente, as regiões-chave da DMN são as mesmas que possuem as maiores alterações fisiopatológicas características da doença. A região medial dos lobos temporais é fortemente afetada pelo acúmulo de ENFs, o que está associado à neurodegeneração (Braak e Braak, 1991). O giro do cíngulo posterior, por sua vez, está dentre as primeiras áreas cerebrais a mostrar acúmulo de βA nas imagens de PET obtidas após injeção intravenosa do composto B de Pittsburgh (Buckner *et al.*, 2005) – uma técnica que permite a visualização *in vivo* dos núcleos de βA das placas senis. Além de ser uma área susceptível ao depósito de placas senis, o giro do cíngulo posterior e suas regiões adjacentes estão dentre as que apresentam uma substancial alteração no metabolismo da glicose. Exames com FDG-PET mostraram que elas possuem uma redução nesse metabolismo, interpretada como uma diminuição na funcionalidade, não totalmente explicada pela atrofia existente na região (Nestor *et al.*, 2003; Kuntzelmann *et al.*, 2013). Assim, devido às alterações estruturais apresentadas por atrofia, bem como ao depósito de βA e à redução no metabolismo de glicose presente nas suas regiões, a DMN tornou-se objeto especial de estudo no campo da DA.

Sob o ponto de vista cognitivo, estruturas anatômicas que integram a DMN processam a memória episódica, entre outras funções (Miller *et al.*, 2008). Alterações na conectividade dessa rede podem, por sua vez, estar relacionadas ao declínio cognitivo de sujeitos com CCLa e DA. De fato, há alguns anos

constatou-se que pacientes com DA possuem uma menor ‘desativação’ da DMN durante tarefas de atenção (Pihlajamaki e Sperling, 2009), evento necessário para um correto engajamento nessas tarefas. Também, observou-se uma menor conectividade funcional nessa rede durante o repouso em pacientes com DA (Sorg *et al.*, 2007), mas resultados controversos para sujeitos com CCLa (ver, por exemplo, Qi *et al.*, 2010; Gili *et al.*, 2011). Dentre as outras redes neurofuncionais, entretanto, pouco se sabe sobre a integridade e significado clínico na DA, apesar de sua conhecida importância no processamento cognitivo.

Estruturalmente, sabe-se que a DA afeta também tratos de substância branca em diversas regiões, incluindo não apenas os feixes que conectam a DMN (Zhu *et al.*, 2013), mas também outros tratos (Acosta-Cabronero *et al.*, 2010). Há aproximadamente uma década, constatou-se que danos específicos no feixe que liga o giro do cíngulo posterior com a região medial frontal – ou seja, o fascículo do cíngulo, estão associados com uma pior performance cognitiva em pacientes com DA (Fellgiebel *et al.*, 2005). Desde então, relativamente pouca pesquisa foi feita a respeito da integridade anatômica da DMN envolvendo outras métricas além de anisotropia fracionada e difusividade média, bem como seus correlatos cognitivos, especialmente em sujeitos com CCLa.

Desse modo, ao longo desses anos de doutorado, nos concentramos principalmente na análise da integridade de redes neurofuncionais em pacientes com DA e CCLa – sobretudo a DMN. Porém, inicialmente, almejando obter uma visão mais geral da integridade das substâncias cinzenta e branca em pacientes com DA e CCLa, estudamos também métodos mais tradicionais de avaliação, como volumetria e morfologia.

A organização desse trabalho se encontra em forma de capítulos (artigos produzidos e/ou publicados), em ordem temporal:

Capítulo 1: Análise de volumetria manual de regiões mediais temporais, corpo caloso e tálamo em pacientes com DA leve, bem como seus correlatos cognitivos;

Capítulo 2: Revisão da literatura sobre da neuroimagem estrutural na DA;

Capítulo 3: Análise da conectividade funcional da DMN, rede de Controle Executivo, Linguagem e processamento Visuoespacial em pacientes com DA, bem como seus correlatos cognitivos;

Capítulo 4: Análise da conectividade estrutural da DMN em pacientes com DA e CCLa, bem como seus correlatos cognitivos;

Capítulo 5: Análise das conectividade funcional e amplitude do sinal BOLD da DMN em pacientes com DA e CCLa, bom como seus correlatos cognitivos;

Capítulo 6: Análise longitudinal das alterações de substância branca e cinzenta em pacientes com DA, bem como seus correlatos com dados fisiopatológicos e cognitivos;

Capítulo 7: Revisão da literatura e proposição de um modelo integrando memórias autobiográficas, DMN e a manutenção do *self* em pacientes com DA;

Capítulo 8/Outros: Revisão da literatura sobre as alterações de redes neurofuncionais em doenças neurodegenerativas, abordando desde aspectos moleculares até a compreensão dessas doenças como ‘redepatias’ e seus correlatos fenotípicos. Aluna como segunda autora.

OBJETIVOS

Conforme descrito na Introdução, o principal objetivo desse trabalho foi investigar as conectividades estrutural e funcional das redes neurofuncionais - com ênfase na DMN. Os resultados produzidos ao longo desses anos, entretanto, não se restringem a ele. Na sessão OBJETIVOS seguiremos a linha de raciocínio da INTRODUÇÃO, enfatizando a análise dessas redes.

Geral

- Avaliar a integridade das redes neurofuncionais em pacientes com DA leve e CCLa comparados a idosos saudáveis.

Específicos

- Comparar a conectividade funcional das redes neurofuncionais entre pacientes com DA leve, CCLa e idosos normais;
- Verificar se há correlação entre a conectividade funcional das redes e escores cognitivos em testes de memória episódica, linguagem, habilidades visuoespaciais e funções executivas.
- Comparar a integridade anatômica (conectividade estrutural) dos tratos de substância branca da DMN em pacientes com DA leve, CCLa e idosos normais, através de DTI;
- Verificar se há correlação entre a conectividade estrutural da DMN e escores cognitivos em testes de memória episódica, linguagem, habilidades visuoespaciais e funções executivas.

Capítulo 1

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Volumetric Brain Changes in Thalamus, Corpus Callosum and Medial Temporal Structures: Mild Alzheimer's Disease Compared with Amnestic Mild Cognitive Impairment

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Key Words
Alzheimer's disease · Amnestic mild cognitive impairment · Thalamus · Corpus callosum · Volumetry

Abstract
Background: It is widely known that atrophy of medial temporal structures is present in the mild stage of Alzheimer's disease (AD) and amnestic mild cognitive impairment (aMCI). However, structures such as the thalamus and corpus callosum are much less studied. ***Methods:*** We compared the volumes of the entorhinal cortex, hippocampus, thalamus and the corpus callosum in 14 controls, 14 patients with mild AD and 15 with aMCI and correlated these volumes with neuropsychological data. MRI was obtained at 2 T followed by manual segmentation. ***Results:*** We found atrophy in hippocampi and thalami of MCI patients compared to controls, and in the bilateral entorhinal cortex of aMCI compared to AD patients. All the structures showed atrophy in AD patients compared to controls, including the corpus callosum. ***Conclusions:*** Our study confirms that thalamic areas are atrophied in aMCI, and the corpus callosum might represent a good structural marker for mild AD. Those areas were associated with cognitive functions already described in the literature.

Introduction

Alzheimer's disease (AD) is characterized by cognitive decline, changes in behavior, and impairment in social and occupational functioning as a result of a slow and progressive degeneration of brain structures, such as the hippocampus, entorhinal cortex and associative cortices. Among other factors, this condition arises due to an excessive deposition of insoluble plaques of β-amyloid pro-

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tein and neurofibrillary tangles secondary to abnormal phosphorylation of tau protein. Amnestic mild cognitive impairment (aMCI) is considered a possible prodromal AD, and is defined as a decline in memory functions without significant social or occupational impairment [1].

The most striking cognitive deficit both in magnitude and prevalence in AD and aMCI is the initial change in episodic memory, manifested clinically by difficulty in retaining new information. Neuropsychological assessment usually shows decreased recall of verbal and nonverbal information. Progressive changes in language are typical, as well as disturbances of visuospatial skills, praxis, gnosis and executive functions, that appear in the course of the disease.

It is widely known that atrophy of medial temporal (MT) structures such as the hippocampus and entorhinal cortex may be present even in the mild stage of AD and possibly in aMCI. It has also been fully demonstrated that the anatomical and functional abnormalities in these structures lead to an important impairment of episodic memory. However, the anatomy of other relevant structures for cognition, such as the thalamus and white matter tracts are much less studied, as well as the clinical impact of their possible dysfunctions. There are few studies evaluating these structures in AD, particularly at the early phase, or in aMCI patients. The majority of the studies have focused on the neocortex and the hippocampus and its memory process, while the thalamus has received less attention [2]. Like in thalamic areas, findings are also inconsistent when atrophy of the corpus callosum (CC) occurs in AD [3]. Knowing that such structures may contribute to cognitive deterioration, and with the promising emergence of new drugs that may modify the progression of the disease, it is imperative to further study diagnostic methods and detect the disease in a very early stage. Studying anatomic and functional details of several brain structures that may be affected in the early stages of this pathology may help to clarify this complex clinical condition.

In this study, we evaluated the anatomy of MT regions (entorhinal cortex and hippocampus), the thalamus (a subcortical structure highly relevant for cognition) and the largest bundle of white matter, also related to cognitive functions, the CC, both in normal elderly and patients with mild AD and aMCI. Also, in order to better understand the contribution of these structures in the genesis of cognitive symptoms, we studied the correlation between these volumes and neuropsychological tests.

Materials and Methods

Study Population

The study was approved by our local ethics committee and all patients signed an informed consent form prior to any procedure. It has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

We studied 14 individuals with mild AD, 15 with aMCI and 14 normal subjects matched for age and education. Patients were evaluated by a trained clinical neurologist at the Unit for Neuropsychology and Neurolinguistics at the University Hospital – UNICAMP. We performed routine laboratory workup (vitamin B_{12}, folate, syphilis serology, thyroid hormones) and brain computed tomography prior to inclusion in the study. All individuals were over 50 years.

To establish the diagnosis of aMCI, we performed a standardized clinical evaluation which included a detailed interview with the patient and the caregiver, a neurological examination, and the Cambridge Mental Disorders of the Elderly Examination [4]. MCI diagnosis followed the criteria of the International Working Group on Mild Cognitive Impairment [1]. All patients with aMCI had preserved activities of daily living, a minimum compromise in performing complex instrumental functions, evidence of decline over time with objective cognitive tasks, and Clinical Dementia Rating (CDR) [5] of 0.5 with an obligatory memory score of 0.5.

We adopted the DSM-IV criteria for dementia and NINCDS-ADRDA for probable AD and CDR = 1 to classify the mild AD group. For the control group, we included subjects with no cognitive or psychiatric complaints and CDR = 0. We excluded individuals with other neurological or psychiatric diseases, Hachinski ischemic score [6] greater than 4, head injury with loss of consciousness, use of sedative drugs 24 h prior to neuropsychological testing, history of drug or alcohol addiction and prior chronic exposure to neurotoxic substances.

Neuropsychological Evaluation

Global cognitive status was measured using the Mini Mental State Examination [7] and episodic memory was evaluated by the Rey Auditory Verbal Learning Test (RAVLT) [8]. Visual perception was assessed with subtests of Luria's Neuropsychological Investigation (LNI) [9], using items G12, G13, G14 (the patient is asked to examine and name pictures of objects that are scribbled over or superimposed on another picture), and G17 (item from Raven's test) and one item for mental rotation of figures (in both items, the patient is asked to complete a structure, a portion of which is missing, by choosing from various options). Four items from the Ratcliff Manikin Test for mental rotation were also used [10]. Language tests included the Boston Naming Test [11] and verbal fluency (VF) for category (animals). Working memory was assessed by the forward (FDS) and backward digit span (BDS) subtest of the WAIS-R [12]. In addition, we applied the Cornell Scale for depression in dementia [13].

MRI Scanning Protocol, Imaging Processing and Statistical Analysis

We used MR images acquired in a 2-tesla MRI system (Elscint Prestige, Haifa, Israel), 3D acquisition, spin-echo weighted in T_1, with isotropic voxels of 1 mm. Prior to processing, all images were evaluated to exclude artifacts, structural lesions or other neurological diseases. The images were converted from DICOM to

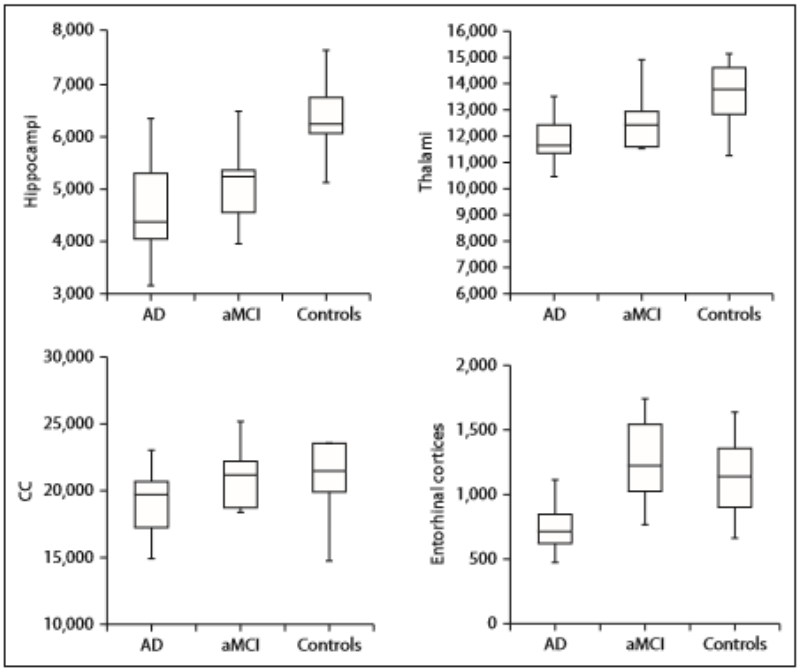

Fig. 1. Box plots for volumes-versus-subject groups for the hippocampus, thalamus, CC and entorhinal cortex. X-axes show the studied groups and y-axes show the magnitude of volumetry. Results of the hippocampi, thalami and entorhinal cortices are mean bilateral volumes.

MINC through N3 software [14] and normalized to Talairach space [15]. We performed manual segmentation of the CC, thalamus, hippocampus and entorhinal cortex using the Display software (www.bic.mni.mcgill.ca). This software automatically performs spatial normalization of the volumetric data by the following formula: structure volume = (patients' structure volume × mean cerebral volume of controls)/patients' cerebral volume. The volumes of individuals with different encephalic perimeter are directly comparable by using this software. Display allows for simultaneous viewing of MR images in the coronal, sagittal and axial planes, contrast adjustment between white and gray matter and the visualization of the same point in the three planes simultaneously.

The borders of the CC were outlined sequentially from medial to lateral. We used the anterior tubercle of the thalamus, which participates in the definition of the ventricular foramen, anteriorly; the pulvinar which projects over the lateral and medial geniculate bodies, posteriorly, and the internal capsule laterally. Initially, for the segmentation of the hippocampus, we used the sagittal sections, going from lateral to medial. In the sagittal plane, lower, upper and posterior limits are easily viewable. The anterior limit of the hippocampus, from this segment, is not as well-defined, merging with the amygdala in some spots. We measured absolute volumes, and compared them between groups using the nonparametric Kruskal-Wallis test. Level of statistical significance was established at $p < 0.05$. To better understand the relationship between these anatomical structures and the cognitive performance of the subjects, we performed multiple regressions (stepwise backwards) to evaluate the correlation between neuropsychological tests and anatomical volumes, with explanatory variables and a higher coefficient of determination. For that, we considered the three groups together (healthy controls, aMCI, and mild AD) to increase our data variance and enhance the correlation between cerebral region and psychological function. We used the package SYSTAT 12 (San Jose, Calif., USA, Systat Software Inc.) for statistical analysis.

Results

The AD versus aMCI group showed significant difference only in volume at the bilateral entorhinal cortex ($p < 0.0001$). The AD versus control group, in turn, showed significant volumetric difference in all structures analyzed: right and left hippocampus ($p < 0.0001$ and $p < 0.001$, respectively), right and left thalamus ($p < 0.008$ and $p < 0.002$, respectively), right and left entorhinal cortex ($p < 0.0001$ and $p < 0.031$, respectively) and CC ($p < 0.043$). The aMCI versus control group showed bilateral atrophy in the hippocampus (right: $p < 0.001$ and left: $p < 0.001$), and bilateral thalamus (right: $p < 0.029$ and left: $p < 0.036$; fig. 1).

As evidenced in table 1, the volume of the CC correlates with the FDS, Boston Naming Test and RAVLT's recognition subitem (multiple $R^2 = 0.529$; $p = 0.007$). The volume of the right thalamus is associated with the Mini

Table 1. Multiple regression, neuropsychological test × anatomical structures

Structure	Test	Multiple R^2	p
Corpus callosum	FDS Boston Naming Test RAVLT delayed recall	0.529	0.007
Thalamus (r)	Mini Mental State Examination	0.492	0.001
Thalamus (l)	Age FDS RAVLT delayed recall	0.542	0.005
Hippocampus (r)	Age BDS VF RAVLT delayed recall	0.578	<0.001
Hippocampus (l)	BDS VF RAVLT delayed recall	0.531	<0.001
Entorhinal cortex (r)	LNI visuospatial BDS RAVLT delayed recall	0.544	0.003
Entorhinal cortex (l)	LNI visuospatial RAVLT delayed recall	0.423	0.019

Mental State Examination (multiple $R^2 = 0.492$; $p = 0.001$). The left thalamus correlates with age, FDS and RAVLT's delayed recall subitem (multiple $R^2 = 0.542$; $p = 0.005$). The volume of the right hippocampus is directly linked with age, BDS, VF and RAVLT's delayed recall subitem (multiple $R^2 = 0.578$; $p < 0.001$); the left hippocampus is related to the BDS, VF and RAVLT's delayed recall subitem (multiple $R^2 = 0531$; $p < 0.001$). The right entorhinal cortex is associated with the LNI Visual Perception Test, BDS and RAVLT's delayed recall subitem (multiple $R^2 = 0{,}544$; $p = 0.003$) and the left entorhinal cortex with the LNI Visual Perception Test and RAVLT's delayed recall subitem (multiple $R^2 = 0423$; $p = 0.019$).

Discussion

Our findings confirmed MT structures atrophy in mild AD and even in aMCI, when compared with normal elderly. In addition, we showed that other cerebral structures relevant for cognition, such as the thalamus and CC, may be compromised even in the early phase of the disease. Most of these structures were significantly related to cognitive functions, like episodic and working memories, language and visuospatial skills.

Many MRI-based studies in AD have reported brain atrophy mainly in MT lobe areas [16, 17] and specially demonstrated significant atrophy in the hippocampus of MCI patients [18–21]. In this study, we also found atrophy in such areas in aMCI patients: both the hippocampi and both the thalami showed significant volume reductions in comparison with controls. In a recent study, in fact, the hippocampal area has been the only structure able to differentiate aMCI patients from healthy elderly people, being the one that showed the largest volumetric variation between these two groups [22]. Furthermore, MT regions – among others – were predictive of cognitive decline in aMCI [23]. Although a previous study has not used entorhinal cortex and hippocampal atrophy measurements to separate different stages of the disease [24], both areas have been mentioned as the best structural biomarkers of AD [21, 25, 26].

Comparing aMCI with AD, we did not find any significant volumetric difference in hippocampal structures, although an atrophic process tended to be greater in AD patients. These data agree with Karas et al. [16], who suggest that those areas are the first ones affected, presenting very early signs of the disease. Therefore, they are useful to separate the first stages of aMCI, but not to differentiate the stages where dementia has been reached. Between aMCI and AD stages, both entorhinal cortices showed significant volume reduction. Indeed, these were the only structures showing significant atrophy between the two groups. Studies have shown that the entorhinal cortices are the first to show pathological changes in AD [27]. In our study, we did not find volumetric differences in this area between aMCI patients and controls. This can be explained by the fact that entorhinal cortex boundaries are not easily defined due to anatomical ambiguity, interfering image artifacts and/or both [28]. In addition, aMCI is a heterogeneous clinical syndrome and as such, not all patients in this study have prodromal AD pathology. This fact could decrease the sensitivity to detect early changes, such as entorhinal atrophy. Our results suggest that hippocampal volumes may be more reliable than the entorhinal cortex to differentiate the two stages [28].

The hippocampus and related MT lobe regions play a crucial role in encoding, consolidation, and retrieval of information responsible for episodic memory [29]. The medial temporal lobe richly connects to thalamic nuclei and the retrosplenial cortex, constituting the hippocam-

pal-diencephalic system, whose integrity is also essential for a normal episodic memory performance [30]. Since the MT lobe together with limbic structures and prefrontal cortex are essential for consolidation, retrieval and recognition of information, it is expected to find episodic memory impairment in patients with damage in these areas.

Besides MT structures, the CC also showed volumetric reduction at some stages. Callosal atrophy has already been reported as a structural marker for more advanced stages of AD when compared to healthy normal controls [31–33]. In some other studies, differences in callosal volumetry were found between AD patients and controls, as well as between MCI and AD patients [34] and also between MCI patients and controls [35]. However, we did not find significant CC atrophy comparing aMCI with AD. Wallerian degeneration has been appointed as the main explanation for callosal abnormalities, arising from a primary atrophy in the cortical association areas.

Anterior callosal areas have been said to be more likely to suffer atrophy [36] in later stages of AD, even though posterior areas could also be involved in the progression of the disease [37, 38]. Anterior portions of the CC are responsible for connection between frontal areas, and according to Di Paola et al. [3], atrophy in these portions can affect attention and executive functions, whereas posterior reduction can harm episodic memory functions performed by posterior cortical memory networks. Our results provide further evidence that callosal atrophy is more pronounced in AD than in healthy elderly people, even in the mild phase of the disease. A significant volume reduction was appointed when comparing AD patients with normal controls, but no changes were detected between aMCI patients and controls, supporting that callosal atrophy is detectable when the dementia stage has developed. These findings suggest that the CC might represent a marker for a more widespread neocortical degeneration in AD.

Given that the CC consists of projections that connect both hemispheres, callosal atrophy might have implications for cognitive decline. Communication and language are cognitive domains which require connection between the two hemispheres. As both of them work in parallel during language-related tasks, it is crucial that the connection between them must occur as a neural basis for interhemispheric information interaction [39]. According to Hallam et al. [33], two possibilities could explain the relationship between such atrophy and cognitive impairment. First, cognitive decline is caused by degeneration of the lobes connected through the CC, and it is not caused by callosal atrophy itself. Secondly, callosal size does reflect in some aspects of cognition and thus, in a reduction of interhemispheric interactions. Although this issue still remains controversial, our study shows evidence that callosal atrophy does have an important influence on the genesis of cognitive symptoms: we found a relationship between the CC and language assessment tests such as the Boston Naming Test and VF (where the former is a test of visual confrontation naming and the latter tests semantic fluency). With this in mind, our results show further evidence for the association of callosal atrophy with language impairment seen in AD.

Thalamic structures are also atrophied in AD, as reported in previous studies [2, 22, 40, 41], when compared to healthy elderlies. In fact, we found differences in thalamic volumetry in aMCI patients, corroborating the findings of de Oliveira et al. [34]. An early thalamic involvement has also been reported by other authors [17, 18] and, in a longitudinal study over an 18-month follow-up period, both the converters and nonconverters to AD presented thalamic atrophy [19]. Since the thalamus is an area that can be difficult to evaluate by computational methods due to its topographical location [40] and manual outlining of this structure would be a more reliable method for volumetric measurement [16], our results comprehend a strong source of evidence of an early thalamic atrophy in the disease.

Finally, since the cingulate gyrus, mammillary bodies, parahippocampal gyrus, hippocampus, entorhinal cortex and thalamus are all part of the Papez circuit [42], thalamic lesions might affect memory functioning and lead to cognitive decline in AD patients. Volume of deep gray matter structures has been said to be the strongest predictor of cognitive effects [2], and thalamic silent infarcts were correlated with a worse performance in memory tasks even in normal people [43]. Among these lesions, those occurring in the left thalamus are more likely to affect verbal memory and language, while the right-sided ones can generate more subtle memory decline, without language impairment [44]. In addition, the thalamus, together with MT lobes, neocortical association areas, basal ganglia and basal forebrain compose a brain network whose atrophy associates with the diagnosis of AD [45].

The limitations of our study must be acknowledged. It is worth mentioning the small sample size of participant subjects. Nevertheless, we could find significant values for atrophy in aMCI and AD. An additional limitation is its cross-sectional nature and, since it is suggested that

aMCI is a continuum stage of AD, follow-up studies where patients are compared to themselves over time would be necessary to better confirm some results. Also, we must consider that some of the aMCI subjects may not develop AD.

Conclusions

To sum up, our results show that MT lobe and thalamic structures are affected in aMCI patients when compared to healthy elderlies. Both the hippocampi and thalami showed significant atrophy in controls versus MCI, but comparing MCI versus AD we found significant atrophy only in bilateral entorhinal cortex. The thalamus significantly correlated with memory and attention skills and MT structures correlated with memory, language and visuospatial domains. In AD patients, however, all structures analyzed were atrophied when compared to healthy controls.

The CC, in turn, showed volumetric reduction only between controls and AD patients. The callosal area has shown to be associated with language and memory impairment seen in the disease and our results provide further evidence that callosal atrophy is more pronounced in AD than in healthy elderly people. A significant volume reduction was appointed when comparing AD patients with normal controls, but no changes were detected between MCI patients and controls, suggesting this area may be a structural marker of the disease, when dementia has been reached. Assuming that aMCI may indeed lie halfway between AD and healthy elderlies, we could say that early changes in aMCI involve atrophy of the MT lobes and thalamic areas, while established AD also involves CC atrophy.

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Disclosure Statement

The authors report no conflict of interest.

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Capítulo 2

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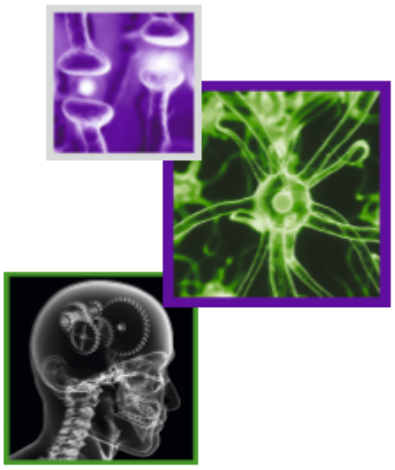
SPECIAL REPORT

Comparing regional brain atrophy in mild cognitive impairment and Alzheimer's disease



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Practice Points

- Structural MRI is essential to the assessment of patients with suspected Alzheimer's disease and mild cognitive impairment.
- Medial temporal lobes are the brain regions that the clinician must pay more attention to; the clinician must also evaluate the patient's hippocampal height and the width of choroid fissure and temporal horn.
- Nonamnestic mild cognitive impairment or atypical Alzheimer's disease may present early atrophy in other brain structures, such as parietal and frontal lobes.
- The analysis of the progression of atrophy over time can help the clinician to decide whether the patient's cognitive problem is likely neurodegenerative or not.
- New structural MRI methods may be very useful in the near future to help the diagnostic process of mild cognitive impairment and Alzheimer's disease, especially measures of cortical thinning and microstructural changes in medial temporal lobes.

SUMMARY Neuroimaging has assumed an active role in the diagnosis of Alzheimer's disease (AD) and other dementias. Structural MRI can estimate changes in specific brain structures relative to normal and pathological aging such as volume, cortical thickness and gray matter density. Several different structural MRI methods can be used to identify neuropathology and point to an early atrophy in medial temporal lobe structures in patients with AD and amnestic mild cognitive impairment, especially in the entorhinal cortex and hippocampus. These alterations in medial temporal lobe structures were also considered evidence for neurodegeneration, even in preclinical AD. However, evaluation in other areas such as ventricular enlargement and precuneus may help the diagnosis, even in the early stages of the disease. Currently, neuroimaging is an excellent tool for increasing diagnostic accuracy, but does not substitute a careful clinical and neuropsychological evaluation. In this article, our objective is to gather information about different structural MRI-based methods that could offer objective measures of brain structures and increase the diagnostic power of mild cognitive impairment and AD.

Neuroimaging biomarkers are extremely relevant to Alzheimer's disease (AD) research. Recently, structural MRI has been included as evidence for neurodegeneration, even in the preclinical stages of AD [1]. This is because the process of brain atrophy seems to be an

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inevitable, inexorably progressive concomitant in the process of neurodegeneration, and brain atrophy – especially in medial temporal lobe (MTL) structures – has a strong correlation with clinical symptoms and neuropathology [2]. Therefore, neuroimaging has changed its role in the diagnosis of AD and other dementias: rather than just excluding other potential causes, it has assumed an active role in identifying specific features of the disease (e.g., patterns of atrophy that are characteristic of the disease).

Structural MRI can estimate changes in specific brain structures relative to normal and pathological aging, such as volume, cortical thickness and gray matter density, and might be used in Phase II and III clinical trials to assess potential disease-modifying drugs. Therefore, the prospect of disease-modifying drugs that can slow or prevent disease progression has caused increased interest in identifying neuroimaging markers of individuals with memory problems and AD.

This has led to the concept of mild cognitive impairment (MCI), a possible prodromal AD. Clinically, subjects with MCI have memory deficits or other cognitive problems that are not severe enough to significantly affect activities of daily living, but have an increased risk of developing dementia [3]. Its prevalence is estimated to be about two- to three-times higher than that of AD [4]. For that reason, positive biomarkers are extremely important in MRI research to detect early brain alterations in the MCI group.

The earliest sites of MRI-based atrophic changes in typical AD are mainly in the MTLs, consistent with early memory deficits and tau deposition [5]. Subsequently, atrophy in other associative cortices occurs, causing other cognitive problems that affect language, executive function, praxis, visuospatial skills and behavioral impairments [6].

However, early-onset AD may present with different clinical and neuroimaging features. There are other possible AD clinical presentations other than amnesic, especially involving visuospatial skills (posterior cortical atrophy syndrome), executive functions/behavioral problems (frontal variant of AD) or linguistic (linguistic variant, most commonly logopenic primary progressive aphasia). In these cases, the pattern of atrophy may differ from 'classic' AD, mainly affecting parietal cortices in the case of posterior cortical atrophy syndrome, frontal lobes in the case of frontal variant and the left posterior temporal cortex and inferior parietal lobule in the case of logopenic primary progressive aphasia. Frisoni *et al.* showed that these atypical clinical presentations are more commonly seen in early-onset AD, with the largest atrophy in the occipital and parietal lobes, although late-onset AD shows the classic pattern of hippocampal atrophy [7].

Whitwell *et al.* studied the neuroimaging correlates of these different pathologically defined AD subtypes based on the distribution of neurofibrillary tangles: typical AD, hippocampal-sparing AD and limbic-predominant AD [8]. Compared with typical AD, hippocampal-sparing AD has more neurofibrillary tangles and atrophy in the cortex and fewer in the hippocampus, whereas the opposite pattern is seen in limbic-predominant AD. Age at onset differed between subtypes, with the youngest ages recorded in the group with hippocampal-sparing AD and oldest in the group with limbic-predominant AD. This difference explains why patients with early-onset AD have atypical clinical syndromes associated with widespread involvement in the association cortex.

In this article, our objective is to gather information about different structural MRI-based methods that could offer objective measures of brain structures and increase the diagnostic power of MCI and AD. Not all of the following methods are available in clinical practice, but they have the potential to become useful in the near future.

Visual rating of brain regions

One of the most useful strategies for the clinician is visual rating of the cerebral patterns of atrophy presented in MRI. This rating method does not involve software or postprocessing of the images. Some different scales can be helpful to assess the whole brain [9] or specific areas (e.g., the MTL atrophy rate [10,11] and the posterior cingulate, precuneus and superior parietal region atrophy rates [12]). MTL structures are better evaluated on coronal images, and even reformatted images from multislice computed tomography can be used in this analysis. Although MTL structures are most commonly affected in the early stages of the disease, other structures – especially in parietal lobes – can be precociously altered, particularly in presenile AD and nonamnestic presentations.

In the sagittal plane, for example, the clinician should rate the width of the posterior cingulate and parieto-occipital sulci. The clinician should also rate the atrophy of the precuneus, in both the axial and coronal planes, the widening of the posterior cingulate sulcus and the sulcal dilation in the parietal lobes. Such rating scales vary from 0 (normal) to 3 (very atrophic), and the examiner must evaluate:

- In the sagittal plane: width of posterior cingulate sulcus and parieto-occipital sulcus, and atrophy of the precuneus;
- In the axial plane: widening of the posterior cingulate sulcus and sulcal dilatation in the parietal lobes;
- In the coronal plane: widening of the posterior cingulate sulcus and sulcal dilatation in the parietal lobes.

In addition, there are other useful visual rating scales that assess the distribution, size and amount of white-matter disease (secondary to cerebrovascular disease) in dementia [13]. The fluid-attenuated inversion recovery sequence is the best option for evaluating white matter hyperintensities and lacunes.

These visual scales are also very useful for comparing the patterns of brain atrophy progression over time, especially in cases where there are uncertainties about the diagnosis. For example, MCI patients who develop AD dementia tend to have a faster rate of atrophy than those who do not [2]. The clinician has to evaluate the anatomy of specific brain structures proportionally to the whole brain.

Volumetry

The most common way to objectively assess brain atrophy is using volumetric MRI. This technique can be performed either in the whole brain or in structures chosen *a priori*, which requires their segmentation. The segmentation step involves defining the anatomical structures by its borders and it can be classified according to the degree of the rater's manipulation: manual, semiautomated and automated techniques. Although some private MRI services provide hippocampal volumes when AD is suspected, the clinician has to be careful when interpretating the results, as the values have to be adjusted for total intracranial volume and be in accordance with normal reference values of the local population.

Manual segmentation

The manual method for segmentation consists of drawing the desired borders directly onto the raw image by the researcher, which means this technique is still very labor intensive (Figure 1). Manual segmentation has to follow a standardized and validated protocol, therefore, there have been attempts to develop a harmonized protocol to overcome the present heterogeneity of manual segmentation [14]. Nonetheless, despite being time-consuming and unsuitable for large samples, it is still considered the gold standard for measuring hippocampal [15] and amygdalar [16] volumes. In general, the values of the measures have to be adjusted for total intracranial volume. Although MTL structures have been much more thoroughly studied [17–19], atrophy of other brain regions can be found, such as ventricular enlargement [14], and thalamus and corpus callosum [20], in patients with mild AD.

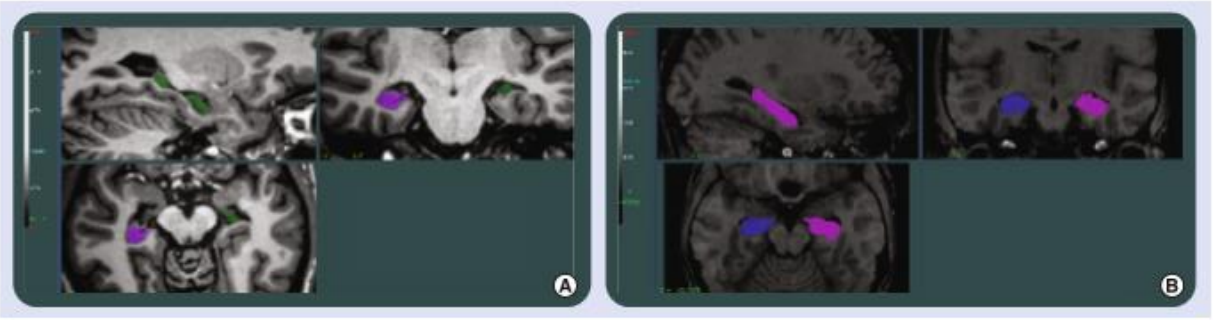

Figure 1. Manual segmentation of the left and right hippocampus in coronal and axial views, and the right hippocampus in sagittal view. The technique is labor intensive as the structures have to be manually delimited. **(A)** Atrophic hippocampus and **(B)** normal hippocampus.

Semiautomated segmentation

Semiautomated techniques consist of automated steps for segmentation with visual rating scales. The advantage of this procedure over the automated technique is that it can be applied individually and can be used in clinical practice. In comparison with manual segmentation, it has been said to be less susceptible to errors [21]. However, it also includes the bias of the rater, a problem shared with the manual technique, but that can be overcome using diagnosis-blind raters.

Regarding MTL structures, hippocampal volumes in the AD group were, on average, 20% smaller compared with a control group [21] and both entorhinal cortex and hippocampal volumes were reduced in MCI and AD [22]. Very early hippocampal atrophy was found elsewhere [23] and subjects with MCI who had phenotypic AD atrophy showed significantly greater 1-year clinical decline, and were more likely to have progression to probable AD [24]. In a study with presymptomatic autosomal dominant mutation carriers for AD, hippocampal volume differences were found 5.5 years before the clinical AD diagnosis [25]. Ventricular enlargement was also reported to occur in MCI subjects in some studies [22,23]. In addition, we can find callosal atrophy already in the very early stage of the disease [26].

Automated segmentation

Owing to the laborious nature of manual volumetric methods, automated tools have been developed to determine alterations in the brain structure using hypothesis- and rater-independent approaches. These have the advantage of being time-saving; however, this method is also susceptible to errors, especially when a more precise differentiation between gray and white matter is needed. In these cases, the errors must be manually corrected. Freesurfer software (Athinoula A Martinos Center for Biomedical Imaging, MA, USA) [101] uses an automated segmentation method described to have high accuracy and good reproducibility and sensitivity, allowing its use for multicenter and longitudinal studies [27]; however, it may have a tendency for overestimation and, thus, be more likely to produce false negatives compared with manual tracing [28].

In automated methods, we can also observe a loss in hippocampal volume in MCI subjects [29] and the rate of hippocampal atrophy has been reported to be higher in MCI converters than the mean value for the group [30].

Cortical thickness

Measuring cortical thickness is the process of defining in millimeters the surfaces of inner (the boundary between white and gray matter) and outer cortices across the entire brain. Among its advantages, it is reliably obtained from single individuals [31], as there is specific software that can properly process cortical thickness information individually (e.g., Freesurfer). In addition, it is visually easier to interpret and localize the atrophy [32].

Global thinning is already apparent by middle age in healthy individuals mainly due to loss of neuronal and dendritic architecture, rather than loss of neurons [33]. For example, in the healthy elderly, regional age-related changes occur predominantly in the frontal and paracentral gyri, but a reduction in cortical thickness can also be observed in the precentral, precuneus and posterior cingulate gyri. Primary cortices also decline with age, but much later in life [34]. Prominent atrophy of prefrontal cortex and relative sparing of temporal and parahippocampal cortex have been stated to be normally thinned by the aging effect [35]. MCI subjects, in turn, present a more pronounced thinning in medial temporal areas [32,36] and some regions of the frontal and parietal cortices [32] when compared with controls.

Most studies report no single structure to be able to separate AD patients from controls, but, instead, there is a more widespread gray matter loss in the AD group (Figure 2) [37]. Nevertheless, the temporal lobe is the area most commonly reported to be atrophic in AD patients, but structures such as parietal regions [32,37–39], the anterior and posterior cingulate regions, frontal lobe [38,39] and the visual associative areas [37] have also been reported.

Voxel-based morphometry

Voxel-based morphometry (VBM) is the most widely published automated postprocessing method and involves a voxel-wise comparison of the local concentration of gray matter between two groups of subjects (Figure 3). The importance of this approach is that it gives a comprehensive assessment of anatomical differences throughout the whole brain without an *a priori* hypothesis, avoiding the bias introduced by the rater in manual techniques. The preprocessing steps

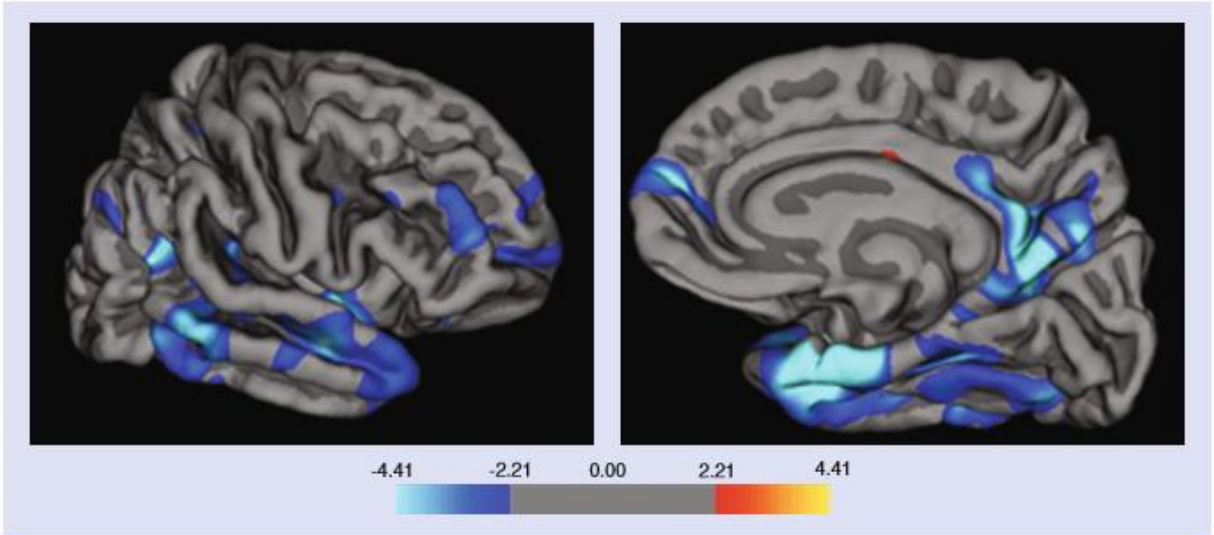

Figure 2. Differences in cortical thickness between mild-to-moderate Alzheimer's disease and normal aging. Image produced using Freesurfer (Athinoula A Martinos Center for Biomedical Imaging, MA, USA) software [101].

involve spatially normalizing all the images to the same stereotactic space (a customized template based on the average of the sample is recommended in neurodegenerative diseases due to the high degree of atrophy), the segmentation of the gray matter from the normalized images and smoothing.

The limitations of this technique include the fact that it is affected by the variability among individuals and that errors may be introduced by the preprocessing steps. Different individuals have unique gyral structures and certain structures have convoluted cortices, which are more difficult to register than simpler structures [40]. However, a recent VBM technique using SPM8 software (FIL Methods Group, London, UK) [102] plus diffeomorphic anatomical registration using exponentiated Lie algebra [41] that improves intersubject registration has been reported to be more powerful than a conventional optimized VBM technique [42]. In mild AD patients, atrophy was found in MTL structures and other regions such as the inferior parietal lobule, inferior and superior frontal gyri, anterior cingulated gyrus, caudate nucleus, precuneus and middle frontal gyrus [43]. Amnestic MCI showed a similar pattern of atrophy, although less intense than that of the mild AD group, mainly in thalami and parahippocampal gyri. In addition, the authors found white matter atrophy in mild AD patients in periventricular areas and the corpus callosum, and adjacent to associative cortices. Although this method is good for differentiating groups, it still lacks an established statistical model for determining individual risk for a single subject.

Deformation-based morphometry

Deformation-based morphometry, a less common automated technique, requires a high-dimensional normalization of the images. Compared with the previous method, this technique does not require segmentation of the brain into different tissue compartments [44] and completely eliminates the anatomical differences between the brains, whereas VBM preserves local differences [4]. A study performed using this approach found atrophy in MTL, the neocortical associative areas, and the thalamus and basal ganglia in AD patients, besides corresponding CSF enlargement; in MCI individuals, nonetheless, no difference was found compared with controls [45].

Magnetization transfer ratio

Magnetization transfer ratio (MTR) has been proposed as a method of detecting structural changes (e.g., histological alterations) that conventional MRI techniques cannot [46]. This happens because conventional T_2 relaxation times are orders of magnitude shorter than currently achievable minimum echo times [47]. Decreased values of MTR indicate demyelination [48], whereas an increased MTR

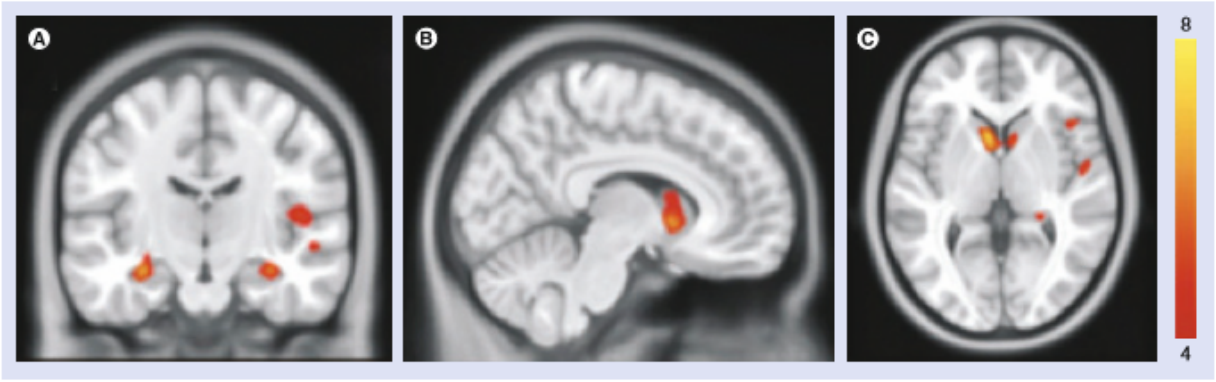

Figure 3. Voxel-based morphometry results showing significant differences in gray matter density between mild Alzheimer's disease and normal aging (corrected for multiple comparisons).

value is associated with amyloid-β accumulation, tau hyperphosphorylation and the absence of neurodegeneration [49]. MTR has been mostly used to detect changes in myelination [49], but this technique is also sensitive to gray matter abnormalities [47]. This technique has been proposed since not all subjects with MCI have volumetric brain changes and a decline in MTR measurements is detectable, even in the absence of volumetric loss [47].

Some studies have demonstrated a reduction in MTR, suggesting microstructural changes in brain tissue already in the MCI phase [47,50]. Such alterations occur mainly in the MTL [51], but structural changes were generally a widespread phenomenon also found in the frontal lobe [52]. In AD patients, MTR alteration was also found all over the brain [53,54], but alterations in specific regions, such as the hippocampus [53,55], cingulate, and parietal [48,55] and prefrontal areas [48], were also reported.

Hippocampal subfields

It is widely known that the AD process exhibits topographic selectivity within temporal lobe structures, especially affecting hippocampal and entorhinal cortex volumes. However, the hippocampus is a complex and heterogeneous structure, and the effects of AD pathology can vary in its different parts. The hippocampus is composed of histologically distinct regions such as the hippocampus proper (consisting of CA1, 2, 3 and 4 subfields), dentate gyrus (DG) and subiculum, and each of these different subfields contribute differently to the memory process [56]. The segmentation of hippocampal subfields in addition to the whole hippocampal volume may provide additional information about AD progress and detect MCI individuals early, and segmentation can be performed either automatically or manually. Such methods, however, could represent a disadvantage for some centers, since this type of segmentation requires high-resolution images of at least 3 Tesla (but perform better with a 7-Tesla acquisition) and the anatomical knowledge of the hippocampus to accurately delimitate the subfields. In addition, because it is hard to delineate its boundaries, the view of an expert would be necessary to accurately delimitate them.

Cognitively normal elderlies themselves present a normal atrophic process in some areas: CA1, CA3, DG [57] and subiculum [58], but the use of hippocampal subfield atrophy to detect early AD is still very controversial. In MCI subjects, for example, atrophy in CA1–2 [57,59], subiculum and CA2–3 [60], CA1, CA4/DG [56], CA1 and subiculum [61], and CA3/dentate and CA1 [62] have been observed, when compared with controls. A longitudinal study reported that excess CA1 and subicular atrophy can be observed in normal individuals predestined to decline to MCI, and when such a phase is reached there is a progressive involvement of these areas, as well as a spread to CA2–3 [63]. In AD patients, in turn, atrophy in the subiculum, CA1 and CA1–2 has been observed [57,59]. Furthermore, in a MCI-converter group, the CA1 subfield had the highest atrophy rate during a follow-up period [58].

The neurofibrillary pathology is known to start in the entorhinal cortex and then spreads to the subiculum, and CA1, 2, 3 and

4 subfields [64]. In cognitive terms, the CA1 area is involved in consolidation and late retrieval, whereas CA3 and DG in encoding and early retrieval [65]. This pattern of expansion (starting in the entorhinal cortex and then spreading to CA1) could explain the very early involvement of CA1 reported in most studies and subsequently the other subfields, as well as its effects in the cognitive domain. In addition, it could contribute to differentiating MCI subjects or cognitively at-risk normal subjects, depending on the atrophic process evolution.

Diffusion tensor imaging

Diffusion tensor imaging is a technique that detects changes in microstructural integrity of neuronal fiber tracts (i.e., the cerebral white matter) by quantitative measures. It is based on the properties that in unconstrained media (e.g., cerebrospinal fluid), diffusion is isotropic (zero), but in cerebral white matter, the direction of water diffusion occurs in favor of movement parallel to the longitudinal axis of the fibers, resulting in high-diffusion anisotropy [66]. The most reported diffusion tensor imaging-derived measures are fractional anisotropy (FA) and mean diffusivity (MD), where FA describes the direction of the fiber tracts and MD determines the overall diffusivity. The more anisotropic the media, the closer FA is to 1 [67], and it indicates a more dense packing of axonal fibers [68]. Therefore, in the neurodegeneration process, FA values tend to be lower than in normal subjects.

White matter alterations tend to occur rather globally than locally in the healthy aging [69] and, overall, the AD pathology leads to higher values of MD and lower values of FA in some specific regions (although varied). For example, changes have been observed in the limbic and commissural tracts [70], uncinate fasciculus, and tracts in the brain stem and cerebellum [71], posterior cingulum [72–74], parahippocampal area [71,74,75] and hippocampus [74,76] in MCI individuals. Either increased MD or decreased FA values have also been reported in AD patients in the parahippocampal area [71,74,77], cingulum (Figure 4) [66,71–74,77,78], corpus callosum [66,71,77,79], prefrontal gyrus, precuneus and middle occipital gyrus [66], limbic region [70], uncinate fasciculus, fornix, inferior and superior longitudinal fasciculus, and tracts in the brain stem and cerebellum [71]. For individuals at risk of AD, the studies have reported decreased white matter integrity, predominantly in tracts with direct or secondary connections to MTL structures [80–82].

Conclusion & future perspective

Neuroimaging has assumed an active role in identifying specific features of AD, despite only excluding other potential causes. In this review, we intended to describe some clinical characteristics of MCI and AD, and illustrate well-known and new MRI methods that measure regional brain atrophy. The analysis of regional brain atrophy in AD and MCI is very important for both research purposes (especially

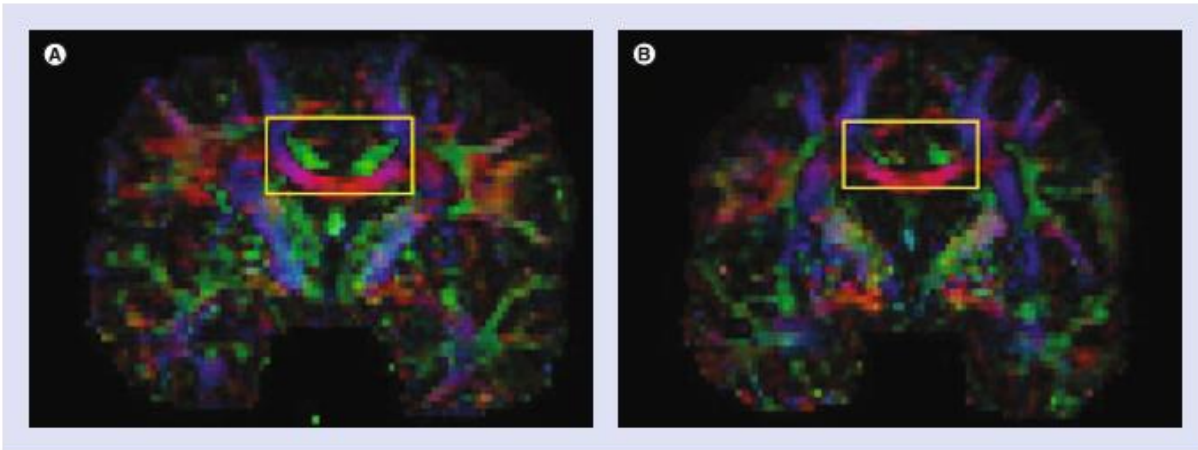

Figure 4. Color-coded orientation maps from diffusion tensor imaging measurement. (A) Cognitively normal subject and **(B)** an Alzheimer's disease patient. Colors indicate the main direction of fiber tracts in left–right (red), superior–inferior (blue) and anterior–posterior (green) directions. Yellow square: the cingulate bundle.

for the search for a neuroimaging biomarker) and also in the clinical setting, providing a more objective support for the diagnosis. With many promising disease-modifying candidate drugs under development, there is significant value in tracking changes with anatomical precision. Volumetry of hippocampus and measurement of whole-brain volume are already used as secondary end points in clinical trials, and cortical thickness and hippocampal subfields are promising candidates as well, as they can be quantitatively assessed.

Currently, neuroimaging is an excellent tool for increasing diagnostic accuracy, but it does not substitute a careful clinical and neuropsychological evaluation. However, advances in structural MRI technology may change this scenario in the future. Hippocampal volumetry is currently the best-established imaging biomarker for AD, but methods such as diffusion tensor imaging and hippocampal subfields have demonstrated promising results and deserve more attention. Some of these new MRI techniques allow detecting subtle structural changes in brain tissue that are not usually detectable by volumetric methods. Moreover, multimodal analyses combining these different measures may help improve sensitivity and specificity in the detection of structural alterations, even before dementia development.

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Default Mode, Executive Function, and Language Functional Connectivity Networks are Compromised in Mild Alzheimer´s Disease

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Abstract: Alzheimer´s disease (AD) is characterized by mental and cognitive problems, particularly with memory, language, visuospatial skills (VS), and executive functions (EF). Advances in the neuroimaging of AD have highlighted dysfunctions in functional connectivity networks (FCNs), especially in the memory related default mode network (DMN). However, little is known about the integrity and clinical significance of FNCs that process other cognitive functions than memory. We evaluated 22 patients with mild AD and 26 healthy controls through a resting state functional MRI scan. We aimed to identify different FCNs: the DMN, language, EF, and VS. Seed-based functional connectivity was calculated by placing a seed in the DMN (posterior cingulate cortex), language (Broca´s and Wernicke´s areas), EF (right and left dorsolateral prefrontal cortex), and VS networks (right and left associative visual cortex). We also performed regression analyses between individual connectivity maps for the different FCNs and the scores on cognitive tests. We found areas with significant decreases in functional connectivity in patients with mild AD in the DMN and Wernicke´s area compared with controls. Increased connectivity in patients was observed in the EF network. Regarding multiple linear regression analyses, a significant correlation was only observed between the connectivity of the DMN and episodic memory (delayed recall) scores. In conclusion, functional connectivity alterations in mild AD are not restricted to the DMN. Other FCNs related to language and EF may be altered. However, we only found significant correlations between cognition and functional connectivity in the DMN and episodic memory performance.

Keywords: Alzheimer's disease, cognition, default mode network, functional connectivity, functional networks, resting-state networks.

1. INTRODUCTION

Alzheimer's disease (AD) is a clinical syndrome characterized by multiple cognitive problems, including difficulties in memory, language, visuospatial skills (VS), and executive functions (EF) as a result of a slow and progressive degeneration of brain structures. Besides these anatomical degenerations, recent advances in the neuroimaging of dementia have highlighted concurrent dysfunctions in functional connectivity networks (FCNs), which may also be involved with the main clinical symptoms of the disease. In fact, most dementia processes are described as having specific network degeneration or deregulation [1] and it has become more clear that the clinical manifestations of AD are associated with abnormal functional integration of different brain regions by disconnection and not only gray matter atrophy [2].

FCNs can be described as groups of cortical and subcortical regions that are spatially distinct but functionally related. These FCNs can be obtained by the analysis of spontaneous modulation of the BOLD ("blood oxygenation level dependent") signal, in the absence of an experimental paradigm or any other explicit stimulus. This analysis is known as intrinsic functional connectivity or "resting-state" fMRI and this method can detect interregional fluctuations of the spontaneous BOLD signal. Some studies suggest that AD is in part caused by a disconnection within cognitive networks and a failure to integrate the functionality of some regions into an efficient network [3]. Such disconnectivity, in fact, has been demonstrated using either structural or functional MRI [4-7].

The default mode network (DMN) is the most studied FCN in AD and there are several studies showing a breakdown in the DMN connectivity, even during mild stages of the disease [8, 9]. This is possibly due to an overlap occurring between its areas and amyloid plaques deposition, an early pathologic feature of the disease [7]. The DMN's function is not completely understood, but it is generally considered that this network increases its activity when a person is not focused on activities directed at the external environment. Also, its key anatomical regions, which include the precuneus, posterior cingulate cortex (PCC), inferior parietal, hippocampus and medial prefrontal cortex (MPFC), may play an important role in episodic memory functions [10].

Little is known, however, about the integrity and the clinical significance of other FCNs that also process cogni-

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tive information, which are invariably affected in AD. Therefore, in this study, we aimed to map different FCNs – the DMN, language, EF, and VS – in mild AD patients, by analyzing low-frequency fluctuations in resting-state functional connectivity MRI. In addition, we also verified if there were any significant correlations between these FCNs and cognitive scores. We hypothesized that mild AD patients would already present connectivity alterations in the FCNs described above, when compared with the healthy elderly, and also that FCNs would be related to changes in cognition.

2. MATERIALS AND METHODS

2.1. Subjects

Participants were recruited in the Neuropsychology and Dementia outpatient clinic (UNICAMP University Hospital). Twenty-six healthy elderly subjects (20 women) and 22 mild AD patients (16 women) were evaluated in this study. Experienced attending doctors and neuropsychologists diagnosed the patients who met the National Institute of Neurological and Communicative Disease and Stroke and Alzheimer's disease (NINCDS-ADRDA) criteria for a diagnosis of probable AD [11]. Examination of each subject included medical history, neurological examination, neuropsychological assessment, Clinical Dementia Rating (CDR) [12], Hachinski ischemic score [13] (≤ 4), and standard laboratory tests, including B12, folate and thyroid hormone levels, and syphilis serology. We only included patients who were classified as CDR 1. The exclusion criteria included a history of other neurological or psychiatric diseases, previous head injury with loss of consciousness, drug or alcohol addiction, prior chronic exposure to neurotoxic substances, and a Hachinski ischemic score > 4. All patients were using anticholinesterasics: donepezil (11 patients), rivastigmine (7 patients), and galantamine (4 patients). Eight patients were using low doses of selective serotonin reuptake inhibitors (fluoxetine, sertraline, or citalopram).

Controls were identified as cognitively normal individuals who did not have any neurological or psychiatric disorders. These individuals did not take psychoactive medication and they had normal Mini Mental Status Examination (MMSE) scores and structural images without any abnormalities, considering age and education. Memory complaints or neurological deficits were not observed in the healthy elderly.

2.2. Neuropsychological Evaluation

An experienced neuropsychologist, blinded to the MRI data, performed the neuropsychological evaluations. Global cognitive status was measured using the MMSE [14] and episodic memory was evaluated by the Rey Auditory Verbal Learning Test (RAVLT) (subitems encoding, delayed recall, and recognition) [15]. Visual perception was assessed with the following tests: subtests of Luria's Neuropsychological Investigation (LNI) [16], using items G12, G13, G14 (the patient is asked to examine and name pictures of objects that are scribbled over or superimposed on another picture), and G17 (item from Raven's test), one item for mental rotation of figures [17], and a copy of the Rey-Osterrieth Complex Figure Test [18]. EF was assessed by Trail Making Test B [19] and the Stroop test [20]. Language tests included the Boston Naming Test (BNT) [21], semantic verbal fluency (SVF) for category (animals), and phonological fluency for letters (FAS) [22].

Statistical data analysis was performed using the SPSS software (version 20). We performed independent sample t-tests for inter-group comparisons of the demographic and cognitive scores. On occasions where data violated the assumptions of parametric tests, we performed the Mann-Whitney test. Results were considered to be statistically significant when $p < 0.05$.

2.3. Data Acquisition

All imaging was performed on a 3.0 T MRI Philips Achieva scanner. Functional MRI were acquired while at rest, where subjects were instructed to keep their eyes closed and to avoid initiating attention-demanding activity. The following protocol was applied to each subject: a) structural: sagittal high-resolution, T1-weighted, gradient-echo images were acquired with TR/TE=7/3.2 ms, a field of view (FOV) of 240×240, and isotropic voxels of 1 mm^3 and b) functional images were acquired during rest: axial T2*-weighted images had TR/TE=2000/30 ms, a FOV of 240×240, and isotropic voxels set to 3×3×3 mm^3. For each participant, we acquired 10 minutes of Echo Planar Image data. The total of all the remaining images was equal to 300 volumes with 40 axial slices each.

All subjects underwent MRI scanning in the same week that the neuropsychological assessment was performed.

2.4. Data Processing

Functional images were pre-processed by applying slice-time and motion-correcting algorithms and removing linear trends. Data pre-processing also included smoothing with a 6 mm FWHM Gaussian kernel, bandpass filtering (0.008-0.1 Hz) and spatially normalized to standard space (MNI152). Six parameters of head motion, three related to head translation, and three associated to the head rotation (yaw, pitch, and roll), as well as cerebrospinal fluid and white matter time series, were regressed with the functional data to remove variance associated with these variables. All of these steps were performed with AFNI (http://afni.nimh.nih.gov/afni) and FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) software.

In this study, we performed a seed-based analysis, placing a seed (radius = 3mm) in: a) the PCC to identify the DMN (MNI coordinates: 0,-51,15), b) the left hemisphere: Broca´s (-48,15,0) and Wernicke´s (-48,-36,12) areas, to identify language networks, c) right (45,18,48) and left (-45,18,48) dorsolateral prefrontal cortices (DLPFC) to identify EF networks, and d) right (36,-84,-3) and left (-36,-84,-3) secondary associative visual cortices, to identify VS. Specifically, for each subject, the average time-course of voxels within each seed was extracted by generating a reference time series. Each time series was then correlated with all the voxels for each subject. Subsequently, r-scores of each voxel where then transformed using Fisher's r-to-z method, so that these data could be used in parametric statistical analyses.

2.5. Statistical Analysis

Differences in connectivity between controls and patients regarding the different FCNs were estimated by using FSL's Randomize software [23]. For all analyses, the null distributions of the non-parametric t-values were estimated using a permutation-based method (1,000 permutations) [23]. We used randomize's Threshold-Free Cluster Enhancement method and p values were corrected for multiple comparisons by using family-wise error rates.

We also used FSL's Randomise to verify if there were positive or negative correlations between FCN maps and cognitive performance: DMN x episodic memory tests (RAVLT's encoding, delayed recall, and recognition), language network x language tests (BNT, FAS, and SVF), EF networks x EF tests (TMT B and the Stroop test), and VS network x visuospatial tests (LNI visuospatial subitems and the Rey-Osterrieth Complex Figure Test). Since the groups were not matched for education, we included the number of years of formal study as a nuisance variable. For these multiple regressions, we considered the two groups together (normal aging and mild AD) to increase our data variance and enhance the correlation between functional connectivity and cognitive function; we assumed that the more connected the network was, the better the cognitive performance and vice versa. Statistical significance was set at a threshold of $p < 0.05$ and the false discovery rate was corrected for multiple comparisons.

All subjects who participated in this study provided written informed consent. This study was approved by the Medical Research Ethics Committee of UNICAMP University Hospital. Local ethical committee approval and written informed consent (either from the subjects or from their responsible guardians, if incapable) were obtained before study initiation, according to the Declaration of Helsinki.

3. RESULTS

There were no significant differences in gender ($p = 0.83$) and age ($p=0.19$) between mild AD and control subjects, but AD patients were less educated than controls ($p < 0.05$) (Table **1**). AD patients performed worse than the healthy elderly in all cognitive tests.

Regarding differences in connectivity between the groups, we found that mild AD patients showed decreased connectivity in DMN and Language Networks (Wernicke´s area) (Fig. **1**, Tables **2** and **3**). The opposite pattern was found in the EF network (right DLPFC), which showed increased connectivity in the mild AD group in relation to controls (Fig. **2**, Table **4**). We did not find differences in connectivity between the groups regarding VS network (right or left), Broca's area and left DLPFC.

Concerning the correlation between cognition and FCNs, statistically significant results were only observed between DMN connectivity and episodic memory (delayed recall) scores (Fig. **3** and Table **5**). Other RAVLT's episodic memory subitems such as encoding and recognition were not related to DMN connectivity. All the other analyses showed no significant correlations (language networks x BNT, FAS and SVF; right and left DLPFC x TMT B and the Stroop test; right and left VS network x LNI visuospatial subitems and the Rey-Osterrieth Complex Figure Test).

DISCUSSION

In this study, we sought to investigate the possible alterations in different brain FCNs in mild AD patients and to verify if these alterations were correlated with cognitive performance. We hypothesized that some FCNs would already be altered in AD, even at a mild phase. Indeed, our findings showed that: (1) mild AD patients demonstrate alterations in some FCNs: increased connectivity in the EF network and decreased connectivity in the DMN and language networks, (2) the connectivity of the VS network, however, did not differ between mild AD patients and the control patients, and (3) DMN connectivity was correlated with episodic memory performance (delayed recall).

These results show that connectivity problems are one of the most prominent characteristics of mild AD and that these connectivity disruptions are not only restricted to the DMN. However, we did not find significant correlations between other FCNs and the previously hypothesized cognitive function tests such as naming, semantic and phonemic fluency, VS, and EF. In the light of these results, we discuss the relevance of connectivity breakdown in AD, especially in the DMN, and the consequent disruption of the complex interactions between these different networks, which could be partly responsible for these clinical symptoms.

Among all FCNs investigated in the AD field, the DMN is undoubtedly the most studied. One reason is because this network includes cerebral regions affected early in the course of the disease like the PCC, the medial temporal lobes, and MPFC. Atrophy, amyloid deposition [24], and disruption of metabolism [25] are problems that have been widely described to occur in parietotemporal areas, components of the DMN. In our study, mild AD patients had decreased connectivity in the DMN and this finding is in agreement with other studies stating diminished connectivity in this network [26], suggesting that loss of functional connectivity within the DMN is one of the earliest findings in AD. In fact, individuals who are mildly cognitively impaired already present reduced network-related activity (while the respective volumes remained unaffected), suggesting effects of early degeneration [8]. Consequently, DMN functional connectivity alteration has been proposed as a biomarker for mild AD, adding to findings of gray-matter atrophy.

Regarding cognitive issues, it is known that the DMN mediates cognitive functions. However, despite the amount of knowledge about the DMN's physiology and anatomy specifically, the cognitive functions of this network are still poorly understood. It has been said that it is not only the DMN that contributes to cognitive performance, but also the interaction among different networks (i.e., internetwork connectivity) [27]. In our study, we found that DMN connectivity was related to a delayed recall subitem of RAVLT, suggesting further evidence of the DMN's role in episodic memory performance. In other words, mild AD patients' difficulties in memory processing could be explained by DMN connectivity loss, in addition to medial temporal lobe atrophy commonly found.

Table 1. Demographic, functional and neuropsychological data. Data presented as mean±SD. MMSE: mini-mental status examination; RAVLT-COD: encoding of Rey auditory verbal learning test (sum of A1+A2+...+A5); RAVLT -A7: delayed recall of Rey auditory verbal learning test; RAVLT-REC: Rey auditory verbal learning test true recognition (i.e., recognition minus false positives); BNT: Boston naming test; SVF: semantic verbal fluency; FAS: phonological fluency for letters; LNI: visuospatial perception item of Luria's neuropsychological investigation; TMT-B: Trail Making Test B. *Pearson Chi-square; **Mann Whitney test was applied due to lack of normality.

	AD	Controls	p
Total (female)	22 (16)	26 (20)	0,83*
Age (years)	73.40 ± 5.67	70.04±6.61	0.19**
Education (years)	5.95 ± 5.17	10.22±5.55	<0.05
MMSE	18.86 ± 4.68	28.59±1.86	<0.0001
EPISODIC MEMORY TESTS			
RAVLT-COD	18.13±6.75	47.54±8.06	<0.0001
RAVLT-A7	0.50 ± 0.67	9.04±2.41	<0.0001
RAVLT-REC	-3.85 ± 4.89	11.95 ± 2.53	<0.0001
LANGUAGE TESTS			
BNT	29.53±13.80	52.04 ± 4.93	<0.0001
SVF	9.31±4.78	17.63±4.99	<0.0001**
FAS	18.42±12.51	33.27±11.72	<0.0001
VISUOSPATIAL SKILL TESTS			
Rey complex figure (copy)	13.24.04±14.57	34.88±3.81	<0.0001
LNI	14.72±2.93	18.09±1.19	<0.0001
EXECUTIVE FUNCTION TESTS			
TMT-B	278.73±56.60	138.04 ± 95.49	<0.0001
Stroop Test- Congruent (seconds)	79.42 ± 43.75	56.04±17.60	0.001
Stroop Test- Congruent (errors)	0.47 ± 0.92	0.04±0.21	0.62
Stroop Test- Incongruent (seconds)	179.95 ± 78.59	102.09±27.35	<0.0001**
Stroop Test-Incongruent (errors)	33.52±21.98	2.86±3.73	<0.0001

Distinct areas of the brain compose the DMN, including the PCC, MPFC, the medial temporal lobe, and the inferior parietal cortex. The activity of the entire network activity is associated with the absence of spontaneous speech and a propensity for mind wandering [28], although each of the component areas has a different cognitive function. For example, PCC activity is associated with self-referential process and autobiographical memory, the MPFC is associated with social functions, the medial temporal lobe relates to episodic memory, and the inferior parietal cortex is associated with semantic processing and attention [29]. The fact that this network is deactivated by memory tasks and some of its components are targeted by amyloid deposition in AD highlights the role of the DMN in episodic memory. In fact, AD patients show problems deactivating this network properly [30] and consequently engaging in cognitive-demanding tasks such as encoding and recalling novel information. Another interpretation that has been proposed to explain the association between memory and the DMN is that the activation of the medial temporal lobe could suppress activity within this network [4]. Either way, the relationship of the DMN and memory processing is once more supported by our results, where hypoconnectivity was correlated with lower delayed recall scores in mild AD patients.

The DMN is one of the first FCNs affected by the disease, as stated above, which causes cognitive damage and possibly behavioral alterations. However, the DMN is not the only network affected and the mechanism in which the pathology advances to other FCNs is not known. In this study, the DMN showed alterations in connectivity, but the language and EF networks did as well. Although we did not find significant correlations with the respective cognitive tests, such alterations could be clinically observed in neuropsychological assessments, where patients performed worse in both language and EF tests. Two different hypotheses have been proposed to explain the spread of the pathology

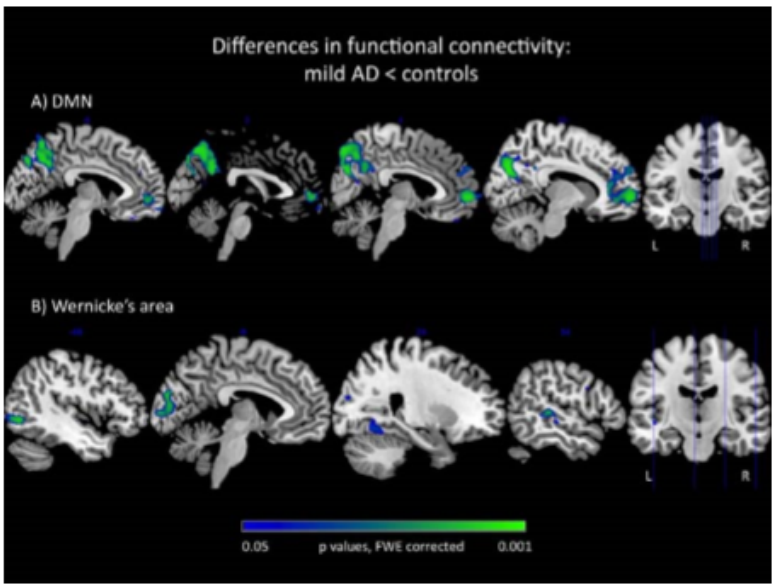

Fig. (1). Areas of significant decrease in functional connectivity in mild AD patients in comparison to normal aging: A) DMN; B) Wernicke's area. $p < 0.05$, family-wise error corrected.

Table 2. Regions of most significant differences in DMN functional connectivity between mild AD patients and controls (AD < Controls). We included just clusters with more than 50 voxels.

Area	Number of voxels	MNI coordinates (Peak of difference)			p
		X	Y	Z	
Right medial frontal gyrus	863	9	57	-3	0.006
Right cuneus	776	12	-72	24	0.005
Left superior frontal gyrus	232	-30	24	48	0.017
Right superior frontal gyrus	146	18	27	48	0.011

Table 3. Regions of most significant differences in Wernicke's area functional connectivity between mild AD patients and controls (AD < Controls). We included just clusters with more than 50 voxels.

Area	Number of voxels	MNI coordinates (peak of difference)			p
		X	Y	Z	
Left calcarine gyrus	360	-6	-96	3	0.012
Left middle occipital gyrus	84	-45	-69	-3	0.007
Right middle temporal gyrus	58	63	-36	3	0.011

throughout other FCNs: one hypothesis states that a sequence of progressive misfolding tau proteins spreads to the postsynaptic neurons, degenerating different networks [31], whereas the other hypothesis purports that the DMN starts a disordered neural communication that spreads to other FCNs [32].

In the healthy aging population, connectivity in the DMN and dorsal attention system are much more affected than the visual system [33]. According to our results, this fact is also observed in AD patients. Whereas the DMN, language, and EF networks all showed alterations in connectivity – either hypo or hyperconnection – the VS network did not differ

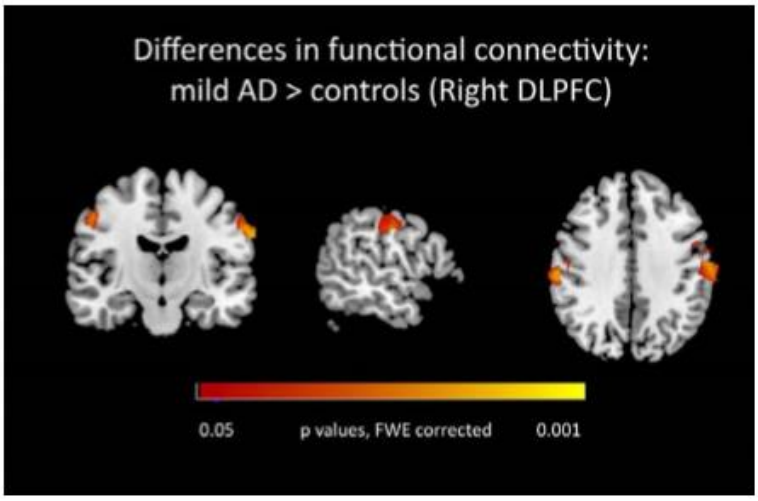

Fig. (2). Areas of increased functional connectivity in mild AD patients in relation to controls in EF network (seed placed at Right DLPFC). $p < 0.05$, family-wise error corrected.

Table 4. Regions of most significant differences in EF network functional connectivity between mild ad patients and controls (AD > Controls). We included just clusters with more than 50 voxels.

Area	Number of voxels	MNI coordinates (Peak of difference)			p
		X	Y	Z	
Left inferior frontal gyrus	196	-56	17	27	0.01
Right Postcentral gyrus	117	60	-21	36	0.017

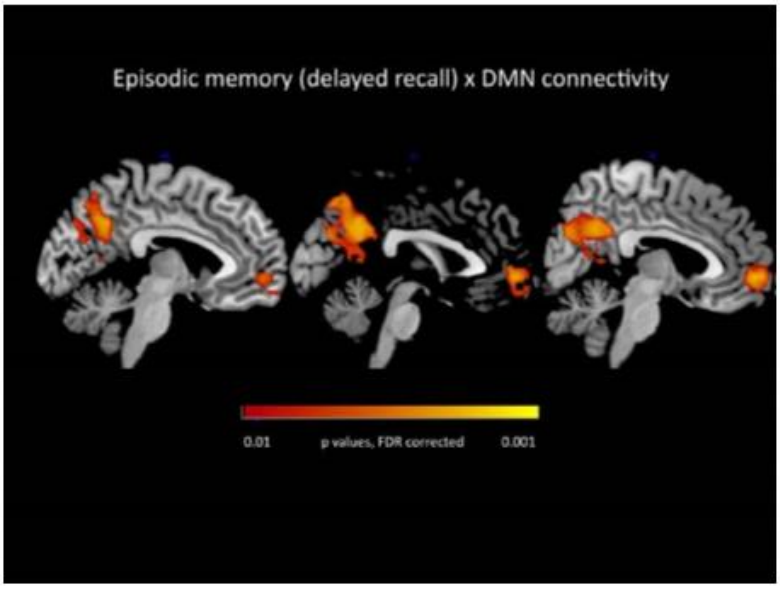

Fig. (3). Areas of significant correlation between DMN functional connectivity and RAVLT's delayed recall subitem. $p < 0.01$, false discovery rate corrected.

Table 5. Regions of most significant correlations between DMN connectivity and episodic memory scores (RAVLT's Delayed Recall), considering the two groups together. We included just clusters with more than 50 voxels.

Area	Number of voxels	MNI coordinates (Peak of correlation)			p
		X	Y	Z	
Right middle orbital gyrus	3300	30	66	-12	0.001
Left anterior cingulate cortex	2458	-15	-48	0	0.001
Left fusiform gyrus	896	-33	-39	-21	0.001
Right inferior temporal gyrus	853	48	-12	-42	0.001
Right parahippocampal gyrus	308	30	-33	-18	0.002
Left middle cingulate cortex	150	0	-30	33	0.001

between controls and patients. The progression of symptoms in AD has been related to the topographic progression of neurofibrillary tangles composed of hyperphosphorylated tau, which first affects the medial temporal lobe and PCC and lastly affects the sensorimotor and occipital cortex [34]. Such progression has been supported by the theory of retrogenesis, which says that early myelinating regions are less susceptible to damage, due to the presence of larger diameter axons and a higher oligodendrocyte-to-axon ratio [35]. The visual cortex in the occipital lobe is an early myelinating area and therefore is not affected until the late stages of the disease, whereas the medial temporal lobe is affected in the earlier stages of the disease. In our study, the VS network was the only network that was not affected in mild AD patients, further supporting the idea that the associative visual areas are affected late by the physiopathology, as opposed to the other networks that are affected at an earlier stage of the disease. It is important to state that in this study we did not include the visual variant of AD, or posterior cortical atrophy patients. If we had, we would probably have found alterations in VS network connectivity. Despite this fact, our AD patients performed worse than the controls in visual perception and constructive praxis, which could mean they may have problems in other aspects of VS, possibly mediated by parietal structures such as angular or supra marginal gyri. Possibly, if we had put the seed in these regions, we might have found different results.

The failure of the DMN regions to interact at a high level of coordination and efficiency could establish the cognitive deficits presented in our AD patients. In other words, the whole network activity and integration plays a fundamental role in cognitive functioning, not only within specific network areas. Negative connectivity between the DMN and EF networks (which showed hyperconnection) is an interesting phenomenon and has already been observed in other studies (see, for example, [36]). These FCNs interact in a complex way that is not yet fully understood. Disruptions of these networks and the complex relationships among them contribute to specific patterns of cognitive and behavioral impairments clearly observed in AD. For example, some authors suggest a close interaction between the DMN, the Salience network, and the EF network. The Salience network, composed of structures such as the fronto-insular cortex and the anterior cingulated cortex, among others, can detect emotionally relevant stimuli, which can originate either internally or externally from the environment [37]. This network is functionally related to the DMN and some authors have proposed that the salience network plays a role in switching brain states from the DMN to the external task-related activity mode [38]. In contrast, the EF network is equipped to operate on identified salience. Such operations require directing attention to pertinent stimuli as behavioral choices are weighed against shifting conditions, background homeostatic demands, and context [37].

Some authors have showed an anticorrelation in connectivity between the DMN and the Salience network in AD [26, 39]. Although we did not evaluate the salience network in the present study, a previous paper of our group including mild to moderate AD patients also found similar results. In addition, that study also showed that these patients have an enhanced detection of environmental stimuli [40]. In the present study, we found an increased connectivity in the EF network that may represent a way to deal with the dysfunctional salient stimuli detected by a hyperconnected salience network. Although we did not find significant correlations between executive tests and EF network as other authors did [36, 37], our AD patients clearly demonstrated executive dysfunctions, which could mean that enhanced connectivity in the EF network is not enough to improve cognitive performance.

Finally, the limitations of our work have to be acknowledged. We did not adjust our data to gray-matter measures. The question whether cortical atrophy could be responsible for alterations in connectivity is not completely known. Another issue without conclusive outcomes is whether connectivity problems precede or are a consequence of atrophy. Some authors demonstrated that disconnection precedes atrophy in the PCC [41] and that asymptomatic carriers of APOE 4 have a reduction in PCC connectivity without atrophy [42]. In addition, other studies showed that functional connectivity alterations could not be explained by atrophy alone, suggesting that both mechanisms either occur independently [26, 36] or that the FCN regions do not overlap the areas of atrophy [8]. Moreover, our data were corrected for multiple comparisons in a conservative way, a method that reinforces our results.

In conclusion, alterations in FCNs in AD, even in the mild stages, are not restricted to the DMN. Other FCNs related to language and EF are hypoconnected or hyperconnected (an alteration that possibly reflects a compensatory mechanism). However, we found in this group of subjects that DMN connectivity and episodic memory performance are significantly related.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Structural connectivity of the default mode network and cognition in Alzheimer's disease

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ABSTRACT

Disconnectivity between the Default Mode Network (DMN) nodes can cause clinical symptoms and cognitive deficits in Alzheimer's disease (AD). We aimed to examine the structural connectivity between DMN nodes, to verify the extent in which white matter disconnection affects cognitive performance. MRI data of 76 subjects (25 mild AD, 21 amnestic Mild Cognitive Impairment subjects and 30 controls) were acquired on a 3.0 T scanner. ExploreDTI software (fractional Anisotropy threshold = 0.25 and the angular threshold = 60°) calculated axial, radial, and mean diffusivities, fractional anisotropy and streamline count. AD patients showed lower fractional anisotropy ($P = 0.01$) and streamline count ($P = 0.029$), and higher radial diffusivity ($P = 0.014$) than controls in the cingulum. After correction for white matter atrophy, only fractional anisotropy and radial diffusivity remained significantly lower in AD compared to controls ($P = 0.003$ and $P = 0.05$). In the parahippocampal bundle, AD patients had lower mean and radial diffusivities ($P = 0.048$ and $P = 0.013$) compared to controls, from which only radial diffusivity survived for white matter adjustment ($P = 0.05$). Regression models revealed that cognitive performance is also accounted for by white matter microstructural values. Structural connectivity within the DMN is important to the execution of high-complexity tasks, probably due to its relevant role in the integration of the network.

1. Introduction

Recent advances in dementia research have pointed to the hypothesis that neurodegenerative diseases might be considered brain network diseases. Under such a model, pathology (most often misfolded proteins) originates in a specific region and spreads, transported along axons, preferentially to interconnected regions (Reid and Evans, 2013). It has been suggested that dementia due to Alzheimer's disease (AD) is caused partly by the disconnection within neural networks and a failure to integrate the functionality of some regions into an efficient network (Bokde et al., 2009).

Amongst the altered networks in AD, the most reported is the Default Mode (DMN), a network that overlaps with amyloid plaque deposition, an early pathologic feature of the disease (Matthews et al., 2012). This network includes the posterior cingulate cortex (PCC), the adjacent precuneus (pCu) and retrosplenial cortex (RSC), the medial temporal lobes (MTL), medial prefrontal areas (MPFC), and inferior parietal cortex, and it deactivates during most externally oriented tasks early in the course of the disease. Disruption of metabolism and atrophy are also widely described to occur in these areas (Villain et al., 2008).

Disconnectivity within a network can be the substrate for different cognitive disorders, because cognitive functions rely on the integrity of dynamic communication between interconnected brain regions and circuits such as the DMN. In fact, it has become clearer that the clinical manifestations of AD are associated with the progressive dysfunction of the DMN (Bozzali et al., 2011). In other words, activity and integration in the whole network, and not only in their isolated areas, play a fundamental role in cognitive functioning.

Although impairment of DMN functional connectivity in AD is well established, much less is known regarding white matter (WM) structural connectivity between the main DMN nodes. Damage in the pathways that link these nodes could represent and/or lead to a failure in the communication between them and, consequently, cause cognitive deficits. In neuroimaging, changes in WM pathways

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can be microstructuraly measured by Diffusion tensor imaging (DTI), a highly sensitive technique which detects alterations that would probably go unnoticed with other structural methods. Although many studies have already used this technique in AD (Lo et al., 2010; Teipel et al., 2010), little is known regarding WM contribution to DMN structural damage and its cognitive outcomes.

Therefore, we sought to investigate the integrity of the main DMN's WM tracts and the cognitive consequences of this hypothesized disconnection in the earliest clinical phase of the AD spectrum: mild AD and amnestic mild cognitive impairment (aMCI), a possible prodromal AD. With these goals, we compared WM anisotropic properties of diffusion, non-fractional measures of diffusivity and streamline count in the cingulum and parahippocampal bundles in patients with mild AD, aMCI and healthy controls, and verified if these measures were associated with their cognitive performance on episodic memory, working memory, executive functions, visuospatial skills and language. As far as we are concerned, this is the first report that analyzed a full range of DTI parameters in the DMN tracts, and investigated the relationship of such parameters with cognition. We hypothesized that both aMCI and AD patients would present microstructural alterations in the DMN tracts compared to controls, and such alterations would affect the worse cognitive performance of the patients.

2. Methods

2.1. Subjects

This study included 76 subjects: 25 mild AD patients (18 women, 71.86 ± 5.6 years old), 21 aMCI patients (six women, 69.54 ± 8.5 years old), and 30 controls (20 women, 70.3 ± 6.61 years old). The study was approved by the Medical Research Ethics Committee of University of Campinas, and written informed consent was obtained before study initiation, according to the Declaration of Helsinki. Pre-diagnostic procedures comprised laboratory tests including Vitamin B12, folate and thyroid hormones.

The diagnosis of AD was based on the criteria of the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) (McKhann et al., 1984), and all patients also fulfilled the National Institute of Aging/Alzheimer's Association core criteria for dementia due to AD (McKhann, 2011). Mild AD patients had a CDR (Morris, 1993) score of 1.

Mild cognitive impairment (MCI) refers to a transitional stage between normal aging and AD, including amnestic MCI-single domain (aMCI), where the impairment involves only memory domain. Patients were diagnosed using criteria for amnestic MCI (Winblad et al., 2004). All patients also fulfilled the following core criteria of the National Institute of Aging/Alzheimer's Association for MCI (Albert et al., 2011): (a) symptoms of memory loss, (b) normal activities of daily living, (c) normal general cognitive function, (d) absence of dementia. All aMCI participants had a CDR score of 0.5, with an obligatory and exclusive memory score of 0.5. This classification was performed using a semi-structured interview.

The criteria for healthy elderly were as follows: (a) no neurological and psychiatric disorders, (b) no abnormal finding in conventional brain MR imaging, (c) no cognitive complaints. All cognitively healthy subjects underwent a cognitive examination; none reported subjective symptoms of cognitive impairment. Also, they were required to not be taking neuropsychiatric medications, to not have any major heart disease, diabetes, cardiovascular disease, epilepsy, nor head trauma. All normal subjects had a CDR score of 0.

Exclusion criteria for all subjects were history of other neurological or psychiatric diseases, head injury with loss of consciousness, use of sedative drugs in the last 24 h before the neuropsychological assessment, drug or alcohol addiction, prior chronic exposure to neurotoxic substances and Hachinski ischemic score > 4.

2.2. Neuropsychological evaluation

Global cognitive statuses were measured using the Mini Mental Status Examination (MMSE) (Folstein et al., 1975) and episodic memory was evaluated by the Rey auditory verbal learning test (RAVLT) (subitems encoding, delayed recall and recognition) (Malloy-Diniz et al., 2007). Visuospatial perception was assessed with the following tests: subtests of Luria's Neuropsychological Investigation (LNI) (Christensen, 1975), using items G12, G13, G14 (the patient is asked to examine and name pictures of objects that are scribbled over or superimposed on another picture), and G17 (item from Raven's test), mental rotation of figures (Ratcliff, 1979) and clock drawing (Sunderland et al., 1989). Constructive praxis was evaluated by the copy of The Rey–Osterrieth Complex Figure Test (Osterrieth, 1944). Executive function was assessed by Trail Making Test A and B (Reitan, 1958) and Stroop test, time and number of errors in congruent and incongruent conditions (Stroop, 1935). Language tests included the Boston Naming Test (BNT) (Kaplan et al., 1983) and verbal fluency (VF) for category (animals), and phonological fluency for letters (F.A.S.) (Christensen and Guilford, 1959). Working memory and attention were assessed by the forward (FDS) and backward digit span (BDS) subtest of the WAIS-R (Wechsler, 1987).

2.3. Data acquisition

All MR images were acquired on a 3.0 T MRI Philips Achieva scanner. Foam padding was provided for comfort and to minimize head motion. The following protocol was applied to each subject: a) sagittal high-resolution T1-weighted images (isotropic voxels of 1 mm^3, no gap, TR/TE = 7/3.2 ms, FOV = 240 × 240 mm^2) b) coronal and axial FLAIR (fluid attenuated inversion recovery) T2-weighted images, anatomically aligned to the hippocampus (reconstructed voxel size = 0.45 × 0.45 × 4.00 mm^3, TR/TE/TI = 12,000/140/2850 ms and FOV = 220 × 206 mm, gap = 1 mm); c) coronal IR (inversion recovery) T1-weighted images (reconstructed voxel size = 0.42 × 0.42 × 3.00 mm^3, TR/TE/TI = 3550/15/400 ms and FOV = 180 × 180 mm^2); d) coronal multi-echo (five echos) T2-weighted images (reconstructed voxel size = 0.42 × 0.42 × 3.00 mm^3, TR/TE 3300/30 ms and FOV = 180 × 180 mm^2). For the WM microstructural analysis, a standard DTI protocol was performed (acquired voxel size = 2 × 2 × 2 mm^3 reconstructed with 1 × 1 × 2 mm^3, no gap, TR = 8.5 s, TE = 61 ms, 32 diffusion directions with $b = 1000$ s/mm^2, acquisition matrix = 128 × 128 reconstructed to 256 × 256). All subjects underwent MRI scanning in the same week that neuropsychological assessment was performed. Image processing and neuropsychological examinations were performed by professionals blinded to the clinical data.

2.4. DTI processing and tractography

Tensor calculation and tractography were performed with ExploreDTI software (Leemans and Jones, 2009), University Medical Center, Utrecht, The Netherlands, that uses a deterministic streamline method to obtain fibers, by setting the Fractional Anisotropy (FA) threshold to 0.25 and the angular threshold to 60°. An FA threshold of 0.25 was chosen to avoid voxels that are not part of the WM tract

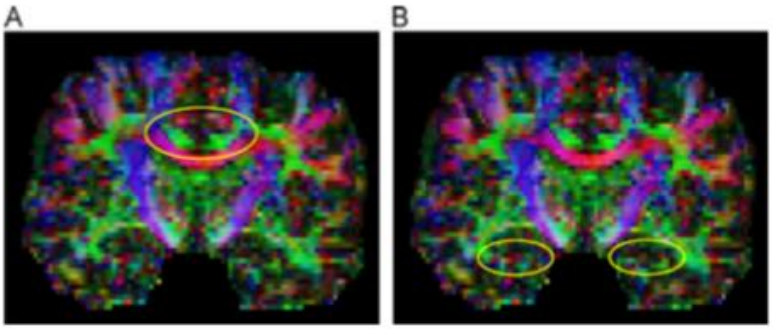

Fig. 1. The color-coded directional maps in coronal view of (A) cingulum bundle and (B) parahippocampal bundle, delimitated by the yellow circles. Colors indicate the main direction of fiber tracts in left–right (red), superior–inferior (blue) and anterior–posterior (green) directions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

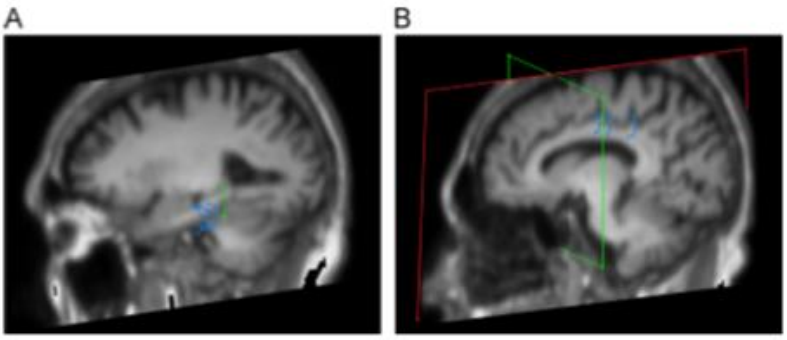

Fig. 2. Illustration of region of interest (ROI) placements in the color-coded map for isolation of (A) the parahippocampal bundle and (B) the cingulum bundle. The blue circles represent the 'SEED's; the green squares represent a 'AND'; the red square represents a 'NOT'. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

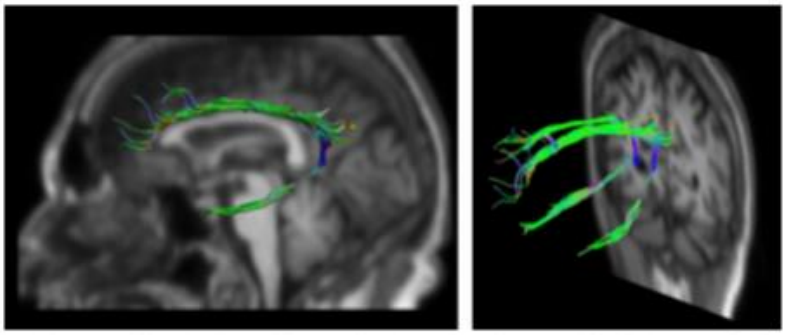

Fig. 3. Illustration of the isolated tracts of the DMN (cingulum and parahippocampal bundles) in a control subject.

(cortex has FA~0.2), minimize the inclusion of voxels with a higher degree of partial volume contamination, and limit the presence of spurious tracts. The color-coded directional maps provided an easy visualization of the WM fiber tracts (Fig. 1), where elliptical regions of interest (ROI) were manually drawn, and tracts were isolated. A 'pilot drawing' was performed for 5 random control subjects, to make sure the strategy was able to isolate the tracts in a satisfactory manner. The strategy was selected according to the following selection criteria: it should have the minimal number of 'SEEDs','AND' and 'NOT' commands (in order to make drawing easier and less susceptible to errors) but still able to extract the fasciculi according to WM atlas (Wakana et al., 2004) and to previous study that isolated DMN fibers in healthy individuals (Greicius et al., 2009). Fig. 2 shows the best strategies that fulfilled those criteria, and which were used for obtaining both tracts. The resultant tract was obtained and there was no longer manual intervention afterwards.

For the DMN tracts, the following WM tracts were selected: (a) bilateral cingulum bundle (tracts connecting PCC/RSC with mPFC); and (b) bilateral parahippocampal portion of the cingulum bundle (PCC/RSC-temporal lobe connection) (Fig. 3). We did not evaluate WM tracts from PCC to the biparietal cortex (another DMN subcomponent) because there are no direct connections between these areas. The strategies (ROIs slices, positions and shapes) were equally applied for all 76 individuals, and visual assessment was performed by an experienced medical physicist.

After the preprocessing and the fiber tracking, a quantitative analysis was performed. The diffusion coefficient along the direction of maximal apparent diffusion (λ1 or axial diffusivity) and the diffusion coefficients along two orthogonal (smaller) directions embedded in the plane perpendicular to the main diffusion direction (λ2 and λ3) were calculated. λ2 and λ3 can be averaged and this value is known as radial diffusivity. Eigenvalues can be also used to estimate the fractional anisotropy (FA) that reflects tract coherence, myelinization and density, through a value between 0 and 1 (where 0 means complete isotropy, without preferential direction of water diffusion; 1 means complete anisotropy, strong preferred direction of water diffusion). Furthermore, the average diffusivity, known as mean diffusivity (MD) can be inferred from the overall dimensions of the diffusion ellipsoid (Acosta-Cabronero et al., 2010). Streamline count, in turn, results in a total number of fibers connecting two regions. To sum up, we evaluated 5 DTI measures: FA, mean, radial and axial diffusivities and streamline count.

2.5. White matter volume

To evaluate if macrostructural WM damage could influence DTI measures in our subjects, we also measured total WM volume and included its values as potential confounders. FreeSurfer software (http://surfer.nmr.mgh.harvard.edu) was used for extraction of subcortical segmentation (Fischl et al., 2002, 2004), and WM volumes were obtained for each participant. The standard data processing stream in FreeSurfer involves intensity normalization for removal of the B1 bias field, skull stripping, and Markov random field modeling for segmentation and gray matter volume labeling, incorporating atlas-based anatomical class priors. Individual structure volumes are in turn computed from labeled voxels and normalized to total intracranial volume.

2.6. Statistical analysis

Statistical data analysis was performed using the SPSS (version 20; SPSS Inc., Chicago, IL, USA). We first performed the Kolmogorov–Smirnov to test for normality, and as all data followed a normal distribution, we performed parametric tests. Once this was completed, two types of analyses were conducted, as explained below. Results were considered to be statistically significant when $P < 0.05$.

- Initially, a WM volume comparison between the groups was performed, in order to verify if there was any important atrophy in either aMCI or mild AD patients compared to controls. For that, we performed a General Linear Model analysis, adjusting for sex and age, Bonferroni correction for multiple comparisons.
- Then, to verify whether there were any differences both in structural connectivity and neuropsychological performance amongst the three groups, we performed one way ANOVA, Bonferroni adjustment for multiple comparisons. The same analysis was then rerun, but controlling for WM atrophy (added as a covariate in a General Linear Model analysis), Bonferroni adjustment for multiple comparisons.
- To examine associations of WM connectivity with neuropsychological outcomes, a series of multiple linear regression analyses were performed, with and without adding the DTI parameters. At this stage, all individuals were combined into one unique group, in order to analyze the extent in which WM microstructural abnormalities affect cognitive functions. At this point of the analysis, we were not focused on how the disease affects the relation between DTI values and cognitive outcomes. Instead, we used Alzheimer's as a model of disease that affects DTI measures which, in turn, affects cognitive performance. First, we performed linear regression analysis in a stepwise fashion not including DTI values (but only sex, age and education as confounders). Then, FA, streamline count, mean, axial and radial diffusivity measures of the cingulum and parahippocampal fasciculi were

additionally entered as independent variables. Stepwise analyses were conducted with a P value for entry of 0.05 and a P value for removal of 0.01.

3. Results

3.1. Demographics and neuropsychological evaluation

Table 1 displays demographic and neuropsychological scores/statistics for healthy controls, aMCI and AD subjects in the sample.

Table 1
Demographics and neuropsychological evaluation of healthy controls, aMCI and AD groups.

	Controls	aMCI	mild AD	ANOVA
Age	70(7)	70(9)	72(6)	$P=0.613$
Education	9.2(5.3)	9.2(5.5)	6.2(4.5)	$P=0.122$
% *females*	66%	52%	76%	$P=0.374^{*}$
MMSE	28.3(1.9)	25.5(2.4)	19.3(4.3)	$P<\mathbf{0.0001}^{a,b,c}$
RAVLT-Encod	46.9(8.2)	31(6.7)	19.5(7.12)	$P<\mathbf{0.0001}^{a,b,c}$
RAVLT-A7	8.8(2.4)	2.9(2.1)	0.4(0.7)	$P<\mathbf{0.0001}^{a,b,c}$
RC-FP	11.5(2.8)	3.7(5.7)	−3.5(5.8)	$P<\mathbf{0.0001}^{a,b,c}$
FDS	4.8(1.8)	4.7(1)	3.6(1.5)	$P=0.036$
BDS	4(1.2)	3.7(1)	1.7(1.5)	$P<\mathbf{0.0001}^{b,c}$
Stroop C Time	54.2(17.4)	57.6(12.1)	78.4(29.5)	$P=\mathbf{0.003}^{b,c}$
Stroop C-Errors	0(0)	0(0)	0.6(1)	$P=0.02$
Stroop I-Time	107.3(27.3)	147(47.1)	191(76.9)	$P<\mathbf{0.0001}^{b,c}$
Stroop I-Errors	2.5(3.4)	8.9(9.8)	30.5(20.7)	$P<\mathbf{0.0001}^{b,c}$
VFC	17.2(4.9)	14.1(5.2)	8.7(4.3)	$P<\mathbf{0.0001}^{b,c}$
F.A.S	31.4(11.1)	27.6(11.9)	16.7(9.9)	$P<\mathbf{0.0001}^{b,c}$
LNI	18.1(1.24)	17.4(1.7)	14.8(3.1)	$P<\mathbf{0.0001}^{b,c}$
Clock drawing	9.2(1.6)	8.5(2.3)	5.6(2.7)	$P<\mathbf{0.0001}^{b,c}$
Rey copy	34.8(4.1)	32.6(3.8)	15.2(14.1)	$P<\mathbf{0.0001}^{b,c}$
TMT-A	67.5(18.2)	91.2(56.7)	220.9(93.9)	$P<\mathbf{0.0001}^{b,c}$
TMT-B	152.3(95.2)	184.2(103.1)	292.6(32.1)	$P<\mathbf{0.0001}^{b,c}$
BNT	51.5(4.7)	45.2(13.3)	32.2(9.9)	$P<\mathbf{0.0001}^{b,c}$

Notes: data presented as average(standard deviation). MMSE: mini-mental status examination; RAVLT-Encod: encoding of Rey auditory verbal learning test; RAVLT-A7: delayed recall of Rey auditory verbal learning test; RC-FP: Rey auditory verbal learning test true recognition (i.e., recognition minus false positives); FDS: forward digit span; BDS: backward digit span; Stroop C: Stroop test Congruent; Stroop I: Stroop test Incongruent; VFC: verbal fluency categorical; F.A.S: phonological fluency for letters; LNI: visuospatial perception item of Luria's neuropsychological investigation; Rey copy: copy of the Rey–Osterrieth Complex Figure Test; TMT-A: Trail Making Test A; TMT-B: Trail Making Test B; BNT: Boston naming test. *Chi-square test. a, b and c: Statistically significant values after Tukey post-hoc test between (a) CTR X aMCI, (b) CTR X AD and (c) aMCI X AD.

There were no differences across groups regarding age, years of education and gender. As expected, AD patients performed worse than the aMCI group in all cognitive tests, except in the Stroop (errors in congruent condition) and FDS tests. When compared to controls, AD patients performed worse in all tests. aMCI patients performed worse than controls only in memory tests and MMSE.

3.2. White matter volume

Mean WM volume across the control group was 388207.43 mm^3 ($\pm$53885.61); 391659.16 mm^3 ($\pm$64085.40) mm^3 for the aMCI group and 363415.29 mm^3 ($\pm$57575.71) for the AD group. There was no difference in WM volume between the groups: controls X aMCI ($P=0.83$), controls X mild AD ($P=0.11$) and aMCI X mild AD ($P=0.13$).

3.3. DTI values across groups

ExploreDTI results of 21 aMCI subjects for anisotropic properties of diffusion, non-fractional measures of diffusivity and streamline count did not show any statistically significant difference when compared with healthy controls. Only AD patients differed from controls in terms of DTI values of the cingulum and parahippocampal bundle. Results are shown in Table 2. According to the table, AD patients presented lower values of FA, streamline count and radial diffusivity in the cingulum; and lower values of radial and mean diffusivities in the parahippocampal bundle relative to controls.

After controlling for WM total volume, FA ($P=0.003$) and radial diffusivity ($P=0.05$) of AD patients in the cingulum remained significantly different than controls, but streamline count did not ($P=0.132$). FA of AD patients was also significantly lower than aMCI subjects ($P=0.049$) after correcting for WM atrophy. In the parahippocampal bundle, only radial diffusivity survived after the adjustment ($P=0.05$), but MD did not ($P=0.198$).

3.4. Neuropsychological evaluation X DTI values

Stepwise multiple regression models were constructed to determine the extent in which DTI values of the cingulum and parahippocampal bundle (independent variables) influence in cognitive testing (dependent variable).

Table 2
Differences between healthy controls, MCI and AD patients regarding DTI values.

	Cingulum		
	Controls	**aMCI**	**mild AD**
FA	0.516 (0.018)	0.510 (0.023)	0.50055 (0.02046)^{a,A,B}
MD	0.00076 (0.00003)	0.00076 (0.00004)	0.000778 (0.000033)
Streamline count	90.31 (36.63)	65.2 (19.01)	64.19 (29.88)^{a}
Axial diffusivity	0.001243 (0.000041)	0.001237 (0.000060)	0.001251 (0.000005)
Radial diffusivity	0.000521 (0.000049)	0.000525(0.000037)	0.000542(0.000030)^{a,A}
	Parahippocampal		
	Controls	**aMCI**	**mild AD**
FA	0.451 (0.031)	0.433 (0.026)	0.432 (0.030)
MD	0.00083 (0.00004)	0.00086 (0.00005)	0.00086 (0.00005)^{a}
Streamline count	61.47 (36.07)	49.26 (40.28)	47.95 (22.92)
Axial diffusivity	0.001271 (0.000058)	0.001302 (0.000089)	0.001299 (0.000074)
Radial diffusivity	0.000606 (0.000042)	0.000644 (0.000044)	0.000642 (0.000046)^{a,A}

Notes: Data presented as average(standard deviation). FA: Fractional Anisotropy; MD: Mean diffusivity.
[a]Statistically different from controls. $P<0.05$.
[A]Statistically different from controls, after adjusting for white matter total volume. $P<0.05$.
[B]Statistically different from aMCI, after adjusting for white matter total volume. $P<0.05$.

Table 3
Models of stepwise regression analysis with and without DTI values as predictors for performance in cognitive tests.

Model	r^2 adjusted not including DTI parameters	r^2 adjusted including DTI parameters	B	Beta	Pearson correlation
RAVLT-A7					
Parahippocampal RD	NA	0.229	−32645.527	−0.38*	−0.402*
Cingulum streamline count			0.039	0.314*	0.342*
RALVT-Encod					
Education	0.151	0.310	0.998	0.370**	0.409**
Cingulum FA			198.83	0.306**	0.402**
Cingulum streamline count			0.103	0.245**	0.315**
FDS					
Education	0.119	0.220	0.125	0.407*	0.370
Cingulum FA			−23.158	−0.314*	−0.266
BNT					
Education	0.178	0.227	1.016	0.419*	0.441*
Parahippocampal RD			−66498.43	−0.254**	−0.289**
VFC					
Education	0.333	0.471	0.675	0.585**	0.597**
Parahippocampal AD			−23522.963	−0.293**	−0.291**
Cingulum streamline count			0.49	0.269**	0.254**

Notes: RAVLT-A7: delayed recall of Rey auditory verbal learning test; RAVLT-Encod: encoding of Rey auditory verbal learning test; FDS: forward digit span; BNT: Boston naming test; VFC: verbal fluency categorical. r^2 adjusted for the RAVLT-A7 model is not available, as only DTI parameters were significantly entered in the model. *$P < 0.05$; **$P < 0.001$.

Table 3 shows that some DTI measurements from both cingulum and parahippocampal bundles are able to predict performance in memory, attention, working memory and language domain tests. In all other models not listed above (with the remaining neuropsychological data), only Education was able to predict performance on cognitive tests. In MMSE model, no DTI variable was able to predict performance on this test. Unexpectedly, we found an anticorrelation between cingulum FA and FDS performance. Fig. 4 shows the distribution of controls, aMCI and AD subjects in relation to DTI parameters and cognitive performance in the statistically significant tests of the regression analysis. For the models detailed in Table 3, r^2 shows us that the DTI parameters included in the models do have some influence in cognitive performance tests. In other words, the outcome – neuropsychological performance, is accounted for by DTI values.

4. Discussion

In the present study, we compared WM connectivity amongst healthy elderly subjects, aMCI and mild AD patients, and examined the utility of anisotropic properties and non-fractional measures of diffusivity to predict cognitive functioning. With regards to differences in DMN WM integrity amongst the three groups, the aMCI group did not show any statistically significant difference when compared with healthy controls, neither for the parahippocampal, nor the cingulum bundle. Mild AD patients, however, had alterations in the cingulum FA, streamline count and radial diffusivity; and parahippocampal mean and radial diffusivities, showing that in our study only mild AD patients, and not aMCI subjects, present microstructural damage in the DMN tracts relative to controls.

AD has long been primarily considered a disease of gray matter damage, yet convergent evidence has suggested that WM neuropathological change is also an important component and influences cognition. Although gray matter areas operate in concert with each other in the mediation of cognitive activities, constituting neural networks such as the DMN, WM is what links these areas into coherent neural assemblies. Consequently, when the DMN WM tracts are damaged, it produces dysfunctions that reflect the uncoupling of the neural network even when they are not macroscopically destroyed (Filley, 2001). As cognitive functions depend critically on normal communication between widely dispersed regions, integrity of the WM tracts is a necessary condition for normal brain functioning, and damage in DMN nodes can cause cognitive impairment due to the isolation of the hippocampus and other essential areas of the memory system (Salat et al., 2010). PCC, medial temporal and frontal regions, without intact tracts connecting them, are segregated and isolated areas that do not perform their functions properly, unbalancing the whole system.

This model of understanding the disease, based on circuits, claims that the progression of symptoms in AD is related to the topographic progression of atrophy and neurofibrillary tangles (which first affects medial temporal areas and the PCC, and lastly the frontal, sensorimotor and occipital cortex) (Pievani et al., 2011). Since the first affected areas in AD compose the DMN, we can suggest that the propagation of the disease occurs along it, following vulnerable pathways, and those clinical symptoms are a result of the progressive involvement of the network. Some interesting theories such as the 'prion-like' mechanism of transmission have been reported to explain the spread of the disease along long-range circuits (Frost and Diamond, 2010; Goedert et al., 2010). Within this context, the recent neural networks model makes necessary the introduction of a view based on connected brain areas, and inclusion of the WM in this theory is essential to expand our understanding of these networks. Our study has brought further evidence to this network model, showing that the microstructural connectivity of the tracts is important to integrate DMN's areas. When such integration is damaged, the entire network is affected, influencing many cognitive domains.

To better evaluate DMN WM integrity, besides the most traditional DTI measurements (FA and MD) we also evaluated other potential relevant techniques because FA might not be the most appropriate measure to detect microstructural alterations in early AD. Other non-fractional measures of diffusivity might be more sensitive to detect degeneration of tracts (Acosta-Cabronero et al., 2010). Furthermore, crossing fibers such as the corpus callosum also produce variation in FA (Beaulieu, 2002). In our study, examination of the nature of WM damage of AD patients compared to controls revealed that besides encountering a difference in the FA of the cingulum, there was also a noticeable

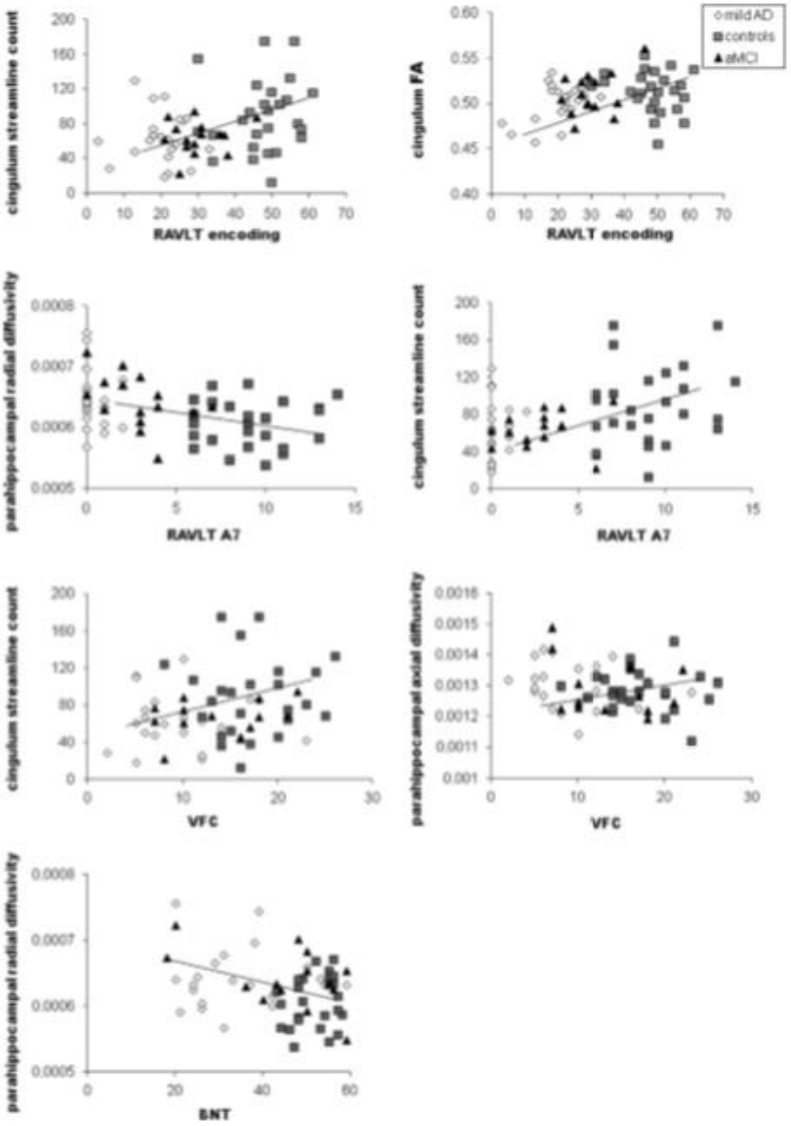

Fig. 4. Scatter plots showing the relationship between DTI parameters and cognitive tests among control subjects, aMCI and AD patients. RAVLT A7: delayed recall of Rey auditory verbal learning test; VFC: verbal fluency categorical; BNT: Boston naming test.

difference in the radial diffusivity and streamline count of this tract, as well as mean and radial diffusivities in parahippocampal tracts. Alterations in radial diffusivity are related to the disruption of myelin integrity in the demyelination process and the increased freedom of cross-fiber diffusion in WM; whereas an intact axial diffusivity is consistent with the presence of intact axons (Song et al., 2002). Interestingly, we found that only radial and not axial diffusivity was increased in both parahippocampal and cingulate bundles, suggesting a demyelination process but with no damage in axons. However, the detailed biophysical mechanism underlying alterations in directional diffusivity coefficients remains to be elucidated, and further study is required to confirm whether demyelination without axon degeneration in fact occurs in AD. In addition to that, we must emphasize that in our work, some DTI measurements were accounted for by WM total volume, as some results did not survive after correction for WM atrophy. Despite our patients did not have significant difference in total WM volume, the existing atrophy caused a partial effect in the results.

Innumerous studies have reported that macrostructural WM changes (either severity or volume) are negatively associated with cognition (Brickman et al., 2011; Meier et al., 2012). WM alterations in the form of hyperintersities detected by T2-weighted MRI, however, can mean that damage has accumulated enough to appear on conventional MRI, a situation that is not always true for AD subjects. A patient with a normally appearing scan in the subcortical area can have microstructural alterations that can lead to severe cognitive impairment. In our analysis, neither aMCI nor mild AD patients presented WM lesions, hyperintensities – as showed by individual visual assessment, and statistically insignificant atrophy when compared to controls. Hence, our study

further demonstrated that WM microalterations, detected with the DTI method, could also give rise to cognitive deficits in episodic memory, working memory, language and attention domains.

The parahippocampal and posterior cingulate tracts are increasingly gaining attention in the AD field, because those areas are the key structures (together with parietal areas) of the DMN and the memory system. Some whole-brain DTI studies, for example (Rose et al., 2006; Wang et al., 2012), found that damage in parahippocampal WM is correlated with memory function in aMCI patients. Seed-based DTI-studies have also reported a correlation between microstructural alterations either in the hippocampus or the posterior cingulate and cognitive functions (Hong et al., 2013; Metzler-Baddeley et al., 2012), but as far as we are concerned, there is a lack of studies correlating cognition with a full range of DTI measures – other than FA and MD, in an attempt to predict the cognitive performance of patients with WM microalterations.

In the present work we found that DTI measures are indeed associated with cognitive performance even when controlling for the effects of age and sex. DMN microstructural alterations are responsible, to some extent, for results in episodic memory, working memory, attention and language domains. DMN regions, in fact, are known to be involved in different high-level cognitive functions. For instance, PCC has been related to control of attentional focus (Leech and Sharp, 2013) and working memory (Sambataro et al., 2010); the medial temporal lobe is mainly engaged in episodic memory (Greicius and Menon, 2004); and the mPFC in the decision-making process (Wallis, 2007) and sustaining attention (Langner and Eickhoff, 2013). However, our study showed that microstructural damage in the tracts that connect those areas cause deficits not only in one cognitive domain, but in many domains for which DMN regions are responsible.

It is already known that other factors such as DMN functional connectivity (Hampson et al., 2006), cortical atrophy (Bakkour et al., 2013) and thinning (Wirth et al., 2013), among others, are also related to cognitive performance in AD. WM damage, however, is increasingly gaining attention in the AD field, and new studies have reported its relation to cognition, such as mentioned above. Human networks are best known by their key gray matter structures, and the WM tracts by which they are connected are less well studied. Even less is known about the relationship between alterations in the tracts by which they are interconnected and the consequences of its alterations in cognition. In this context, one of the major findings of this study is that the structural connectivity between the gray matter nodes of the DMN is important to the execution of high-complexity tasks, because it has an important role in the integration and communication of the network.

There are weaknesses in the current study that are important to be highlighted. As we did not evaluate AD biomarkers (beta-amyloid and total and phosphorylated tau), our aMCI patients will not necessarily be AD converters. This is a possible explanation for our failure to detect WM alteration in this group. The replication of our findings longitudinally, showing a possible progression of WM impairment, and the evaluation of AD biomarkers could have produced better results. Also, it is important to note that even if the adjusted r^2 adjusts for the inflation of type I errors (caused by the number of variables in the equation), such correction disappears when we compare the models with each other (and then, the new threshold of significance would change, generating a slight likelihood of inflation of such errors). It is also important to note that even though we did not include WM total volume in the regression models, subcortical atrophy could also lead to cognitive problems. Because there was no difference in WM volume between the three groups, and the main objective of the present work was to assess the influence of DMN microstructural alteration in the cognition, we decided not to consider WM volume in the analysis. In future work, it would also be interesting to compare DTI parameters within the DMN with tracts that do not comprise this network, enabling us to compare neurodegeneration of DMN tracts compared to others.

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SPECIAL SECTION ON ALZHEIMER'S DISEASE

Differences and the Relationship in Default Mode Network Intrinsic Activity and Functional Connectivity in Mild Alzheimer's Disease and Amnestic Mild Cognitive Impairment

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Abstract

There is evidence that the default mode network (DMN) functional connectivity is impaired in Alzheimer's disease (AD) and few studies also reported a decrease in DMN intrinsic activity, measured by the amplitude of low-frequency fluctuations (ALFFs). In this study, we analyzed the relationship between DMN intrinsic activity and functional connectivity, as well as their possible implications on cognition in patients with mild AD and amnestic mild cognitive impairment (aMCI) and healthy controls. In addition, we evaluated the differences both in connectivity and ALFF values between these groups. We recruited 29 controls, 20 aMCI, and 32 mild AD patients. To identify the DMN, functional connectivity was calculated by placing a seed in the posterior cingulate cortex (PCC). Within the DMN mask obtained, we calculated regional average ALFFs. Compared with controls, aMCI patients showed decreased ALFFs in the temporal region; compared with AD, aMCI showed higher values in the PCC but lower in the temporal area. The mild AD group had lower ALFFs in the PCC compared with controls. There was no difference between the connectivity in the aMCI group compared with the other groups, but AD patients showed decreased connectivity in the frontal, parietal, and PCC. Also, PCC ALFFs correlated to functional connectivity in nearly all subregions. Cognitive tests correlated to connectivity values but not to ALFFs. In conclusion, we found that DMN connectivity and ALFFs are correlated in these groups. Decreased PCC ALFFs disrupt the DMN functional organization, leading to cognitive problems in the AD spectrum.

Key words: functional MRI; episodic memory; dementia; amnestic mild cognitive impairment; Alzheimer's disease

Introduction

Dementia due to Alzheimer's disease (AD) is a complex clinical condition that affects the brain in different levels, such as molecular functioning, neurotransmitter systems, and anatomic and functional organization, leading to problems in cognition, behavior, and social independence. Amnestic mild cognitive impairment (aMCI) is thought to be a possible prodromal AD, in which patients have objective memory problems, with little-to-no impairment in social and occupational independence.

Recently, increasing attention has been given to the use of functional magnetic resonance imaging (fMRI) to explore the normal functional organization of the brain, as well as in neurologic diseases, such as AD. The functional analysis of a brain "at rest," that is, without a specific external experimental stimulus (rsfMRI), can inform us about intrinsic brain activity, as well as the level of synchronization of different cerebral regions (functional connectivity). Intrinsic brain activity, measured by the amplitude of low-frequency fluctuations (ALFFs) in BOLD signals, which are usually between 0.01 and 0.08 Hz, concerns the fluctuations in neural activity across time, while functional connectivity describes the linkage between the neural activities of different regions across spatially distinct parts of the brain (Northoff, 2013).

Among the rsfMRI approaches used to study the brain, the functional connectivity analysis is the most widely reported (Brier et al., 2012; Song et al., 2013; Xia et al., 2014). Many studies have shown that functional connectivity in the default mode network (DMN) is impaired in AD patients (Agosta et al., 2012; Greicius et al., 2004; Xia et al., 2014), probably contributing to cognitive and clinical symptoms (Balthazar et al., 2014; Weiler et al., 2014). The function of DMN is not completely understood, but is generally

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considered a distributed system for self-related cognitive activity that is activated when a person is not focused on activities directed to the external environment (Buckner et al., 2008; Menon, 2011). Also, the key anatomical regions of DMN, which include the precuneus, posterior cingulate cortex (PCC), inferior parietal, hippocampus, and medial prefrontal cortex, may have an important role in episodic memory, a cognitive function invariably disrupted in AD.

Although being a relatively new technique, the ALFF analysis throughout the brain has also brought some interesting results (yet controversial). For example, for MCI subjects, parietal regions can have either decreased (Zhao et al., 2014) or increased (Wang et al., 2011) values. Temporal regions are mostly described as having lower values in these patients (Zhao et al., 2014), but are also reported to have the opposite pattern (Liang et al., 2014; Wang et al., 2011), and the same happens to the frontal regions (Han et al., 2011; Wang et al., 2011; Zhao et al., 2014). For AD patients, in turn, the studies bring mainly decreased ALFF values for all analyzed areas (Liu et al., 2014b; Xi et al., 2012). It is interesting to note, also, that the areas that comprise the DMN are reported as having the highest ALFF values in controls at rest (Zuo et al., 2010), and the ones that are mostly affected in AD patients (Liu et al., 2014b).

The single-level evaluation of the DMN, however, does not permit us to conclude from which network subregion the breakdown in connectivity comes from (Zang et al., 2007). Also, one may suppose that as long as the BOLD signal varies similarly for two regions—even when both are decreased or increased—the correlation between those two regions will remain. In other words, those regions may be synchronized but with pathologic intrinsic activation, and it is within this context that the ALFF measures can shed some light in fMRI research.

Many studies have investigated the DMN synchrony (connectivity) and a few others have explored the DMN intrinsic activity (ALFFs) but, as far as we know, none have explored the relationship between them in AD and aMCI. Recent studies, for instance, have reported an overlap between changes in regional ALFFs and functional connectivity in several brain regions in stuttering (Xuan et al., 2012) and seasonal affective disorder subjects (Abou Elseoud et al., 2014), which supports a relationship between ALFFs and functional connectivity. In this study, we aimed to explore the differences in DMN regional spontaneous activity, measured by ALFFs, between aMCI subjects, mild AD patients, and healthy elderly subjects, and to examine its relationship with connectivity. To best evaluate the regional differences, we divided DMN into four subregions: the ventromedial prefrontal cortex, medial parietal cortex (PCC + precuneus), inferior parietal lobe, and medial temporal lobe. We also aimed to analyze the possible correlations between global cognition and episodic memory performance of these groups with DMN intrinsic activity and functional connectivity.

Methods

Subjects

Participants were recruited in the Neuropsychology and Dementia outpatient clinic of the Universidade Estadual de Campinas (UNICAMP) University Hospital. Eighty-one subjects were evaluated in this study: 29 healthy elderly subjects (controls), 20 aMCI patients, and 32 mild AD patients. Experienced attending doctors and neuropsychologists made the diagnosis of the AD patients according to the National Institute of Aging and Alzheimer's Association criteria for a diagnosis of probable AD (McKhann, 2011). Examination of each subject included medical history, neurological examination, neuropsychological and neuropsychiatric assessment, Clinical Dementia Rating (CDR) (Morris, 1993), a Hachinski ischemic score ≤ 4, and standard laboratory tests, including B12, folate, and thyroid hormone levels, and syphilis serology. The study included only AD patients who were classified as CDR 1.

aMCI patients were diagnosed using the core criteria of the National Institute of Aging/Alzheimer's Association for MCI (Albert et al., 2011). All aMCI participants had a CDR score of 0.5 (with an obligatory memory score of 0.5). This classification was performed using a semistructured interview.

Controls were identified as cognitively normal: they did not exhibit any neurological or psychiatric disorders or require psychoactive medication; they demonstrated normal Mini Mental State Examination (MMSE) scores, considering age and education (Brucki et al., 2003); and their structural images were without any abnormalities. Memory complaints or neurological deficits were not observed in the control group.

Exclusion criteria for all subjects included the following: a history of other neurological or psychiatric diseases or a head injury with loss of consciousness, use of sedative drugs in the last 24 h before the neuropsychological assessment, drug or alcohol addiction, prior chronic exposure to neurotoxic substances, and a Hachinski ischemic score of >4. This study was approved by the Medical Research Ethics Committee of UNICAMP, and written informed consent (either from the subjects or from their responsible guardians, if incapable) was obtained from all participants before study initiation, according to the Declaration of Helsinki.

Neuropsychological evaluation

An experienced neuropsychologist, who was blinded to MRI data, performed the neuropsychological evaluations. Global cognitive status was measured using the MMSE (Brucki et al., 2003; Folstein et al., 1975) and episodic memory was evaluated by the Rey Auditory Verbal Learning Test (RAVLT) (subitems: encoding, delayed recall, and recognition) (Malloy-Diniz et al., 2007). Visual perception was assessed with subtests of Luria's neuropsychological investigation (Christensen, 1975). Evaluation of language included the Boston naming test (Kaplan et al., 1983), verbal fluency for category (animals), and phonologic fluency (FAS) (Christensen and Guilford, 1959). We assessed constructive praxis with the Rey-Osterrieth complex figure test (Osterrieth, 1944); executive function with the trail making test A and B, the Stroop color-word test (congruent and incongruent) (Stroop, 1935), and the clock drawing test (Sunderland et al., 1989); and working memory with the forward and backward digit span subtest of the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1987).

RsfMRI data acquisition

All imaging was performed on a 3.0 T Philips Achieva MRI scanner. Foam padding was provided for comfort and to minimize head motion. Functional MR images were

acquired while at rest; subjects were instructed to keep their eyes closed, to avoid initiating goal-directed, attention-demanding activity during the scanning sessions, and to remain awake. The following protocol was applied to each subject: (1) functional images: axial T2*-weighted images (TR/TE = 2000/30 ms, FOV = 240 × 240, and isotropic voxels set to 3 × 3 × 3 mm^3); for each participant, we acquired 6 min of EPI data, which corresponds to 180 volumes with 40 axial slices each. (2) Structural images: (a) sagittal high-resolution T1-weighted (gradient echo images, TR/TE = 7/3.2 ms, FOV = 240 × 240 and isotropic voxels of 1 mm^3); (b) coronal and axial fluid-attenuated inversion recovery (FLAIR) T2-weighted images, anatomically aligned at the hippocampus (TR/TE/TI = 12,000/140/2850 ms, FOV = 220 × 206, voxels reconstructed to 0.45 × 0.45 × 4.0 mm^3, and the gap between slices set to 1 mm); (c) coronal IR (inversion recovery) T1-weighted images (TR/TE/TI = 3550/15/400 ms, FOV = 180 × 180, and voxels reconstructed to 0.42 × 0.42 × 3.00 mm^3); and (d) coronal multi-echo (five echos) T2-weighted images (TR/TE = 3300/30, FOV = 180 × 180, and voxels reconstructed to 0.42 × 0.42 × 3.00 mm^3).

All subjects underwent MRI scanning in the same week that neuropsychological assessment was performed.

Functional connectivity analysis

Functional images were preprocessed by applying slice-time and motion correction algorithms and by removing linear trends. Data preprocessing also included smoothing, with a 6-mm full width at half maximum (FWHM), and bandpass filtering (0.01–0.08 Hz). For spatial normalization, structural images were first linearly registered to the MNI152 (standard space) with a 12-parameter affine transformation. The resulting image was again registered to the MNI152 space, now using a nonlinear warping algorithm. Functional data was initially registered to the structural image using a 6-parameter affine transformation, and then warped to standard space using the transformations calculated for the structural image. Six movement parameters (three translational and three rotational) were included as nuisance regressors aiming to directly correct to head motion noises. Also, participants were instructed to keep their eyes closed, relax, and move as little as possible. Foam pads were used to reduce head movements and scanner noise. The global signal time series of the cerebrospinal fluid and white matter were also included in the model, increasing the regression power to confounding signal components that arise from physiological events. All of these steps were performed using AFNI (http://afni.nimh.nih.gov/afni) and FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) software.

To identify the DMN, seed-based functional connectivity was calculated by placing a seed in the posterior cingulate cortex (0, −51, 15; MNI; seed radius = 3 mm). The PCC has already been reported as a key node of the DMN (Greicius et al., 2003) and used as a seed region previously (Weiler et al., 2014; Wells et al., 2013). Specifically, for each subject, the average time course of voxels within this seed was extracted to generate a reference time-series. Each time series was then correlated with all the voxels within the brain for each subject. Subsequently, *r*-scores of each voxel were then transformed using Fisher's *r*-to-*z* method, so that these data could be used in parametric statistical analyses to obtain whole cortical statistical *z*-score maps.

To calculate the DMN *z*-average, we used a mask of DMN based on our control subjects' statistical maps (*z*-score). These images were created according to the study methodology, with a seed placed on the PCC. All maps were used to create an average image that was smoothed (FWHM = 6 × 6 × 6 mm^3) and binarized using a minimum threshold of 0.3 (*z*-score value). This template was used as reference to define the DMN regions of each volunteer and to extract their average connectivity values. The volunteer's connectivity maps were not thresholded and all voxels with positive connectivity scores (higher than zero) that overlapped with the DMN template were included to calculate the average values. Additionally, the binarized DMN template was divided into four distinct subregions: the ventromedial prefrontal cortex, medial parietal cortex (PCC + precuneus), inferior parietal lobe, and medial temporal lobe; for this analysis, these subregions are referred to as "frontal," "PCC," "parietal," and "temporal" subregions, respectively. These DMN subregion masks were used to overlap each statistical map and to calculate the average *z*-score value to each defined DMN region (Fig. 1).

ALFF analysis

The ALFF preprocessing was performed with FSL processing tools, by using the script from the 1000 Functional Connectomes Project (www.nitrc.org/projects/fcon_1000). The preprocessing routine was based initially on the removing of the image spikes, removing of very low and high frequencies (0.01–0.08 Hz), linear-trend removing, spatial smoothing (FWHM = 6 mm), and scaling to the grand mean, which aimed to put all time series on a common scale. The time series were transformed to frequency domain using fast Fourier transform (parameters: taper percent = 0, fast Fourier transform length = shortest) to obtain the signal power spectrum. Then, this power spectrum was square-rooted and then averaged across 0.01–0.08 Hz at each voxel. ALFFs were calculated as the sum of the amplitudes in the low-frequency band. Subsequently, these data were transformed to *z*-score maps and normalized to standard space (MNI152) (Zuo

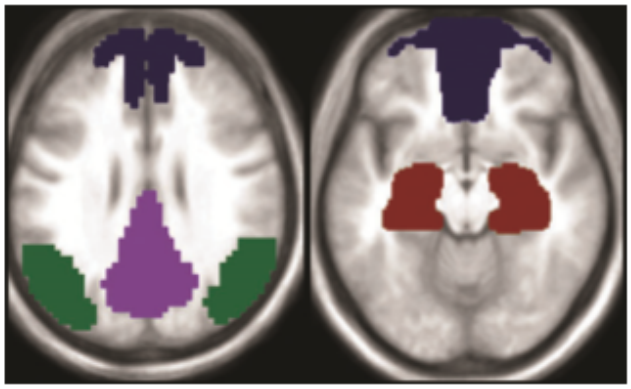

FIG. 1. Default mode network (DMN) subregion mask used to overlap each statistical map and to calculate the average *z*-score amplitude of low-frequency fluctuations (ALFFs) and functional connectivity measures. Dark blue corresponds to the ventromedial prefrontal cortex; purple corresponds to the medial parietal cortex (PCC + precuneus); green corresponds to the inferior parietal lobe; and red corresponds to the medial temporal lobe.

TABLE 1. DEMOGRAPHICS AND NEUROPSYCHOLOGICAL EVALUATION OF HEALTHY CONTROLS, aMCI, AND AD GROUPS

	Controls	*aMCI*	*Mild AD*
Age	70.5 (6.81)	67.9 (6.95)	72.8 (6.67)
Sex (female)	21 (72%)	13 (65%)	24 (75%)
Education (in years)	10.6 (5.08)	10 (5.2)	7.4 (5.12)
MMSE	28.66 (1.67)	25.94 (2.62)[a]**	19.86 (3.87)[a]**,[b]**
RAVLT-encoding	44.9 (7.7)	30.25 (7.02)[a]**	19.9 (6.5)[a]**,[b]**
RAVLT-A7	8.59 (2.23)	2.75 (2.02)[a]**	0.59 (0.78)[a]**,[b]**
RC-FP	11.66 (2.39)	4 (5.93)[a]**	−3.45 (5.99)[a]**,[b]**
Forward digit span	5.03 (1.78)	4.94 (1.12)	3.72 (1.51)
Backward digit span	4.11 (1.29)	3.81 (1.05)	2.03 (1.50)[a]**,[b]**
Stroop C time	56.62 (16.62)	55.29 (11.57)	73.21 (29.51)[a]**,[b]**
Stroop C errors	0.08 (0.27)	0.07 (0.27)	0.42 (0.87)
Stroop I time	106.92 (28.52)	135.79 (42.46)	179.10 (74.70)[a]**,[b]**
Stroop I errors	2.8 (3.52)	7.79 (8.39)	28.79 (20.37)[a]**,[b]**
Semantic verbal fluency	17.11 (4.47)	12.93 (5.32)	9.17 (4.69)[a]**,[b]**
Phonological verbal fluency	33.07 (10.82)	27.73 (11.79)	19.24 (10.53)[a]**,[b]**
Luria's neuropsychological investigation	18.29 (1.15)	17.88 (1.41)	15.31 (3.04)[a]**,[b]**
Clock drawing	9.5 (1.35)	9.53 (1.30)	6.39 (2.81)[a]**,[b]**
Rey complex figure copy	34.96 (3.43)	32.37 (4.76)	18.02 (14.18)[a]**,[b]**
Trail making test-A	65.14 (17.75)	81.13 (54.20)	198.14 (98.50)[a]**,[b]**
Trail making test-B	136.79 (93.85)	161.47 (82.55)	286.93 (42.38)[a]**,[b]**
Boston naming test	52.04 (4.76)	47.67 (10.42)	36.45 (10.75)[a]**,[b]**

Data presented as average (SD) except for sex.
[a]Significantly different from controls.
[b]Significantly different from amnestic mild cognitive impairment (aMCI).
*$p < 0.05$.
**$p < 0.001$.
aMCI, amnestic mild cognitive impairment subjects; mild AD, mild Alzheimer's disease patients; MMSE, Mini-Mental Status Examination; Stroop C, Stroop test congruent; Stroop I, Stroop test incongruent; RAVLT, Rey auditory verbal learning test; RAVLT-A7, delayed recall of Rey auditory verbal learning test; RC-FP, Rey auditory verbal learning test true recognition (i.e., recognition minus false positives).

et al., 2010). The same DMN mask described previously was used to calculate regional average z-score ALFFs of DMN and its subregions.

Statistical analysis

Statistical data analysis for functional connectivity and ALFF z-values of DMN subregions between groups, demographic and neuropsychological evaluation, and significance of correlations was performed using SPSS (version 20; SPSS, Inc., Chicago, IL) software. We first performed the Kolmogorov–Smirnov test to determine normality and as our data did not follow a normal distribution, we performed nonparametric tests. To examine the relationship between both functional analyses, we performed simple linear regressions between functional connectivity and ALFF z-values for each of the DMN subregions. At this stage, to increase our data variance, all individuals were combined into one unique group (normal aging, aMCI, and AD groups). In addition, simple linear regressions were performed among the MMSE and RAVLT (subitems: encoding, delayed recall, and recognition) scores, functional connectivity, and ALFF z-values. Results were considered to be statistically significant when $p < 0.05$.

Results

Demographics and neuropsychological evaluation

Table 1 displays demographic and neuropsychological scores/statistics for healthy controls, aMCI patients, and

TABLE 2. VALUES OF THE AMPLITUDE OF LOW-FREQUENCY FLUCTUATION AVERAGE z SCORES IN CONTROLS, aMCI, AND AD PATIENTS

	Controls	*aMCI*	*Mild AD*
Frontal ALFF z	0.3798 (0.1907)	0.5016 (0.3859)	0.4229 (0.2255)
Temporal ALFF z	0.6052 (0.1692)	0.4518 (0.2594)[a]*	0.6243 (0.2212)[b]*
Parietal ALFF z	0.3856 (0.1895)	0.4863 (0.2881)	0.347 (0.1990)
PCC ALFF z	0.8093 (0.3400)	0.8025 (0.3241)	0.5697 (0.2346)[a]*,[b]**
DMN ALFF z	0.7093 (0.0877)	0.8008 (0.2728)	0.7238 (0.0631)

Data presented as average (SD).
[a]Significantly different from controls.
[b]Significantly different from aMCI.
*$p < 0.05$.
**$p < 0.001$.
ALFFs, amplitude of low-frequency fluctuations; DMN, default mode network; PCC, posterior cingulate cortex.

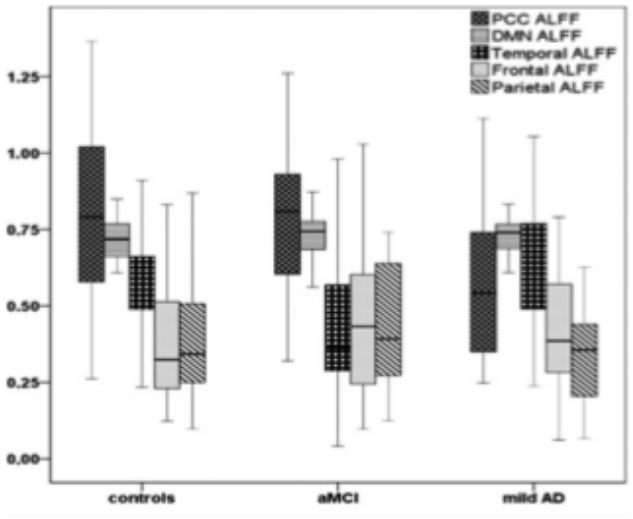

FIG. 2. Box plot displaying medians, minimum, and maximum values of ALFF *z* data for the DMN and its subareas across controls, amnestic mild cognitive impairment (aMCI), and mild Alzheimer's disease (mild AD) patients.

mild AD patients in the sample. There were no differences across groups regarding age, years of education, and gender. aMCI patients performed worse than controls, and mild AD patients performed worse than healthy controls and aMCI patients in all tests.

ALFF z data

Compared with the healthy elderly subjects, the aMCI patients exhibited significantly decreased ALFF *z*-values in the temporal region ($p=0.012$). Compared with the AD patients, the aMCI patients exhibited greater values in the PCC region ($p=0.007$) but lower ALFF *z*-values in the temporal region ($p=0.005$). Only the mild AD group PCC exhibited a significantly lower standardized ALFFs when compared with control ($p=0.007$) (Table 2). Distribution of ALFF *z*-values across the three groups is demonstrated in Figure 2.

Functional connectivity data

There was no significant difference between the connectivity in the aMCI group compared with controls, nor in the aMCI group compared with mild AD patients. The frontal ($p<0.001$), the PCC ($p=0.004$), and parietal ($p=0.014$) regions in mild AD patients showed decreased connectivity with the seed region when compared with the control group (Table 3). Distribution of functional connectivity data across the three groups is demonstrated in Figure 3.

Relationship between functional connectivity and ALFF values

Significant results were observed mainly in the PCC subarea. For example, PCC ALFF *z*-value correlated to the frontal connectivity ($r=0.289$, $p<0.05$), to the parietal connectivity ($r=0.272$, $p<0.05$), and, as expected, to the PCC connectivity ($r=0.487$, $p<0.001$). Among the other subregions, also the parietal region showed a correlation between its ALFF *z*-value and the PCC ($r=0.297$, $p<0.01$) and parietal connectivities ($r=0.221$, $p<0.05$). No other subregion ALFF *z*-value correlated to any other subregion functional connectivity.

Relationship between cognitive performance and ALFF values

Simple linear regressions failed to show any statistically significant association between any score of the cognitive tests (MMSE and RALVT subitems: encoding, delayed recall, and recognition) and ALFF values.

Relationship between cognitive performance and functional connectivity values

Simple linear regressions showed a statistically significant association between the scores of the MMSE ($r=0.281$, $p<0.05$) and RALVT subitems encoding ($r=0.323$, $p<0.001$), delayed recall ($r=0.256$, $p<0.05$), recognition ($r=0.278$, $p<0.05$), and frontal connectivity values. Temporal region connectivity was correlated to RAVLT subitems encoding ($r=0.320$, $p<0.001$) and recognition ($r=0.237$, $p<0.05$). PCC connectivity was associated with RAVLT encoding subitem ($r=0.266$, $p<0.05$). Please see Figure 4 for individual-group behavior.

Discussion

This study aimed to explore the differences in regional intrinsic activity (ALFFs) throughout DMN subregions between aMCI patients, mild AD patients, and controls, and to examine its relationship to the functional connectivity. We also aimed to analyze whether the memory performance and cognitive global status of our subjects were more related to DMN ALFFs or with functional connectivity values. Regarding the ALFF analysis, our results can be described as follows: (1) aMCI patients had lower values than controls in the temporal region and, surprisingly, lower than mild AD patients as well; (2) mild AD patients presented lower values

TABLE 3. VALUES OF FUNCTIONAL CONNECTIVITY IN CONTROLS, AMCI, AND AD PATIENTS (AVERAGE *z* SCORES)

	Controls	*aMCI*	*mild AD*
Frontal connectivity	0.348 (0.093)	0.299 (0.108)	0.259 (0.107)[a]*
Temporal connectivity	0.212 (0.078)	0.182 (0.069)	0.176 (0.054)
Parietal connectivity	0.368 (0.107)	0.350 (0.103)	0.306 (0.123)[a]*
PCC connectivity	0.508 (0.120)	0.472 (0.124)	0.411 (0.105)[a]*
DMN connectivity	0.249 (0.045)	0.244 (0.063)	0.221 (0.060)

Data presented as average (SD).
[a]Significantly different from controls.
*$p<0.05$.

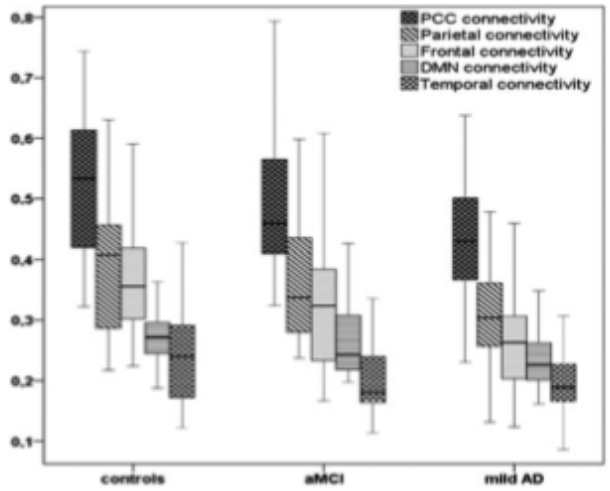

FIG. 3. Box plot displaying medians, minimum, and maximum values of connectivity data for the DMN and its subareas across controls, amnestic mild cognitive impairment (aMCI), and mild Alzheimer's disease (mild AD) patients.

in the PCC region compared with both controls and aMCI. Regarding functional connectivity data, (3) the aMCI group showed no significant difference compared with controls, nor with AD patients; (4) mild AD patients exhibited decreased connectivity in the frontal, parietal, and PCC regions compared with controls; and (5) MMSE and memory scores were not related to ALFF values, but only to the level of connectivity between regions (in which the connectivity between the frontal region with the PCC showed association with memory and MMSE tests). Based on these results, we can state that DMN connectivity problems in AD are associated with decreased functional activity in the medial parietal ("PCC") region.

In the present study, the aMCI group showed significantly decreased ALFF values in the temporal region compared with the healthy elderly group, which likely reflects the neurophysiological alterations widely known to occur in this region, such as neurofibrillary tangles (Thangavel et al., 2009). Compared with the AD group, the aMCI patients presented higher ALFF values in the PCC region, and unexpected lower values in the temporal region. We could speculate

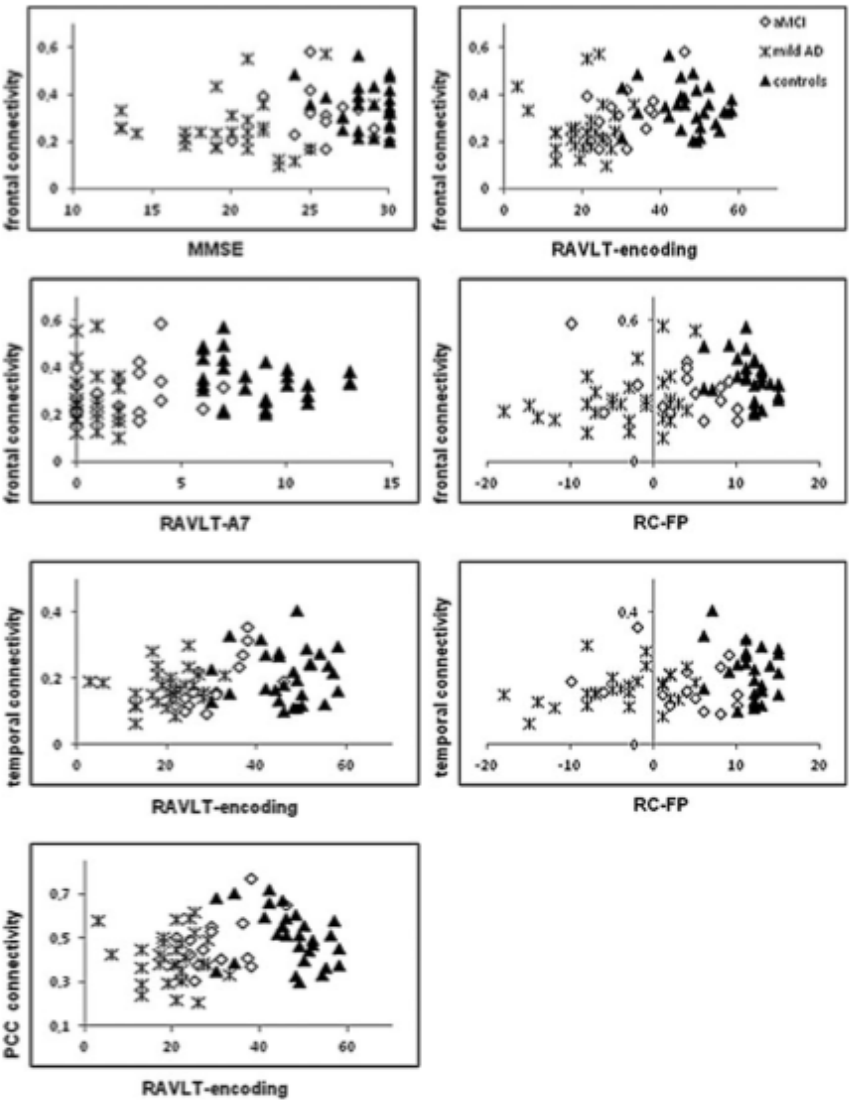

FIG. 4. Graphs showing the relationship between cognitive scores and subregions connectivity values. MMSE, Mini-Mental State Examination; RAVLT-encoding, Rey auditory verbal learning test subitem encoding; RAVLT-A7, delayed recall of Rey auditory verbal learning test; RC-FP, Rey auditory verbal learning test true recognition (i.e., recognition minus false positives).

that mild AD patients present a compensatory mechanism that increases the activity of the remaining neurons in the medial temporal region, which does not happen in the aMCI phase. Though not statistically significant, it is also interesting to note that aMCI patients exhibited greater ALFF values in relation to controls in the frontal and parietal regions, as well as the whole network, suggesting an incipient imbalance in the DMN activity even in the aMCI phase.

Using rsfMRI ALFF analysis, we found abnormal functional activity in mild AD patients in the PCC region, a region known to be key in the DMN. The DMN is already known as a brain system much like the motor or the visual systems, which contains a set of interacting brain regions that are functionally tightly connected and distinct from other systems within the brain. This network is more active during passive tasks than during goal-directed tasks, and is highly associated with reminiscence of past experiences, planning, and autobiographical episodes (Mazoyer et al., 2001). In particular, regions within the DMN show reduction of metabolic activity and atrophy in AD patients (Zhu et al., 2013), and are among the earliest to show abnormal amyloid deposition (Mintun et al., 2006).

Within this context, it is not surprising that the PCC of AD patients showed decreased ALFF values relative to controls, similarly to previous studies (Xi et al., 2012), and may be a reasonable explanation for the cognitive deficits presented in the disease. Interestingly, however, the cognitive scores did not correlate with ALFF values at all, contradicting previous studies (Liang et al., 2014; Liu et al., 2014a; Wang et al., 2011), but only with the connectivity values instead. One possible explanation for our results is that PCC ALFF values are altered in mild AD patients, either due to abnormal amyloid deposition, synaptic dysfunction, or metabolic changes, which leads to a disconnection with the parietal and frontal regions (and the latter causes cognitive problems). Therefore, our results indicate that the cognitive decline in mild AD patients is associated with disrupted functional connectivity between the two main hubs of the DMN, the frontal and the PCC regions, as previous studies have reported (Zhang 2009, 2010). Therefore, rather than the amplitude of DMN regions, the temporal synchrony of them—especially the frontal/PCC connection—is critical for normal cognitive functioning.

Another interesting finding of our study was the association between functional connectivity and ALFF scores, especially in the PCC. Very little is known about the influence of the ALFFs on functional connectivity measures, but a recent study with the healthy elderly population showed that they are in fact related to each other (Di et al., 2013). Likewise, our results showed that the functional connectivity is associated with values of local fluctuation amplitude also in AD. For instance, demented patients exhibited lower ALFF values in the PCC, which in turn was associated with the connectivity with the frontal, parietal, and PCC subareas. In other words, we could suppose that a diminished intrinsic activity in the PCC could lead to a lower connectivity of this area with the frontal and parietal subareas. Also, the parietal ALFF value (although not being statistically lower in AD) had a relationship with the diminished connectivity of the PCC and parietal subareas. Again, we could suppose that the PCC is a core region, important for maintaining the connectivity with many regions, and intrinsic abnormalities in this region may cause disconnection to many others.

There are limitations in the current work that must be highlighted. Because we did not evaluate AD biomarkers (beta-amyloid or total and phosphorylated tau), our aMCI patients may not necessarily be AD converters. This may explain our failure to detect an altered functional connectivity in this group. The replication of our findings longitudinally and the evaluation of AD biomarkers may produce better results.

Conclusions

In the present work, we found that aMCI subjects are characterized by a decrease in intrinsic functional activity in the medial temporal lobe, whereas the dementia stage is characterized by decreased activity in the PCC. Also, the regional BOLD signal amplitude is related to the functional connectivity of some areas, and alteration in the ALFF values of specific regions (such as the PCC) is related to disruption of the synchrony with the frontal and parietal areas. Finally, the cognitive decline observed in mild AD patients is modestly associated with disrupted functional connectivity of some regions (especially the frontal), rather than the intrinsic activity of them. These findings give us some evidence that AD is, among other physiopathological features, a disconnection syndrome.

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Author Disclosure Statement

No competing financial interests exist.

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Following the Spreading of Brain Structural Changes in Alzheimer's Disease: A Longitudinal, Multimodal MRI Study

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Abstract.
Background: Longitudinal MRI studies in Alzheimer's disease (AD) are one of the most reliable way to track brain changes along the course of the disease.
Objective: To investigate the evolution of grey matter (GM) atrophy and white matter (WM) damage in AD patients, and to assess the relationships of MRI changes with baseline clinical and cognitive variables and their evolution over time.
Methods: Clinical, neuropsychological, and MRI assessments (T1-weighted and diffusion tensor [DT]-MRI) were obtained from 14 patients with AD at baseline and after a 16 ± 3 month period. Lumbar puncture was obtained at study entry. At baseline, AD patients were compared to 37 controls. GM atrophy progression was assessed with tensor-based morphometry and GM volumes of interest, and WM damage progression using tract-based spatial statistics and tractography.
Results: At baseline, patients showed cortical atrophy in the medial temporal and parietal regions and a widespread pattern of WM damage involving the corpus callosum, cingulum, and temporo-occipital, parietal, and frontal WM tracts. During follow up, AD patients showed total GM atrophy, while total WM volume did not change. GM tissue loss was found in frontal, temporal, and parietal regions. In addition, AD patients showed a progression of WM microstructural damage to the corpus callosum, cingulum, fronto-parietal and temporo-occipital connections bilaterally. Patients with higher baseline cerebrospinal fluid total tau showed greater WM integrity loss at follow up. GM and WM changes over time did not correlate with each other nor with cognitive evolution.
Conclusion: In AD, GM atrophy and WM tract damage are likely to progress, at least partially, independently. This study suggests that a multimodal imaging approach, which includes both T1-weighted and DT MR imaging, may provide additional markers to monitor disease progression.

Keywords: Alzheimer's disease, grey matter atrophy, longitudinal MRI, progression, tau pathology, white matter tract damage

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INTRODUCTION

Over the last few decades, considerable effort has been spent to improve our understanding of the physiopathological processes of Alzheimer's disease (AD), as well as to develop and validate early biomarkers for its detection [1]. In this context, non-invasive magnetic resonance imaging (MRI) techniques are likely to play an important role [1]. Several studies involving amnestic mild cognitive impairment (MCI) subjects, who are at high risk for developing AD, have shed light on the patterns of cortical atrophy [2, 3] and resting-state functional alterations [4, 5], which can predict the conversion to AD.

Longitudinal MRI studies in AD are one of the most reliable way to track brain changes along the course of the disease [6–9]. Grey matter (GM) atrophy has been shown to progress from the medial temporal lobe to parietal, frontal, and occipital lobes with the highest rates of change occurring in the hippocampus and entorhinal cortex [6–9].

There is the evidence that amyloid-β (Aβ) and tau pathologies, which are the principal extra- and intracellular AD pathological alterations, spread through the brain following a stereotyped sequence, possibly along structural pathways interconnecting cortical regions, gradually reaching unaffected areas from affected ones [10–12]. Diffusion tensor (DT) MRI allows to map microstructural white matter (WM) damage along such anatomical connections [13] and its application has led to the demonstration of WM damage in AD and MCI patients [14]. Longitudinal MRI studies combining volumetric and DT MRI measures may therefore contribute to prove or disprove the network-based degeneration model of AD and elucidate the direction of disease-related pathology spreading.

In this study, we investigated the evolution of GM atrophy and WM damage in AD assessing both cross-sectional and longitudinal MRI data. Specifically, the aims of the study were to track changes of GM volume and WM damage in AD patients over an average period of 16 months, and to investigate the relationships of MRI changes with baseline clinical and cognitive variables and their evolution over time.

MATERIALS AND METHODS

This study was approved by the Local Ethical Committee on human studies and written informed consent from all subjects was obtained prior to their enrolment.

Subjects

Fourteen patients with probable AD [15] were recruited consecutively at the Scientific Institute San Raffaele, Milan, Italy. At baseline, all patients performed clinical, neuropsychological and MRI assessments. Clinical evaluations were performed by an experienced neurologist blinded to the MRI and cerebrospinal fluid (CSF) results. History was taken with a structured interview from patients' relatives. Age at onset was determined based on the estimated date of first symptom presentation as reported by caregivers. AD patients performed a follow up visit (mean follow up duration: 16 ± 3 months) including clinical, neuropsychological, and MRI evaluations. Thirty-seven healthy controls were recruited among spouses of patients and by word of mouth; at study entry, they underwent clinical and cognitive assessments and an MRI scan.

For those patients with available CSF, we included only patients with low CSF $A\beta_{1\text{-}42}$ levels and at least one abnormal AD-like neuronal injury biomarker [15]: 1) medial temporal lobe atrophy on structural MRI; 2) temporoparietal hypometabolism on fluorodeoxyglucose (FDG) positron emission tomography (PET) or hypoperfusion on single photon emission computed tomography (SPECT); 3) increased CSF total Tau (t-Tau); or 4) phosphorylated tau (p-Tau). For those patients without a CSF sample, at least two neuronal injury biomarkers were required in order to be included in the study. Subjects were excluded if they have had any of the following: a family history suggestive of an autosomal dominant disease; medical illnesses or substance abuse that could interfere with cognitive functioning; any other major systemic, psychiatric, or neurological illnesses; and other causes of focal or diffuse brain damage at routine MRI, including lacunae and extensive cerebrovascular disorders. An experienced observer reviewed the severity of the cerebrovascular disease according to a scale for rating age-related WM hyperintensities (WMH) [16]. Subjects were excluded when the Fazekas rating scale [16] was greater than 2.

CSF sample

At baseline, CSF samples were collected from 13 AD patients (one patient refused to undergo lumbar puncture). They were centrifuged at $800\times$ *g* for 5 min to remove cells and stored frozen for protein analysis. $A\beta_{1-42}$, t-Tau, and p-Tau were determined using the Inno-Bia AlzBio3 kit from Fujirebio (Pomezia,

Rome). Normative values are: $A\beta_{1-42}$ ≥500 ng/L, t-Tau ≤450 ng/L, and p-Tau ≤61 ng/L.

Cognitive assessment

Neuropsychological assessment was performed by an experienced neuropsychologist unaware of the MRI and CSF findings, and evaluated: global cognitive functioning with the Mini-Mental State Examination (MMSE) [17]; memory function with verbal and spatial span [18], prose memory test [19], and Rey's Figure Delayed Recall Test [20]; visuo-spatial abilities with the Rey's Figure Copy Test [20]; reasoning and attention functions with the Raven's Coloured Progressive test [21] and the Attentive matrices [22]; and language with the Phonemic and Semantic Fluency tests [19] and Token Test [23].

MRI acquisition

Brain MRI scans were obtained using a 3.0 T scanner (Intera, Philips Medical Systems, Best, the Netherlands). The following sequences were acquired at baseline from all subjects and from AD patients also at follow up: T2-weighted spin echo (SE) (repetition time [TR] = 3500 ms; echo time [TE] = 85 ms; echo train length = 15; flip angle = 90°; 22 contiguous, 5-mm-thick, axial slices; matrix size = 512 × 512; field of view [FOV] = 230 × 184 mm^2); fluid-attenuated inversion recovery (TR = 11 s; TE = 120 ms; flip angle = 90°; 22 contiguous, 5-mm-thick, axial slices; matrix size = 512 × 512; FOV = 230 mm^2); 3D T1-weighted fast field echo (TR = 25 ms, TE = 4.6 ms, flip angle = 30°, 220 contiguous axial slices with voxel size = 0.89 × 0.89 × 0.8 mm, matrix size = 256 × 256, FOV = 230 × 182 mm^2); pulsed-gradient SE echo-planar (PGSE EP) sequence with sensitivity encoding (acceleration factor = 2.5; TR = 8773 ms; TE = 58 ms; 55 contiguous, 2.3-mm-thick, axial slices; acquisition matrix 112 × 88, with an in-plane pixel size = 1.87 × 1.87 mm and a FOV = 231 × 240 mm^2; diffusion gradients applied in 35 noncollinear directions; b factor = 900 s/mm^2). Fat saturation was performed to avoid chemical shift artifacts. All slices were positioned to run parallel to a line that joins the most inferoanterior and inferoposterior parts of the corpus callosum.

MRI analysis

MRI analysis was performed by an experienced observer, blinded to subjects' identity. WMH, if any, were identified on T2-weighted and fluid-attenuated inversion recovery scans. WMH load was measured on T2 scans using the Jim software package (Version 5.0, Xinapse Systems, Northants, UK, http://www.xinapse.com).

MRI preprocessing

Using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/), T1-weighted images were segmented to produce GM, WM, and CSF tissue maps [24]. DT MRI preprocessing was performed using the in the FMRIB software library (FSL) (http://www.fmrib.ox.ac.uk/fsl) tools and the JIM5 software (Version 5.0, Xinapse Systems, Northants, UK, http://www.xinapse.com). The diffusion-weighted data were skull-stripped using the Brain Extraction Tool implemented in FSL. The rotation component was also applied to diffusion-weighted directions. Eddy currents correction was performed using the JIM5 software [25]. The DT was estimated on a voxel-by-voxel basis using DTIfit provided by the FMRIB Diffusion Toolbox. Maps of mean diffusivity (MD), fractional anisotropy (FA), and radial (radD) and axial (axD) diffusivities were obtained.

GM damage

Global MRI measures

At each time point, GM fraction (GMF) was calculated by dividing GM tissue volume by total intracranial volume (TICV, i.e., WM + GM + CSF). In addition, GM tissue maps were linearly registered to the b_0 image, and coregistered segments were superimposed on the MD maps to obtain average GM MD.

Voxel-wise

At baseline, voxel-based morphometry (VBM) was performed using SPM8 and the Diffeomorphic Anatomical Registration Exponentiated Lie Algebra (DARTEL) registration method [24] to detect GM volume alterations, as previously described [26]. Briefly, after the T1-weighted images segmentation (i), the spatial transformation and segmentation parameters were imported in DARTEL; (ii) the rigidly aligned version of the images previously segmented was generated; (iii) the DARTEL template was created and the obtained flow fields were applied to the rigidly-aligned segments to warp them to the common DARTEL space and then modulated using the Jacobian determinants; (iv) the modulated images from DARTEL were normalized to the Montreal Neurological Institute (MNI) template using an affine transformation

estimated from the DARTEL GM template and the a priori GM probability map without resampling (http://brainmap.wisc.edu/normalizeDARTELtoMNI). Prior to statistical computations, images were smoothed with an 8 mm FWHM Gaussian filter. Tensor-based morphometry (TBM) implemented in SPM8 allows to quantify voxel-wise patterns of volumetric change by calculating the gradient of a deformation field necessary to warp one MR image to another. In this study, TBM was used to map progression of regional GM atrophy over time in AD patients. TBM procedure has been performed as follows. A bias correction was applied to the baseline and follow up T1-weighted scans, which were then half-way rigidly co-registered using a home-made procedure in SPM8. A high-dimensional deformation field was used to warp the corrected late image to match the early one. The amount of volume change was quantified by taking the Jacobian determinant of the gradient of deformation field at a single-voxel level. The following formula was applied to the segmented GM image obtained from the first scan and the Jacobian determinant map: [(Jacobian value - 1) x GM]. The resulting image represents a measure of the GM volume change between the first and the second scan. As for VBM, segmentation, DARTEL initial import and DARTEL template creation were re-run starting from the half-way T1-weighted images and the obtained flow-fields were applied to the GM volume images. Normalized images were smoothed using an 8-mm isotropic Gaussian kernel. Normalized, smoothed maps of GM changes over time for each subject were entered into the statistical analysis.

Regions of interests (ROIs)

Using FLIRT in FSL (http://www.fmrib.ox.ac.uk/fsl/fdt/index.html), the AAL template was linearly registered to each subject GM tissue map at both baseline and follow up. Then, mean GM volumes were obtained from each AAL region. In order to reduce the number of variables, we averaged values from different areas to obtain mean volumes of 16 ROIs per each hemisphere: 1) inferior and 2) orbito-frontal_cortices; 3) anterior cingulate cortex; 4) insula; 5) motor cortex; 6) lateral temporal; 7) temporal pole; 8) inferior and superior parietal lobe; 9) posterior cingulate cortex; 10) occipital lobe; 11) cerebellum; 12) hippocampus; 13) caudate nucleus; 14) putamen; 15) pallidum; 16) thalamus. Finally, ROI GM volumes were normalized to TICV.

WM damage

Global MRI measures

At each time point, WM fraction (WMF) was calculated by dividing WM tissue volume by TICV. In addition, WM tissue maps were linearly registered to the b_0 image, and coregistered segments were superimposed on the MD maps to obtain average WM MD.

Voxel-wise

Tract-Based Spatial Statistics (TBSS) version 1.2 (http://www.fmrib.ox.ac.uk/fsl/tbss/index.html) [27] was applied to both baseline and follow up scans, as previously described [26]. Briefly, (i) FA volumes were aligned to a target image; (ii) a mean FA image was then created by averaging the aligned individual FA images, and thinned to create a FA skeleton representing WM tracts common to all subjects, and (iii) individual MD, FA, axD, and radD data were projected onto this common skeleton. Voxel-wise WM change maps were obtained for each subject by subtracting follow up from baseline DT MRI maps (projected onto the same common skeleton).

Tracts of interests

Seeds for tractography of the genu, splenium, and body of the corpus callosum, cingulum, inferior and superior longitudinal fasciculi, and uncinate fasciculus were defined in the MNI space on the FA template provided by FSL, as previously described [28, 29]. Fiber tracking was performed in native DT MRI space using a probabilistic tractography algorithm implemented in FSL (probtrackx), which is based on Bayesian estimation of diffusion parameters (Bedpostx) [30]. Details of the tractography procedure have been previously reported [28, 29]. Maps of MD, FA, axD, and radD were obtained from each tract in the native space. The same tractography procedure was applied to DT MRI data obtained at follow up. Tract maps were obtained for each subject at each time point. Then, linear registrations from baseline FA maps to follow up ones and vice versa were estimated and averaged, and the half way transformation was obtained and applied to both baseline and follow up tract maps. Finally, the overlap between baseline and follow up tracts was estimated, back transformed to the original space and the diffusion values were extracted for each patient.

Statistical analysis

Baseline demographic, clinical, cognitive, and global MRI data

Baseline demographic, clinical, cognitive, and global MRI data were compared between patients and controls using ANOVA models. MRI data comparisons were adjusted for age. Results were considered significant at $p < 0.05$ corrected for multiple comparisons using the false discovery rate (FDR). Analyses were performed using SAS Release 9.3 (SAS Institute, Cary, NC, USA).

MRI changes over time (global, ROI GM and tract of interest DT MRI values)

In AD patients, changes of GMF, WMF, GM, and WM average MD values, as well as ROI GM volumes and DT MRI values of WM tracts of interest were assessed using repeated measurements ANOVA models adjusting for age at first MRI and time elapsed between scans. In addition, GM and WM MRI changes over time were correlated each other and with baseline cognitive and CSF levels and cognitive changes over time, using multiple regression models adjusting for age at first scan and time elapsed between scans. Results were considered significant at $p < 0.05$ corrected for multiple comparisons using the FDR. Analyses were performed using SAS Release 9.3.

VBM/TBM

VBM group comparisons were performed to assess baseline GM volume differences using SPM8 and ANCOVA models, adjusting for age and TICV. In order to assess GM tissue loss in AD patients, GM change maps obtained with TBM entered a one-sample t test in SPM8, adjusting for age at first MRI and time elapsed between scans. VBM findings were related to tract of interest DT MRI changes over time, using multiple regression models in SPM adjusting for age and TICV. TBM findings were related to baseline neuropsychological scores, CSF levels and tract of interest DT MRI values, as well as with cognitive and DT MRI changes during follow up, using multiple regression models in SPM8 for age at first scan and time elapsed between scans. Correlation analysis was restricted to those variables which have been found to be significantly different between patients and controls at baseline or significantly changed over follow up. Results were assessed at $p < 0.05$ family-wise error (FWE)-corrected for multiple comparisons.

TBSS

DT MRI voxelwise statistics were performed using a permutation-based inference tool for nonparametric statistical thresholding ("randomize", FSL [31]). The number of permutations was set at 5000 [31]. Baseline DT MRI maps were compared between patients and controls using ANCOVA models and adjusting for age. In AD patients, TBSS changes over time were assessed using generalized linear models with WM change maps as the unique factor, adjusting for age at first MRI and time elapsed between scans. TBSS changes were related to baseline neuropsychological scores and CSF levels, as well as with cognitive changes during follow up, using multiple regression models in FSL adjusting for age at first scan and time elapsed between scans. Correlation analysis was restricted to those variables which have been found to be significantly different between patients and controls at baseline or significantly changed over follow up. The analyses were thresholded at $p < 0.05$, corrected for multiple comparisons (FWE) at a cluster level using the threshold-free cluster enhancement option [32].

RESULTS

Demographic, clinical, and neuropsychological data

Demographic, clinical, and neuropsychological findings at baseline and follow up visits are reported in Tables 1 and 2. At baseline, patients and controls were similar for age, gender, years of education, Fazekas score, and WMH load. In AD patients, Fazekas score and WMH load did not change over time. Compared to controls, AD patients performed worse in all neuropsychological tests. At follow up, AD patients showed a significant worsening in MMSE and semantic fluency. All patients had decreased $A\beta_{1\text{-}42}$ levels; 7 patients had elevated t-Tau; 12 patients had elevated p-Tau. Six patients had all three CSF biomarkers abnormal, and seven patients had two CSF abnormal levels.

Global MRI measures

At baseline, compared to controls, AD patients showed reduced GMF and WMF, and increased average GM and WM MD (Table 1). At follow up, AD patients showed a significant decrease of GMF (Table 1).

Table 1
Demographic and clinical findings at baseline and follow up visits

	Controls	AD patients (baseline)	AD patients (follow up)	*p* values Controls x AD baseline	*p* values AD: baseline *x* follow-up
n	37	14	14	–	–
Gender (female)	18 (49%)	6 (43%)	–	0.14	–
Education (y)	12 ± 4.4 (5–24)	9.2 ± 5.0 (3–17)	–	0.16	–
Age at disease onset (y)	–	65.4 ± 6.2 (52.9–76.6)	–	–	–
Age at MRI (y)	70.1 ± 7.7 (58.1–81.9)	69.1 ± 5.7 (60.7–79.6)	70.5 ± 5.8 (61.4–81)	0.97	–
Disease duration at MRI (y)	–	3.67 ± 1.35 (2.02–7.8)	5.03 ± 1.29 (3.25–8.54)	–	–
WMH load (ml)	1.3 ± 1.7 (0–4.6)	0.7 ± 0.8 (0.03–2.8)	0.9 ± 1.0 (0.1–4.7)	0.10	0.47
Visual rating WMH (0/1/2/3)	14/19/4/0	7/6/1/0	6/6/2/0	0.85	0.57
GMF	0.40 ± 0.03 (0.35–0.47)	0.36 ± 0.03 (0.33–0.42)	0.35 ± 0.03 (0.31–0.42)	<0.001	0.02
WMF	0.33 ± 0.03 (0.26–0.40)	0.29 ± 0.03 (0.25–0.36)	0.29 ± 0.03 (0.25–0.34)	<0.001	0.30
GM average MD [$\times 10^{-3}$ mm^2 s^{-1}]	1.04 ± 0.04 (0.95–1.12)	1.12 ± 0.05 (1.05–1.21)	1.14 ± 0.06 (1.01–1.29)	<0.001	0.15
WM average MD [$\times 10^{-3}$ mm^2 s^{-1}]	0.79 ± 0.03 (0.75–0.85)	0.84 ± 0.03 (0.79–0.89)	0.85 ± 0.04 (0.79–0.93)	<0.001	0.15
CSF levels (ng/L)	–		–	–	–
$A\beta_{1\text{-}42}$		316.8 ± 132.6 (98–467)			
t-Tau		609.3 ± 504.4 (118–2072)			
p-Tau		107 ± 45.5 (54–218)			
CDR	–	1.0 ± 0.3 (0.5–2)	–	–	–
CDR sum of boxes	–	4.6 ± 1.2 (3.5–7)	–	–	–
ADL	–	6.0 ± 0.0	5.2 ± 1.1 (3–6)	–	0.40

Values are mean ± standard deviation (range) or number (%). $A\beta_{1\text{-}42}$, amyloid $\beta_{1\text{-}42;}$ AD, Alzheimer's disease; ADL, activities of daily living questionnaire; CDR, clinical dementia rating; CSF, cerebrospinal fluid; GM, gray matter; GMF, GM fraction; MD, mean diffusivity; MRI, magnetic resonance imaging; t- and p-Tau, total and phosphorylated tau protein; WM, white matter; WMF, white matter fraction; WMH, white matter hyperintensity. Analyses are adjusted for multiple comparisons using false discovery rate (see text for further details).

Table 2
Neuropsychological features of AD patients at baseline and follow up

	Controls	AD patients (baseline)	AD patients (follow up)	*p* values Controls x AD baseline	*p* values AD baseline x follow-up
MMSE	29.16 ± 1.01	22.29 ± 3.15	17.89 ± 1.72	<0.001	0.01
Memory					
Digit Span (co: 3.75)	5.95 ± 1.22	4.64 ± 0.74	4.15 ± 0.25	0.002	0.69
Rey's Figure Recall (co: 9.47)	18.92 ± 6.36	2.86 ± 3.53	2.22 ± 0.98	<0.001	1
Prose memory (co: 8)	–	2.21 ± 2.42	1.27 ± 0.98	–	0.45
Corsi test (co: 3.75)	5.76 ± 3.63	2.85 ± 1.41	2.69 ± 0.41	0.01	1
Language					
Token test (co: 26.5)	32.55 ± 2.93	26.39 ± 4.28	24.19 ± 1.42	<0.001	0.44
Phonemic fluency (co: 17)	36.13 ± 10.04	18.21 ± 7.4	14.28 ± 3.74	<0.001	0.69
Semantic fluency (co: 25)	40.58 ± 8.95	17.86 ± 8.17	12.65 ± 2.51	<0.001	0.01
Visuo-spatial skills					
Rey's Figure Copy (co: 28.88)	33.84 ± 2.52	13.11 ± 9.44	9.51 ± 2.55	<0.001	0.68
Attention					
Raven's Matrices (co: 18)	29.94 ± 3.64	18.29 ± 9.30	14.21 ± 2.40	0.001	0.45
Attentive matrices (co: 31)	49.44 ± 7.62	31.86 ± 10.55	28.03 ± 4.14	<0.001	0.69

Values are means ± standard deviations. AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; co, cutoff. Analyses are adjusted for a multiple comparisons using false discovery rate (see text for further details).

GM atrophy

At baseline, compared to controls, AD patients showed a distributed pattern of cortical atrophy with a more severe involvement of the medial temporal lobe, inferior parietal and postcentral gyri, and posterior cingulum, bilaterally (Fig. 1A; Supplementary Table 1). At follow up, AD patients did not show additional

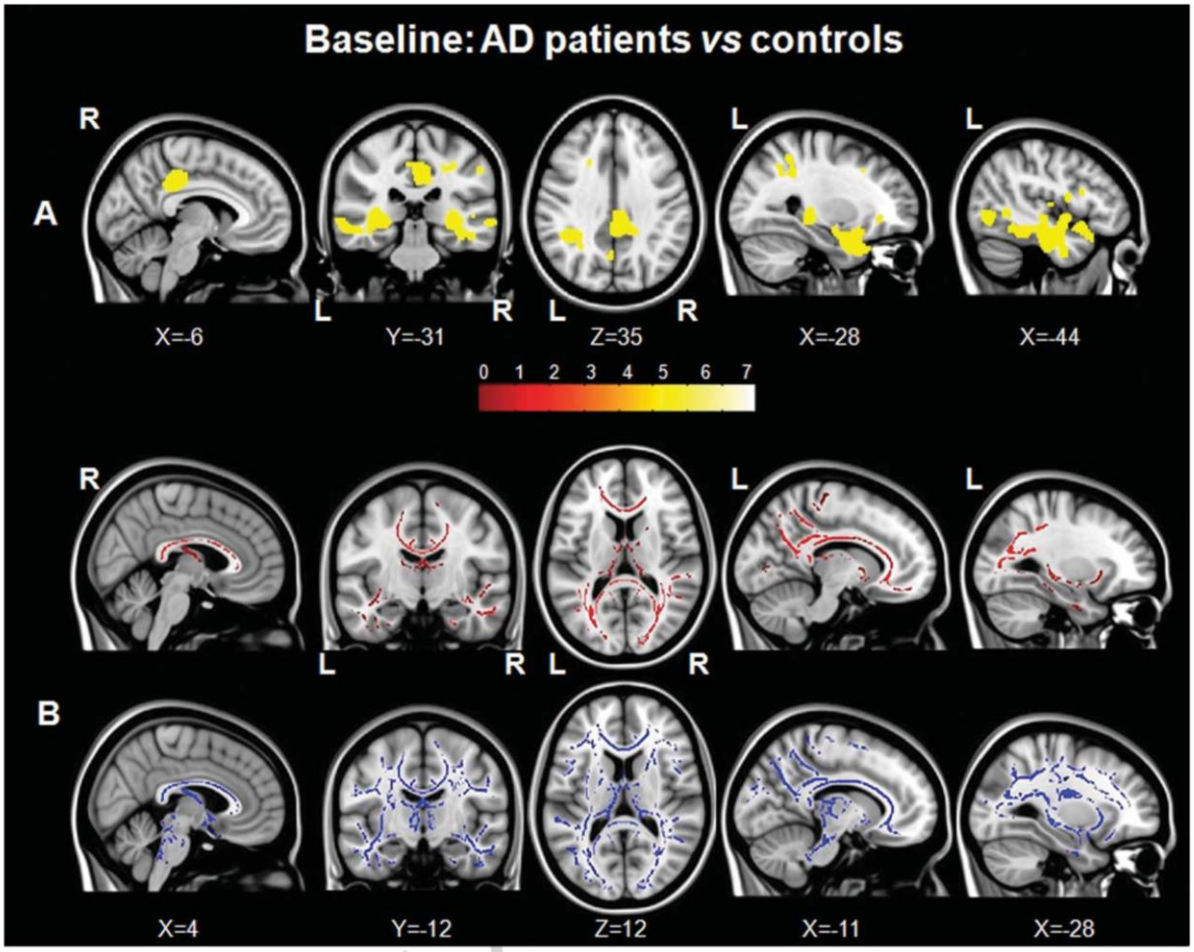

Fig. 1. Baseline comparison between Alzheimer's disease (AD) patients and healthy controls. A) Regions showing grey matter (GM) atrophy in AD patients relative to controls. Colored bar denotes t values, $p<0.05$, family-wise error (FWE)-corrected for multiple comparisons. B) Decreased fractional anisotropy (FA, red) and increased mean diffusivity (MD, blue) in AD patients compared with healthy controls. X, Y, Z are coordinates in the Montreal Neurological Institute space. Results are displayed at $p<0.05$, FWE-corrected for multiple comparisons.

regions of GM atrophy at the voxel-wise level relative to baseline. ROI analysis indicated that AD patients showed volumetric changes over time of the hippocampus, lateral temporal cortex, temporal pole, inferior frontal and orbitofrontal cortices, inferior and superior parietal lobe, posterior cingulate cortex, cerebellum and putamen bilaterally, left anterior cingulate cortex and insula, and right caudate nucleus (Table 3, Fig. 3A).

WM damage

At baseline, compared to controls, AD patients showed a widespread pattern of voxel-wise DT MRI abnormalities including the corpus callosum, posterior cingulum, parahippocampal WM, temporo-occipital, parietal and frontal WM tracts, thalamic radiations, and brainstem (Fig. 1B). TBSS showed that AD patients experienced a progression of WM damage over time in the corpus callosum, internal and external capsule, cingulum, frontal and temporo-occipital WM (Fig. 2). In addition, tract of interest-based analysis indicated that AD patients had a significant worsening of damage to the body and splenium of the corpus callosum, superior longitudinal fasciculus (SLF) bilaterally, right cingulum and left inferior longitudinal fasciculus (ILF) at follow up (Fig. 3B; Table 4).

Relationship between clinical, cognitive, CSF, and MRI variables

Baseline t-Tau levels correlated with the increase of radD ($r=0.69$, $p=0.03$) and MD ($r=0.77$, $p=0.01$)

Table 3
Significant changes of grey matter (GM) volumes at follow up relative to baseline in AD patients

GM volumes	AD patients (baseline)	AD patients (follow up)	p values
Frontal lobe and insula			
Right orbitofrontal	0.37 ± 0.06	0.36 ± 0.06	0.029
Left orbitofrontal	0.37 ± 0.06	0.36 ± 0.06	0.032
Right inferior frontal	0.37 ± 0.06	0.35 ± 0.06	0.038
Left inferior frontal	0.36 ± 0.06	0.35 ± 0.05	0.038
Left anterior cingulate	0.45 ± 0.08	0.43 ± 0.08	0.041
Left insula	0.46 ± 0.08	0.44 ± 0.08	0.331
Basal ganglia			
Right caudate	0.46 ± 0.09	0.44 ± 0.09	0.041
Right putamen	0.28 ± 0.08	0.32 ± 0.11	0.028
Left putamen	0.40 ± 0.12	0.37 ± 0.11	0.028
Temporal lobe			
Right anterior temporal	0.43 ± 0.07	0.41 ± 0.06	0.038
Left anterior temporal	0.44 ± 0.07	0.43 ± 0.07	0.028
Right lateral temporal	0.42 ± 0.06	0.30 ± 0.06	0.028
Left lateral temporal	0.43 ± 0.07	0.31 ± 0.07	0.034
Right hippocampus	0.44 ± 0.07	0.43 ± 0.07	0.028
Left hippocampus	0.44 ± 0.08	0.43 ± 0.07	0.028
Parietal lobe			
Right posterior cingulate	0.32 ± 0.05	0.31 ± 0.05	0.028
Left posterior cingulate	0.37 ± 0.05	0.36 ± 0.05	0.041
Right inferior and superior parietal	0.36 ± 0.05	0.34 ± 0.05	0.028
Left inferior and superior parietal	0.35 ± 0.06	0.34 ± 0.05	0.029
Cerebellum			
Right cerebellum	0.45 ± 0.07	0.44 ± 0.07	0.029
Left cerebellum	0.49 ± 0.07	0.48 ± 0.07	0.038

Values are means $\pm$ standard deviations. Analyses are adjusted for age, time between scans and multiple comparisons using false discovery rate (see text for further details).

values of the right cingulum at follow up (Fig. 4). Low MMSE score at baseline was associated with a greater GM volume loss of the right anterior temporal pole during follow up ($r = 0.80$, $p = 0.04$). In AD patients, left anterior cingulate volume loss at follow up correlated with radD increase of the body of the corpus callosum ($r = -0.78$, $p = 0.03$). We did not find any other statistically significant relationship between clinical, cognitive, CSF, and MRI findings.

DISCUSSION

This study reports brain structural changes in a sample of AD patients over an average period of 16 months. At baseline, AD patients showed the expected pattern of GM atrophy and WM microstructural damage involving regions which are known to be typically hit by the disease, such as the medial temporal, posterior cingulate and inferior parietal cortices, the corpus callosum, and the cingulum [14, 33]. Over follow up, both GM atrophy and WM microstructural alterations progressed. Importantly, WM damage accrual was not related to baseline GM nor to GM tissue loss over time. In addition, AD patients with higher CSF t-Tau levels at baseline showed a greater progression of microstructural WM damage.

We observed that, in GM regions already hit at baseline, such as the hippocampus, posterior cingulate, and temporo-parietal cortices, cortical atrophy further progresses over a period of 16 months in AD patients. These data are in agreement with previous studies, which observed that in the rate of progression of atrophy in these regions is about 2–4% per year [8, 34]. The additional tissue loss that we found in the orbitofrontal, inferior frontal regions, and basal ganglia over time is also in keeping with previous studies [6, 8, 35] and well reflects the trajectory of the neurofibrillary pathology which typically occurs later in these areas [36, 37].

This study revealed that AD patients experienced distributed DT MRI changes of the corpus callosum, cingulum, fronto-parietal and temporo-occipital connections bilaterally over the period of follow up. Previous studies of WM changes in AD have shown abnormalities of the corpus callosum [38] and cingulum [39] over 12 months. Possible mechanisms of propagating WM degeneration within neuronal networks include secondary degeneration in which neuronal loss is followed by axonal damage. Our longitudinal MRI data, however, showed that WM changes over time did not correlate with cortical atrophy neither at the baseline nor at follow up, suggesting, at least partially, independent dynamics of GM and WM changes in AD. Together with previous cross-sectional studies in AD [40], MCI [41, 42], and healthy subjects [43] demonstrating that WM damage can be detected before the development of cortical atrophy and overt dementia, these findings suggest that WM alterations may reflect an additional aspect of AD-related damage over and above cortical pathology. In addition, although we did not include patients with high WMH load and WMH burden did not change over follow up, we cannot exclude an effect of microvascular lesions on the progression of WM intrinsic damage. The interpretation of the lack of a relationship between GM and WM damage needs to be approached with caution, given the different imaging modalities used to investigate these two types of tissue and the small sample of patients included in our study. Future studies should investigate whether cortical thickness measurement is more sensitive to volumetric changes relative to VBM-based approaches. However, the apparent independent progression of cortical and WM changes observed over

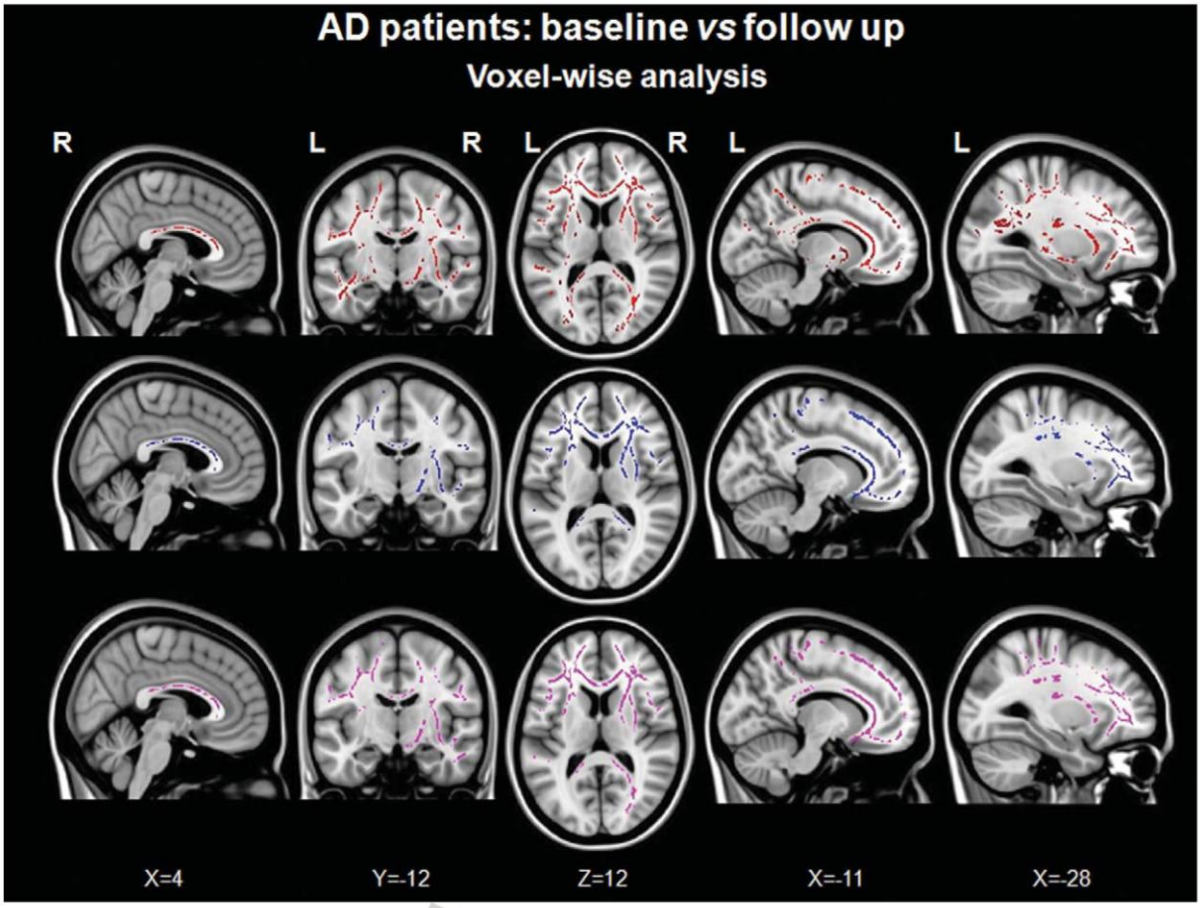

Fig. 2. Voxel-wise progression of white matter damage over an average period of 16 months in patients with Alzheimer's disease (AD). Decreased fractional anisotropy (FA, red) and increased mean (MD, blue) and radial (radD, pink) diffusivities in AD patients at follow up relative to baseline. X, Y, Z are coordinates in the Montreal Neurological Institute space. Results are displayed at $p < 0.05$, family-wise error-corrected for multiple comparisons.

follow up indicates the utility of a multimodal imaging approach which includes both T1-weighted and DT MR images to measure disease progression in AD.

Our results can also be interpreted in the light of the recent notion that WM injury in AD has a central role on the way the disease strikes and progresses. A series of recent studies has provided evidence for a prion-like pathological transmission of Aβ and tau aggregates in AD from neuron to neuron along WM connections [10–12]. Interestingly, in prion diseases, such as Creutzfeldt-Jakob disease, it has been recently demonstrated [44] that WM damage is not simply due to secondary degeneration, but is likely due to a direct effect of prion aggregation. It has also been proposed that factors such as microglial activation may promote neurotoxic and oligodendrotoxic AD pathology spreading through WM tract damage [45]. The corpus callosum and cingulum are the main tracts connecting the crucial nodes of the default mode network (DMN), such as the medial temporal lobes, posterior and anterior cingulate cortices and inferior parietal lobes. We observed DT MRI changes in the SLF and ILF, which link the temporoparietal regions, early affected in AD, with the prefrontal and the occipital cortices, which are typically hit later on in the course of the disease. Pathological, structural, and functional MRI studies have shown that patterns of neurodegeneration in AD converge over time into the DMN and frontoparietal networks, with a preferential involvement of the posterior nodes of these networks [46–48]. Several mechanisms have been postulated to explain the vulnerability of such networks in AD, including a high neural activity and/or metabolic stress of the main cortical hubs enhancing Aβ production, aggregation,

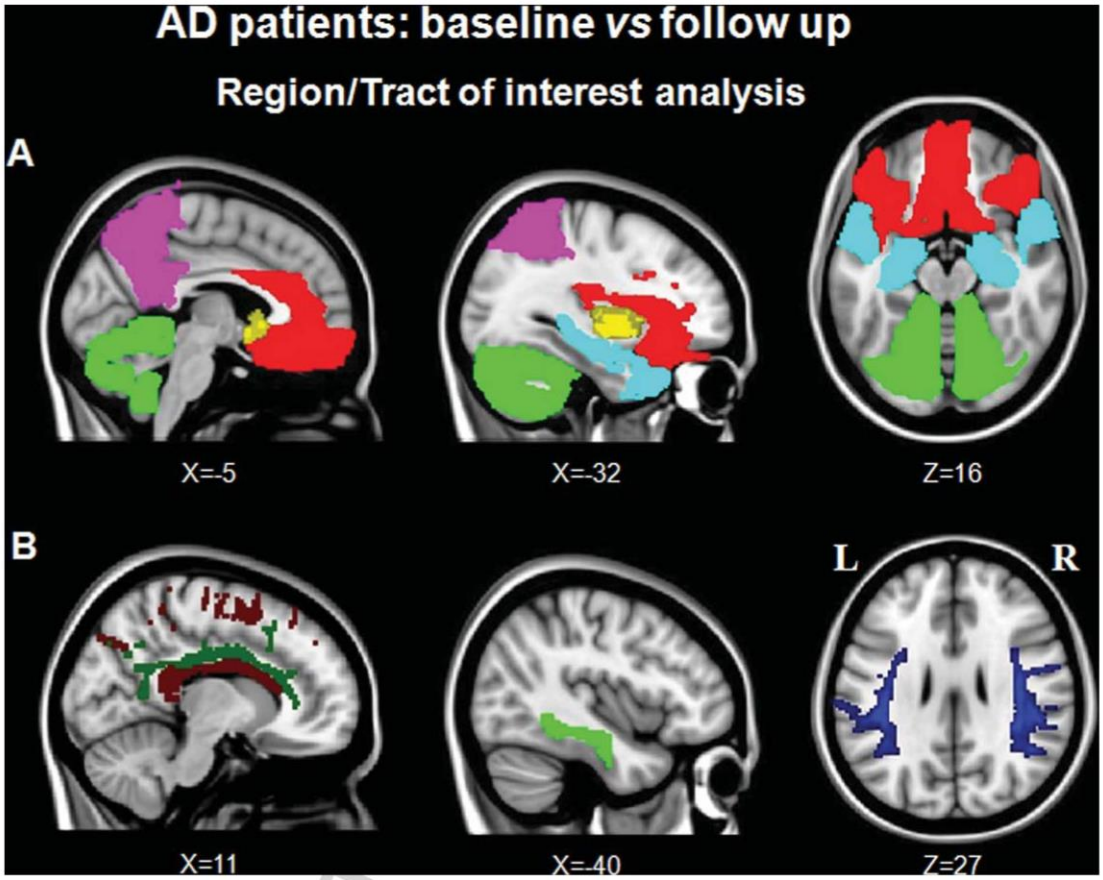

Fig. 3. Region and tract of interest-based progression of grey matter (GM) atrophy and white matter (WM) damage over an average period of 16 months in patients with Alzheimer's disease (AD). A) Regions showing GM volume loss at $p < 0.05$, false discovery rate-corrected for multiple comparisons. Red, frontal lobe; cyan, temporal lobe; violet, parietal lobe; yellow, basal ganglia; green, cerebellum. B) WM tracts showing worsening of microstructural damage in AD patients. Magenta,corpus callosum; dark green, right cingulum; light green, left inferior longitudinal fasciculus; dark blue, right superior longitudinal fasciculus. $p < 0.05$, false discovery rate-corrected for multiple comparisons. X, Y, Z are coordinates in the Montreal Neurological Institute space.

deposition, and neurotoxic effect [46, 49]. DT MRI changes we observed over a 16 month period in our AD sample may contribute to explain how the pathogenic process of the disease spreads from temporoparietal to frontal and occipital brain regions *via* WM tracts.

In this framework, another intriguing finding of this study is the observation that high CSF t-Tau levels at baseline were associated with radD and MD increases of the right cingulum at follow up, while no relation was observed between CSF $A\beta_{1\text{-}42}$ levels and DT MRI changes. Biomarker studies have shown that the relationships between Aβ pathology and downstream processes, such as glucose hypometabolism, brain atrophy, disease severity and progression, are modest at best [1]. On the contrary, there is evidence for a close association between the spatial distribution of tau depositions and clinical evolution of the disease, as well as between postmortem tau burden and cognitive performance during life in AD patients [1]. Although speculative, our findings suggest that, at least at the dementia stage, non-Aβ processes are more likely driving WM degeneration in AD.

It is worth noting that we did not observe any relationship of cortical atrophy and WM damage with cognitive changes over time. Such disappointing findings could be explained by several factors. First, they could be a reflection of the homogeneous cognitive decline within the patient group which may not provide a large enough range of changes sufficient to reach a statistically significant correlation. Intrinsic limitations of existing clinical measures in quantifying the severity of disease or the burden of underlying disease pathology may have also contributed to such a finding. Finally, it could be the case that there is not a

Table 4
Significant changes of diffusion tensor MRI measures at follow up relative to baseline in AD patients

Tracts	DT MRI parameters	AD patients (baseline)	AD patients (follow up)	*p* values
CC	MD	0.98 ± 0.07	1.01 ± 0.08	0.042
	radD	0.67 ± 0.06	0.69 ± 0.07	0.035
CC body	FA	0.50 ± 0.02	0.47 ± 0.03	0.042
	radD	0.67 ± 0.06	0.72 ± 0.10	0.044
CC splenium	MD	0.98 ± 0.06	1.02 ± 0.08	0.042
	axD	1.63 ± 0.07	1.68 ± 0.09	0.035
Right CING	radD	0.70 ± 0.08	0.73 ± 0.08	0.035
Left ILF	radD	0.64 ± 0.04	0.67 ± 0.04	0.044
Left SLF	MD	0.78 ± 0.02	0.80 ± 0.02	0.042
	radD	0.59 ± 0.03	0.61 ± 0.03	0.035
Right SLF	FA	0.40 ± 0.02	0.39 ± 0.02	0.042
	radD	0.62 ± 0.03	0.64 ± 0.04	0.035

Values are means ± standard deviations. MD and radD values are $\times 10^{-3}$ mm^2 s^{-1}. AD, Alzheimer's disease patients; CC, corpus callosum; CING, cingulum; DT MRI, diffusion tensor MRI; FA, fractional anisotropy; ILF, inferior longitudinal fasciculus; MD, mean diffusivity; radD, radial diffusivity; SLF, superior longitudinal fasciculus. Analyses are adjusted for age, time between scans and multiple comparisons using false discovery rate (see text for further details).

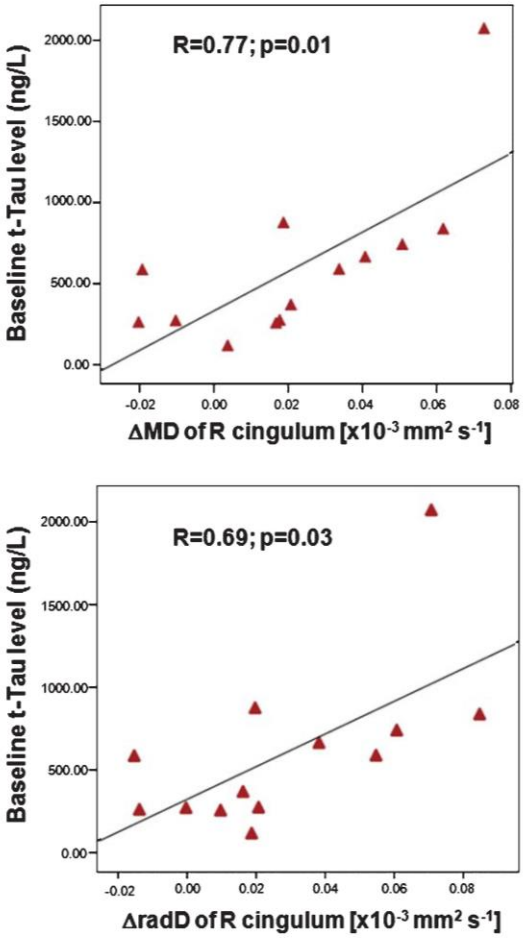

Fig. 4. Relationships between the level of cerebrospinal fluid total-Tau (t-Tau) at baseline and the increase of mean diffusivity ($r = 0.77$, $p = 0.01$) and radial diffusivity ($r = 0.69$, $p = 0.03$) of the right cingulum over an average period of 16 months in Alzheimer's disease (AD) patients. Excluding the outlier AD patient reporting a CSF t-Tau level of 2072 ng/L did not change the results (mean diffusivity: $r = 0.71$, $p = 0.01$; radial diffusivity: $r = 0.62$, $p = 0.03$). t-Tau, total tau; MD, mean diffusivity; radD, radial diffusivity.

one-to-one temporal correspondence between clinical worsening and tissue damage evolution. This is likely due to the fact that tissue damage takes time to translate in clinically detectable findings and may prompt to the use of T1-weighted and DT MRI as powerful tools to detect the disease pathological progression before and beyond the clinical manifestations.

Despite these limitations, a number of factors increases the reliability of our findings. The patient population was clinically, cognitively, and biologically well defined. Furthermore, GM and WM changes have been investigated using sound methodological approaches, which included correction for multiple comparisons and for confounding variables. However, some cautions should be exercised when interpreting our findings, given the relatively small sample size (which reduces the statistical power of our analysis and could lead to false negative findings), and the lack of the neuropsychological and follow up assessment of healthy controls.

In conclusion, according to our and previous studies, we speculate that AD onsets in vulnerable posterior brain regions and spreads forward via WM tracts within the DMN and frontoparietal networks. Such a progression is likely to be driven by tau pathology more than by Aβ. In addition, our results show that, at least at a certain point of AD evolution, the pace of WM microstructural changes may be independent of that of cortical atrophy. This would suggest that a multimodal imaging approach, which includes both T1-weighted and DT MR imaging, may provide additional markers to monitor AD progression after dementia onset.

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SUPPLEMENTARY MATERIAL

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Capítulo 7

Artigo submetido

The self, cortical midline structures and the resting-state: implications for Alzheimer's disease.

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ABSTRACT

Different aspects of the self have been reported to be affected in many neurological or psychiatric diseases such as Alzheimer's disease (AD), including mainly cognitive higher-level self-unawareness. This higher sense of self-awareness is most likely related to and dependent of episodic memory, due to the proper integration of ourselves in time, with a permanent conservation of ourselves (i.e., sense of continuity across time). Reviewing studies in this field, our objective is thus to raise possible explanations – especially with the help of neuroimaging studies, to where such self-awareness deficit comes from in AD patients. We then propose a model that includes not only episodic (and autobiographical memory) impairment, but also the important role of cortical midline structures, the Default Mode Network and the resting state (intrinsic brain activity) for the processing of self-related information.

Key-words: Alzheimer's disease; self-awareness; autobiographical memory; cortical midline structures; Default Mode Network; resting state; intrinsic activity; temporal continuity.

The Self

Understanding the concept of self is a challenge for philosophers and scientists. Even more difficult is to define the self, an issue that humans face long before the emergence of psychology. Although the literature includes countless articles, chapters and books that try to build a suitable concept, there is no coherent body of knowledge that comprises a cognitive explanation of the self.

Phylogenetically speaking, a more primordial concept of self has been raised to define the relationship between the organisms with the environment (Panksepp and Northoff, 2009), the biological urges that make them active creatures reacting to their interoceptive goals towards the world's exteroceptive stimuli. This so called 'core self' is believed to be present among all mammals and was postulated to be a basic neural function composed of viscero-somatic and subcortical structures that, although initially aimless, promoted a higher aspect of self-related process (Northoff and Panksepp, 2008). This 'core self' - or 'minimal self', is the most basic level of the self, and refers to the consciousness of oneself as an immediate subject of experience, temporally and spatially confined to the immediate present (Clare et al., 2011), which does not require language nor working memory, but only a brief short-term memory (Damasio, 1998). It has a relationship with time processing in the sense that the perceptions and actions should be continually sensed by someone, and disruptions in this system (and therefore in the 'minimal self') are commonly seen in schizophrenic patients (Martin et al., 2014).

The 'reflexive self', on the other hand, is intentionality directed towards the act of consciousness itself, such as someone thinking about the act of perceiving something and being the object of awareness (self-referential). The distinction between self-related and self-referential processing (see also Northoff et al., 2006) can also be conceived as the line that demarcates or differentiates the human self from other animals.

Beyond the perception that something is happening to us, humans can also reflect upon the perception that something is happening to us (Mograbi et al., 2009). We are the object of our own attention, we are aware of our existence, we are aware of being aware. We judge thoughts and actions, and identify one's own abilities, attitudes and behaviors, recalling our own experience to assume the existence of comparable experience in others. Due to the capacity of mirror-recognition, chimpanzees and orangutans were claimed to have an extended self, for which a certain kind of subcortical networking was responsible and enough for this process (Northoff and Panksepp, 2008); however recalling from our own experience a sequence of specific events in time and place might be an uniquely human feature (Levine, 2004).

This higher sense of self-awareness is most likely related to and dependent of episodic memory (Fargeau et al., 2010), as every day we have the feeling of being the same person because we remember who we are now and were before. By the proper integration of ourselves in time, we know we are the same person today we were in the past and will be in the future, with a permanent conservation of ourselves. By remembering who we were yesterday, we infer who we are today, and who we will be tomorrow, just the same as we know how we feel today because we remember how we felt yesterday. The term 'autonoesis' (Tulving, 2002) refers to self-knowing and has been used to refer to this type of consciousness that allows us to be aware of subjective time in which events happened. Also called 'autobiographical self' by Damasio (Damasio, 1998), it describes the human capacity of representing ourselves mentally in time and becoming aware of this representation, linking personal experiences with self through consciously recalling. We consider the self partly as a function of time, being in continuous change, but remaining and feeling the same. Hence, self-awareness seems to be closely related to self-continuity, and self-continuity is inseparable from memory. In this context, personal

memories help establish personal identity (D'Argembeau et al., 2014) through the knowledge of ourselves, which in turn emerges from our life history and experiences. The self how we represent in our mind creates a sense of identity, which in turn, can be broken into self-knowledge (for example, knowledge of our own traits) and narratives (our life, experiences, personal history) (Addis and Tippett, 2004).

Clare et al (Clare et al., 2011) have proposed a model in which the self is composed by four different aspects: sensory registration, performance monitoring, evaluative judgment and meta-representation. Whereas the first one involves only simple internal representations ('core self'), the other three require capacities that are more complex. For instance, performance monitoring includes monitoring ongoing task performance and identifying errors; evaluative judgment refers to the individual's awareness of his abilities, symptoms or situation; and meta-representation refers to the ability to consider the perspective of others or other situation (Clare et al., 2011). Klein et al (Klein et al., 2003) have mentioned at least five isolable components for the unified self: episodic memories of one's own life, representations of one's own personality traits, facts about one's personal history (semantic personal knowledge), experience of personal agency and continuity through time, and the ability to reflect on one's own thoughts and experiences.

That said, now imagine a situation where you do not have access to your own life history and there is no continuity in it. All your brain has access is some broken pieces of information, sometimes antique, that may not make sense as a narrative personal history. You do not remember what you did yesterday or even how you felt. It is like reading a book with missing or repeated chapters, making it difficult to follow and understand. This is probably the situation that Alzheimer's disease (AD) patients face, that can affect their narrative self even in earlier phases. That is, AD patients lose self-

awareness in several aspects that involve complex cognitive abilities and allow them to know who they are. Reviewing studies in this field, our objective is thus to raise possible explanations – especially with the help of neuroimaging studies, to where such self-awareness deficit comes from. We shall begin with a phenomenological approach regarding AD phenotype, describing the aspects of the self that have been reported to be altered in these patients. We highlight the importance of self-continuity across time and then we bring neuroimaging findings to support and fit an existing model to AD.

Alzheimer's disease and impairments of the self

Different aspects of the self have been reported to be affected in many neurological or psychiatric diseases such as dementia; although there is no doubt that some aspects of the self might still be present in AD patients even with moderate impairment. Due to the occurrence of moments of clarity following periods of confusion, it can be hard for the family and caregivers to understand how much awareness the patients still present (Clare and Woods, 2005). Regarding the sensory registration level raised by Clare et al (Clare et al., 2011), for example, some studies have demonstrated that advanced patients who are still able to verbally communicate respond to discomfort, pain, noise or change of temperature (Clare et al., 2008). Also, AD patients express a range of affective signals and present and intact and functional emotion system even in the advanced stages (Magai et al., 1996). The awareness of existing is also preserved among AD patients (Gil et al., 2001).

Visual self-recognition in photographs and mirror were also objects of study in the AD field. Although mild patients still present preserved visual self-recognition in photographs (even though they have no memory of the photographic session) (Fazio and Mitchell, 2009), this scenario can change in more advanced phases. Some patients for

example, can misidentify their own reflection in the mirror in later stages. Breen et al (Breen et al., 2001) described two patients who were unable to recognize their own reflection and attempted to communicate with the reflected person. One patient believed that his own reflection was another person who was following him around, anywhere that there was a reflecting surface. While attempting to communicate with the reflected person on numerous occasions without success, he was somewhat perturbed that the person never replied. The second patient said that as the person never replied he could only assume that there was something wrong with himself.

But perhaps the most reported aspect of the self to be impaired in AD is the memory/cognitive impairment awareness, also called anosognosia. It refers to the impaired judgment of AD patients of their own cognitive deficit, behavior or daily activities and is assessed by the discrepant score between the subject's evaluation and the evaluation of someone close. Even in the earlier stages, AD patients present unawareness to recognize their cognitive impairment and medical condition (Clare et al., 2012; Zamboni et al., 2013), have the tendency to overestimate their ability to consider the perceptions (Degirmenci et al., 2013) and present impairment in judging the level of success or failure on daily tasks (Morris and Mograbi, 2013). Anosognosia symptoms also tend to worsen with disease severity (Gil et al., 2001).

Anosognosia is a higher-level process because includes judgments, attributions and reflection. Salmon et al (Salmon et al., 2005) interpreted it as an impairment to see oneself through a third person perspective and Agnew and Morris (Agnew and Morris, 1998) have related it to a failure to update the self-awareness system in relation to those of others. It can be related to memory in the sense that the subject should compare the current memory functioning with his past memory functioning (Clare et al., 2011); but Hannesdottir and Morris (Hannesdottir and Morris, 2007) have proposed

a neuropsychological model to distinguish anosognosia secondary to memory/executive dysfunction from primary anosognosia (which instead is caused by a break in the long-term and self-awareness system itself). We will come back to this issue later in the text. Although some studies (Maki et al., 2012) have used the term 'impaired self-awareness' to refer to anosognosia specifically – therefore, in a simplistic way, impaired self-awareness in AD goes beyond cognitive self-evaluation.

In clinical settings and among their families, for example, caregivers and close ones often report AD patients are no longer themselves and display behaviors from past self-identities. For example, a woman with severe dementia presented retrievable knowledge of her personality traits and those of her daughter, but the knowledge of her personality was from before the onset of the disease (Klein et al., 2003). The authors also report a deep change in the patient's personality as the disease progressed – although the patient was unaware of this transformation. In addition, AD patients are often said to be absent-minded and disconnected from the present (Globerman, 1995). They lose strength, quality and direction of identity and individuality (Addis and Tippett, 2004) and as the disease progresses, they also lose the ability to define themselves as they once did (Basting, 2003).

Regarding trait adjectives self-knowledge, in which participants are required to describe their own personality (e.g., "are you patient?") or the personality of a close friend (e.g., "is John patient?") results are interesting. One study found that AD patients presented less accurate self-representations than healthy older adults (both when making the judgments from their own perspective and from the perspective of their relatives) (Ruby et al., 2009), and another study found that demented patients had worse performance only when they were asked to judge themselves, but not when evaluating a close person (Zamboni et al., 2013). Studies involving this kind of methodology, in which

patients are asked to evaluate self adjectives either from their point of view or from someone else's, allow us to investigate the meta-representational level of awareness presented in AD patients. When participants are required to answers such questions, they should be able to self-transport to another point of view (projecting into this new perspective) and make judgments about themselves.

Meta-representation, however, is not restricted to the ability of seeing oneself from a third person perspective, specifically. Being able to watch oneself from another point in time is a process that also requires a new perspective view. For example in a functional MRI study (fMRI) (D'Argembeau et al., 2008), participants were instructed to reflect on their own traits and those of a close other, for both their present life period and a past life period. The authors found that thinking about the past self and thinking about the other person were associated with similar levels of activity in the same brain areas. Interestingly, the effect of temporal distance was symmetrical between the past and the future events (D'Argembeau et al., 2010). This process of recollecting past events, also called autobiographical memory (ABM), will be described apart in this text due to its importance in AD.

Autobiographical memory: the feeling of being continuous in time

Recollection of ABMs has been likened to mentally traveling back in time and re-experiencing one's past, a phenomena that requires a subject to travel (for instance, 'the self', Tulving, 2002). Time travelling, in this sense, means the ability to escape the influence of the current mental state or the ability to maintain different mental states simultaneously. It has also been called episodic future thinking (Atance, 2005), memory for the future (Ingvar, 1985), pre-experiencing (D'Argembeau and Van der Linden, 2004), and imagination (Decety and Grezes, 2006). Such process requires the involvement of a

high order cognitive ability named meta-representation (or self-projection (Buckner and Carroll, 2007), sense of subjective time (Tulving, 2002), self from the inside (Suddendorf and Corballis, 1997), in which the subject shifts from the perspective of the immediate present to alternative perspectives, such as the past or the imagined future.

The successful access to our life history creates in oneself the sense of unit and continuity, and is experienced as part of one's past. This high level of memory and consciousness - i.e. autobiographic recall, relies on the self-knowing awareness or autonoietic ability and occurs in an organized way. Pieces of information such as 'what', 'when' and 'where' combine and permit one to coherently access his past, ensuring the maintenance of one's identity in an environment that frequently changes. Self and ABM walk together and depend on each other: in providing autobiographical information about our own past, episodic memories may provide the basis for personal identity; also, one may also need an awareness of self in the present in order to be able to relate memory representations to experiences of one's self in the past (Suddendorf and Corballis, 1997). A failure of recollection of ABM, on the other way, might lead us to an insecure sense of identity (Markowitsch and Staniloiu, 2013).

The semantic aspect of the ABM, specifically, contains the knowledge and facts of our past, including knowledge of our identity, our personal characteristics, and the facts supporting awareness of personal past events, including awareness that an event has occurred to us. Semantic memory relies on factual general external information of the world, such as who is the president of our country. Personal semantic memory allows us to state the name and location of the school we attended, for example, or where we lived during childhood. The episodic aspect of the ABM, on the other hand, allows us to consciously re-experience past information, such as the feelings and emotions during events. Episodic ABM transports us to particular information that is spatially and

temporally located, empowering oneself with autonoietic consciousness (Wheeler et al., 1997). Episodic memory gives rise to the notion of mental time travel, because episodic memory means the ability to reconstruct particularities of specific events that have happened to the individual, which will be the substrate for future imagining. In other words, one needs autonoietic awareness and meta-representation ability to mental time travel, and mental time travelling for ABM recollection. The mental reconstruction of past events and construction of future may be responsible for the understanding of continuity between past and future, which allow one to understand that past and future are on the same mental dimension.

Interestingly, younger adults produce more details in ABM recall than do older adults and, as they get older, adults tend to recollect fewer details about happenings, locations, perceptions, and thoughts, but provide a more integrative approach to the interpretation of past experiences (Levine et al., 2002). This pattern of switching from strong episodic personal recollection to a more semantic and integrated way of recollecting things that naturally happens with ageing, is magnified in early stages of AD (even though semantic memory is also impaired in the early stages of this disease). This shift pattern, however, does not affect the sense of the self in healthy older adults, as opposed to what happens with AD patients (Martinelli et al., 2013a). Individuals with amnestic mild cognitive impairment (aMCI), who are at risk for developing AD, already present reduced specificity of ABM recall (Donix et al., 2010; Berna et al., 2012) mainly to recent life memories (Leyhe et al., 2009), and exhibit a compromised capacity to generate vivid, self-referential visual imagery and re-experience the original emotion of events (Irish et al., 2010). Also, although the semantic aspect of the ABM could still be relatively intact (Barnabe et al., 2012) or even increased (Murphy et al., 2008) in aMCI

individuals, the fact that the episodic aspect of ABM is altered in these patients is much more accepted (Tramoni et al., 2012).

In severe stages of the disease, when episodic and semantic memories are lost, the patients lose their expanded, autobiographical self, and seem to be confined to their "core self" or "primordial self", at the same time that they lose the ability to master temporal operations and to conceive time (objective time) as homogeneous and common to every phenomenon (Damasceno, 1996). In more advanced stages of the disease, when the dissociation between object and subject´s actions is about to be dissolved, what seems to remain of the "core self" is the ability to judge the time of his/her own actions (for example, as "longer" or "shorter") and the feelings and emotional reactions to situations (for example, of boring waiting, expectation, unsatisfied effort or failure) - similarly to what was observed by Piaget in children before one year age (Piaget, 1952).

In recent years, an increasing number of neuroimaging studies have suggested that remembering the past and imagining the future rely on common neural processes, involving similar networks and cognitive capacities (Okuda et al., 2003; Botzung et al., 2008; Conway, 2009). Thus, it is not surprising that amnesic patients are impaired at imagining new experiences, lacking spatial coherence and richness, and result fragmented imaginative constructions (Hassabis et al., 2007). aMCI subjects also produce fewer episodic and event-specific details for both past and future events, and have difficulty constructing scenarios that have never happened (Gamboz et al., 2010), and the same is true for AD patients (Addis et al., 2009). Interestingly, the greater the impairment of notion of time in AD patients, the more they behave as they used to do in the past (Harrison et al., 2005), suggesting a strong role of ABMs in the formation and updating of the self and identity.

These results lead us to assume that the sense of self-continuity shrinks in degrees in AD patients (which gets smaller and smaller thus covering shorter time durations/segments), and their time construction/projection abnormally shifts towards the past. A severely impaired episodic memory domain removes the sense of personal continuity of AD patients in their daily lives (Zakzanis and Leach, 2002), which might prevent them updating the self-knowledge store, leading the patients to express earlier and antique self-knowledge (Klein et al., 2003). All these findings suggest that AD patients lose the ability to time travel and, as they became unable to form new memories, they start losing the knowledge of who they are in the present and the ability to properly project themselves into alternative situations. By doing so, such patients may assume identities associated to the poorly accessible personal information, and gradually lose their narrative, autobiographical self as they lose their episodic memory. However, even in the most severe cases of amnesia, the subject keeps intact his/her experiential 'core self', self-consciousness (awareness of itself), self-givenness, and self-referentiality, differently from what occurs with the narrative self - which is highly dependent on the sense of continuity.

It is important, however, to distinguish mental time travelling from anticipatory behavior, as for example in conditioned reactions. Despite the evidence that the ability to imagine past and future events may not be uniquely human but may also be present in chimpanzees (Zentall, 2006), this imagination seems not going far beyond the context of the present. There is indeed evidence of episodic-like memory in animals, particularly when this ability is important for survival, regardless of their having autonoietic consciousness. Scrub jays, for example, can remember when and where they cached a variety of foods that differ in the rate at which they degrade; they make temporal generalizations about when perishable items should degrade (thus, showing elements of

semantic memory), without this performance being explained by familiarity or operant conditioned learning (Clayton et al., 2001a; Clayton et al., 2001b). Similarly, great apes seem to be confined to a present time that is limited by their current drive states, and apparently do not demonstrate the understanding of what food to catch for example, or even where, when and why to catch it (Suddendorf and Corballis, 2007). We can make a similar comparison in human ontogeny, in which the development of self-awareness is usually assessed in terms of the child's ability of mirror recognition: the recognition of one's own body does not reflect the recognition of one's own experience. However, we should be cautions in rejecting that great apes, dolphins, elephants, domestic dogs and even scrub jays could have some degree of autonoietic consciousness, since it is a private, first-person perspective, subjective experience, which cannot be reduced or equated to any neural (neuroimaging) signature. It is believed that episodic memories phylogenetically evolved from semantic memories, appearing long latter in the human evolution, while other animals present only a well-developed system for semantic memory (Tulving, 2002). Questions such as 'where have we come from?', 'what are we?', 'where are we going?' require time travelling and consequently, subjective self-awareness, a cognitive ability which emerges together with episodic memories (Atance, 2005). Due to the involvement of higher cognitive systems for consciously re-experiencing events, episodic recollection is not firmly established before the age of four (Levine, 2004).

Time travelling requires a self originated from the inside, an understanding of continuity and existence across time, an integration of past and future regardless the present and the external environmental needs. Evolutionarily speaking, internally generated processes are extremely adaptive because they allow the organism to address unresolved problems over long periods of time (Binder et al., 1999), and increases the

organism's chances of future survival by allowing the imagination of different scenarios beyond the organism's instinct. One function of episodic memories is to keep an adaptive record of recent goals, and they need to be specific enough to provide the appropriate information when assessed (Conway, 2009), and by consciously imagining different scenarios, the organism is able to consider whether a particular situation could be faced or avoided if encountered (Addis et al., 2007). Whereas simple episodic memory provides us information to guide decisions, mental time travel leads us towards more restrained choices, which in the long term are advantageous (Boyer, 2008). Time travelling is a uniquely human characteristic (Suddendorf and Busby, 2003) and phylogenetically might be merged with the enlargement of more complex societies, allowing social interactions that goes beyond cooperation and kinship. The emergence of meta-representation and consequently time travelling is associated with the prominent expansion of the frontal lobes in hominid evolution, rather than being exclusively dependent on medial temporal lobe structures (Wheeler et al., 1997).

The role of hippocampus in autobiographical recollection can be visualized in semantic dementia patients – which do not present hippocampal atrophy and recall recent personal incidents better than AD patients do (Hou et al., 2005; Irish et al., 2011). Amnestic patients, on the contrary, have deficits recalling recent past experiences and present the inability to conceive and imagine their personal future (Corkin, 2002). Given how closely imagined experiences match episodic memories, the absence of this function mediated by the hippocampus may also affect the ability to vividly re-experience the past. ABM deficits and autonoietic unawareness are among the major complaints of AD patients and could thus be related to hippocampal atrophy (Thomann et al., 2012), a well know feature of even preclinical AD patients. However, KC, a widely studied patient who suffered an accident that caused deep amnesia and changed his personality, was able to

relearn his trait self-knowledge (Tulving, 2002; Rosenbaum et al., 2004) and although he presented no autonoietic consciousness, his self-knowledge was represented abstractly (Tulving, 1993). These findings suggest that, although necessary for ABM recollection, a unified sense of self and sense of continuity go beyond personal memories, and a more complex system may be behind the process of self awareness.

Regarding the sense of continuity above described, Northoff (Northoff, 2013) proposed a model to complement the current bidimentional view of consciousness (composed by level and content) in which the spatiotemporal continuity ('form') represents a third dimension. In his models, the 'form' allows one to put together and organize the discrete points of time and space, providing a bridge to temporospatial gaps and as a result, one experiences a temporal continuum between the different contents. This third level or 'form' links different discrete points in time and space by cerebral low frequency fluctuations and functional connectivity (i.e. intrinsic brain activity). Thus we could suppose that damage in the hippocampus alone may not be enough to cause self-awareness problems in AD patients. In the next sections, we shall discuss in further details these assumptions.

The cortical midline regions

The self and its different components are not "located" in a single place in the brain, but may instead depend on distributed neural systems that include both cortical and subcortical structures (Northoff and Panksepp, 2008). Neuroimaging studies have shown that both the experience of past and the construction of future events activate a common network that are traditionally associated with diverse forms of self-projection processes such as time travelling (Addis et al., 2007; Botzung et al., 2008). This common network, which is an anatomical-functional unit of cortical midline structures (CMSs) (Northoff

and Bermpohl, 2004), is a fundamental component in generating a model of the self (Gallagher, 2013) since it is engaged in the processing of self-referential stimuli (Northoff et al., 2006; Genon et al., 2014; Huang et al., 2014).

According to the CMSs model of the self monitoring, the information of recent past experiences and imagination of the future is processed in medial temporal lobe region, including the hippocampus (Addis et al., 2007; Botzung et al., 2008), and then is represented in the prefrontal cortex, which is the entrance door to the CMSs. Once there, the self-related information is processed, decisions are made under conditions of uncertainty (when multiple possible answers are available) and, together with the memory system, a feeling of rightness is provided (Gilboa, 2004). The posterior midline regions - more specifically the posterior cingulate cortex (PCC), together with the medial prefrontal cortex (MPFC), have an important role in the integration of these stimuli (Northoff and Bermpohl, 2004; Northoff et al., 2006). The PCC has been related to the individual's own self-beliefs and is associated with a first-person perspective (Ochsner et al., 2005), but has also been linked to more social processes and monitoring of the environment (Qin and Northoff, 2011). The structural maturation between PCC and MPFC has been related to the development of self-related and social-cognitive functions that emerge during adolescence (Supekar et al., 2010). The posterior parietal regions, although not being part of the CMSs, works in conjunction with these regions, in the sense of acting as a convergence zone that binds episodic feature ensembles within the neocortex, linking episodic memories to each other (Shimamura, 2011). These regions are responsible for the access to the full set of details associated with a particular experienced event, and patients with lesions in parietal areas have reduced sense of re-experiencing a past event (Berryhill, 2012). The MPFC is activated during the processing of an individual's own self-beliefs and the individual's perception of how others view one

(Ochsner et al., 2005). The function of the ventral MPFC (vMPFC) specifically, might be to represent personal value or significance to self-related contents (and here, self-related contents are not restricted to external environment, but also internally generated contents) (D'Argembeau, 2013). The CMSs are, thus, responsible for linking internal and external stimuli and integrate these stimuli in the emotional and autobiographical context of one's own person.

The MPFC has also been associated with making judgments and semantic self knowledge. For example, Kelley et al (Kelley et al., 2002) found that judgments about the self lead to greater activations in this regions than judgments about others, and Denny et al (Denny et al., 2012) reported that the ventral portion specifically, might be more related to self judgments, whereas the dorsal portion might be responsible for making judgments about others. An increased activation in the MPFC when reflecting about one's own traits (compared to the traits of others) has also been observed in several subsequent studies (Johnson et al., 2002; Gutchess et al., 2007; Jenkins et al., 2008). Regarding trait adjectives self-knowledge, in which participants are required to describe their own personality (e.g., "are you patient?") or the personality of a close friend (e.g., "is John patient?") results are interesting in AD. One study found (Ruby et al., 2009) that not only did AD patients present less accurate self-representations than healthy older adults (both when making the judgments from their own perspective and from the perspective of their relatives), but also activated different areas when compared to control groups. For example, AD patients predominantly activated the intraparietal sulci for the self judgment, a region associated with familiarity judgment (indicating that patients use more than recollection when assessing their personality). Also, when asked to think about the other perspective, patients recruited prefrontal regions (whereas elderly controls normally activated visual associative areas), suggesting they use more reasoning processes than

visual imagery of ABM to project themselves as a third person. Another study found that demented patients had worse performance only when they were asked to judge themselves, but not when asked to evaluate a close person (Zamboni et al., 2013). In addition, patients failed to recruit the MPFC when asked to make self judgments, but not when evaluating others.

The CMSs unit is also remarkably similar to the network involved in the retrieval of ABMs (Cabeza et al., 2004; Gilboa, 2004; Svoboda et al., 2006; Martinelli et al., 2013b) and thinking of future events (Okuda et al., 2003; Botzung et al., 2008). Damage in the MPFC, for example, leads to an inability from the individual to build events in an appropriate temporal sequence (Milner et al., 1985) - which is relevant to the brain basis of self-projection, and an inefficient planning of actions that require foresight (Shallice, 1982; Unterrainer and Owen, 2006). Issues and events of personal life, i.e. episodic ABM, activate more regions such as the vMPFC and posterior cingulate cortex (PCC) than the retrieval of general semantic knowledge (Andrews-Hanna et al., 2010a). D'Argembeau et al (D'Argembeau et al., 2014) reported that when participants were asked to engage in more concrete contents, attempting to actually re-experience the events (i.e. episodic ABM retrieval), CMSs and temporal regions were activated, whereas more lateralized regions were recruited when participants were required to reflect on the personal importance of their memories (i.e. ABM reasoning). The vMPFC activation, in turn, was a differential between the participants who had a higher disposition to engage in self-reflection when they thought about the significance and meaning of the memories.

Although both semantic and episodic self-knowledge are usually interpreted separately, information and details about specific events can help build the beliefs about ourselves and even influence self judgments (D'Argembeau and Salmon, 2012). For example, when asking to judge about personal characteristics (impatience, for instance),

one might remember some occasions when he/she was in fact impatient. This recalling of ABMs thus, somehow 'feeds' and nourish personal self knowledge, and both processes engage common self-referential mechanisms processed by the CMSs (D'Argembeau and Salmon, 2012). In line with this view, a meta-analysis of functional neuroimaging studies has revealed that the CMSs were involved in processing self-referential information across multiple cognitive domains and sensory modalities (e.g., the recognition of one's own body and actions, self-face recognition and the representation of one's own traits) (Northoff et al., 2006). The CMSs unit, therefore, process and integrate mechanisms specialized for creating and refreshing the personal database which, in turn, needs to be updated often for the self renovation too (Klein et al., 2003).

Anosognosia, a common problem observed in AD patients, has also been related to the activation of the CMSs. Ries et al (Ries et al., 2012) for example, found that the recruitment of these regions in MCI subjects was diminished during self-appraisal activities and this activation correlated with the cognitive self-awareness deficit in the subjects. However, we should carefully interpret anosognosia results, since discrepancies might be found due to different imaging techniques, different severities of cognitive and functional impairment, the possible use of medication and concurrent psychiatric disorders and divergent strategies used to diagnose anosognosia (Starkstein, 2014).

The above-mentioned network that may support self-reflection about internal thoughts and feelings is thought to mediate a "default mode of brain function", or DMN. The similarity between the CMSs activation in self-related cognition and the DMN regions most probably reflect a shared neural system for default-mode self-reference (Whitfield-Gabrieli et al., 2011) which has been confirmed with neuroimaging experiments (Andrews-Hanna et al., 2010b; Qin and Northoff, 2011; Araujo et al., 2013). In addition to that, the same regions are involved during mind-wandering (Mason et al.,

2007), lapses of attention in externally oriented tasks (Weissman et al., 2006), and imagining future events (Schacter et al., 2012), which are considered to be self-oriented processes, or forms of spontaneous cognition.

During the last decades, several studies in the AD field have consistently found alterations in the DMN and its regions. And this is not coincidently. Physiopathologically speaking, AD is characterized mainly by the deposition of extracellular and insoluble β-amyloid plaques and intracellular neurofibrillary tangles composed of hyperphosphorilated tau protein (Selkoe, 2001). This deposition occurs mainly in the DMN regions: temporal areas are highly affected by the harmful effects of phospho-tau (Thal et al., 2013), whereas the PCC is among the first regions to show β-amyloid burden (Buckner et al., 2005). Such misfolded proteins also spread to synaptically connected nonadjacent areas in a time-dependant and predictable manner. The spreading of pathology across distant regions makes the neuronal damage go beyond regional alterations and affects parts of the brain that are somehow connected – AD is, due to this reason, commonly called "disconnection syndrome" (Cronin-Golomb, 2010; Vallet et al., 2013). Consequently, specific systems are chronologically targeted and clinical symptoms outreach the primary phenotype (i.e. memory impairment). White matter tracts that connect DMN hubs (such as the cingulum - which links the medial temporal lobe with the PCC and the MPFC, and the corpus callosum) are widely affected (Pievani et al., 2010) and correlate with cognitive performance in AD patients too (Weiler et al., 2014a). Besides being susceptible to amyloid deposition, the PCC and the precuneus also present metabolic alterations of glucose consumption in AD patients (Minoshima et al., 1997; Kuntzelmann et al., 2013).

In addition, demented patients present less activation in temporal areas (Machulda et al., 2003; Sperling et al., 2003; Dickerson et al., 2005) and deactivation in

the DMN (Pihlajamaki and Sperling, 2009; Rami et al., 2012) during cognitive tasks than cognitively normal elderly. During resting-state, DMN regions show diminished connectivity (Agosta et al., 2012), which correlates with a worse performance in episodic memory tests in AD patients (Weiler et al., 2014b) and also in aMCI subjects (Sorg et al., 2007), as well as decreased amplitude of low frequency fluctuations (Weiler et al., 2014b). The higher the deposition of β-amyloid, the greatest the alteration in DMN connectivity, even in cognitively normal elderly who present high burden of β-amyloid – and it is assumed that the effects of altered proteins in AD start years or even decades before the onset of the disease (Sheline et al., 2010; Kikuchi et al., 2011). The connectivity of the DMN, in turn, follows AD severity and continues to decline as the disease progresses (Damoiseaux et al., 2012). Moreover, the miscommunication among the DMN nodes do occur regardless cortical damage (Gili et al., 2011; Petrella et al., 2011), suggesting that functional deficits within the network may precede structural alterations. Graph theoretical analysis also bring a loss of both structural and functional connectivity in DMN regions (Supekar et al., 2008; Tijms et al., 2013), specially an increased average characteristic path length (as a result of loss of connectivity among its areas) (Tijms et al., 2013).

The resting-state

The CMSs, which are the core regions of the DMN, show a particularly interesting neuronal activity when any individual is left alone and undisturbed. These moments were initially called ‘resting-state’ or ‘baseline activity’ because it was said that the brain was resting and thus not engaged at any cognitive task. Also, neuroimaging studies have shown that the MPFC is among the regions having the highest baseline metabolic activities at rest (Raichle et al., 2001), and when external attention is required

or the subject engages in cognitive tasks the DMN activity is attenuated (task-negative network) (Gusnard and Raichle, 2001). At the baseline activity, the brain is in a highly organized spontaneous pattern that consumes most of the brain's energy (Raichle and Mintun, 2006), contrasting to task related activation which accounts for <5% of the total blood oxygen- level-dependent (BOLD) signal and requires only a small percentage of neurons (Biswal et al., 1995). For this reason, it has been said that the rest activity states contain complex, structured brain activity patterns that may support important brain functions. If so, what is the purpose of such intrinsic and organized activity? Although it is tempting to assume that it reflects simply housekeeping functions such as neuronal repair, resting-state functions may go beyond them.

During these passive states, the subjects engage in spontaneous cognition and experience mainly monitoring of external environment and body state (Gusnard and Raichle, 2001), stimulus-independent thought (Buckner et al., 2008), problem-solving (Binder et al., 1999), retrieval and consolidation of past experiences, and planning and preparing for the future (Andreasen et al., 1995; Buckner and Vincent, 2007). It is also interesting to note the human propensity for engaging in these inner thoughts and easily shift attention away from primary tasks. Such easily achievable mental states are characterized by a substantial amount of self-referential thought, making us think that when left undisturbed, one is processing self-related information. Indeed, studies have shown that the CMSs mediate internally-oriented self-relatedness (Schneider et al., 2008) and these regions overlap with the resting-state (Qin and Northoff, 2011).

But what is the nature of the processing of self-related information during rest? Very interesting studies showing the interaction between resting periods and stimuli can shed some light in the present discussion. For instance, adults that were submitted to intense visual training had increased functional connectivity at rest between networks

engaged by the task, which correlated with the degree of perceptual learning (Lewis et al., 2009). Another study investigated the effects of motor learning on resting state activity and reported that the functional connectivity in the motor system increased after the subjects learned a novel task (Albert et al., 2009). Two other studies involving rats trained to run in a maze provided clues about how resting state activity may relate to learning episodes. While the rats were resting after the task, the cells recruited by the task spontaneously fired with the same sequential patterns than when actually performing the task, but much faster and in reverse order (Foster and Wilson, 2006; Diba and Buzsaki, 2007). Although the resting state activity may be responsible for maintaining the systems alertness and prepared for rapid responses when required, the intrinsic neuronal activity and learning-related functional connectivity changes during rest suggest that some form of consolidation may take place during these periods (Vincent, 2009). If the connectivity of the above-mentioned cognitive networks supports the consolidation of previous experience that engaged the network, why not assume the same for other resting-state circuits such as the DMN?

Similarly to the theories that have been raised to explain the DMN function, the resting-state activity has also been related to monitoring the external world. However, the strong association between the rest and self-referential processing makes us think about an off-line consolidation of memories (Miall and Robertson, 2006), leading to a more introspective function of the rest. The MPFC, for instance, exhibits a higher activity when a subject has to refer to internal states compared to external states, and even greater when the subject is at rest (Wicker et al., 2003). Thinking about one's personality traits (D'Argembeau et al., 2005) and the retrieval of episodic ABMs (Andreasen et al., 1995) were associated with common activation in the vMPFC during resting states in positron emission tomography studies. Also, the overlap between the self and resting state found

in a meta-analysis study suggests that self-specificity seems to be encoded in the resting states' neural activity (Northoff, 2012) - i.e., the Default Mode Network processing. For such reasons, the resting state has been considered the ultimate state of inspection of the self, enabling one to represent knowledge pertaining to himself and helping him to maintain a stable self-concept overtime (D'Argembeau et al., 2005).

The formation and updating of the self, similarly to a novel task learned, consists of the linkage and interaction between the mind's intrinsic features and the environment's external stimuli. It cannot be reduced to the internal origin only; neither can be defined with purely external origin (Qin and Northoff, 2011). The resting state is somehow needed to integrate the external stimuli with the inner information, and this interaction is necessary to generate the stimulus-specific activities (Northoff et al., 2010). It is known that the DMN is activated during rest, periods characterized by high self-related processing. It might be that, during rest, the DMN consolidates self-related information through mental activities like time travelling, planning, personal problem solving, self judgments and so on. It might be that our personal experiences – the external stimuli, are linked together and processed during resting periods (Qin and Northoff, 2011) by the DMN, and without them we would not be able to properly piece together our ABMs, compose and understand our life history. It might be that resting periods are necessary for personal events processing, and a healthy DMN is needed for the formation and updating of the self concept.

Diseases such as schizophrenia and depression, for example, affect the individual concept of self, which has been related to alterations in spatiotemporal continuity. While schizophrenic patients can be characterized by spatiotemporal disruption and fragmentation, depressed patients present spatiotemporal dysbalance (Northoff, 2014a). As above exemplified, AD patients also present several alterations in

many aspects of the self, tending to freeze it in time and originating a ‘petrified’ self (Mograbi et al., 2009). Although it is tempting to say that AD patients are not able to update their self-knowledge probably because of their widely known episodic memory deficit, it might be that an inefficient resting state functioning causes those alterations, such as it has been postulated for schizophrenia and depression (Northoff, 2014a).

We brought earlier many findings regarding CMSs and more specifically DMN alterations in functional connectivity, metabolism and amplitude of low frequency fluctuations in AD patients. It has been postulated that the linkage between discrete points in physical time (and thus making one experience a temporal continuum between the different contents, i.e. the ‘form’ of consciousness) is made by low frequency fluctuations and functional connectivity (Northoff, 2013; Northoff, 2014b). Abnormalities in the resting state intrinsic organization can lead to alterations in the way the brain process this different and discrete points in physical time, making one no longer experience temporal continuity in their consciousness (Northoff, 2014a). For AD patients, specifically, it is like their brains are always in a ‘disorganized’ resting-state, lacking a proper conversation between the DMN areas. If the role of the DMN during rest is to properly make self-referential processing, miscommunications between the DMN regions might cause the self-related problems faced by AD patients, because there is no longer updating of the old/internal by the new/external information. The role of hippocampal and other medial temporal lobe structures in episodic memory is undeniable, but the integration of that information into a coherent picture, which is necessary for the updating of the self-referential database, seems more like a network function. The new/external stimuli may encounter an already altered temporospatial continuity when interacting with the brain’s intrinsic activity (Northoff, 2014a). Thus, AD patients lose the ability to update their self knowledge, failing to integrate the self into a coherent and meaningful picture.

CONCLUSIONS

The human sense of self comprises multiple facets or levels, ranging from the consciousness of oneself as an immediate subject of experience to the construction of oneself as a distinct entity with a personal history. AD patients present alterations in many aspects of the self, which include the higher-level self-awareness. Since self-awareness seems to be closely related to self-continuity, and self-continuity is inseparable from memory, it is tempting to say that memory problems could be responsible for the alterations in the feeling of being continuous in time. However, we here assume that self-related alterations presented by AD patients go beyond episodic memory recall made by hippocampus, but comprehend network functions. Feeling of being continuous in time comes from CMSs/DMN processing during resting state periods and, without the correct processing and communication between its regions, there is no proper ABM recall, feeling of self-continuity and narrative self. It is the resting state activity (especially DMN/CMSs processing) that constructs the continuity of time that underlies any subsequent cognitive function including ABM retrieval. Thus, by constructing and feeling temporal continuity (stream of consciousness) we can have self-continuity and ABM recall. The construction of the continuity of time is essential for ABM retrieval, which, in turn, is a precondition for the updating of the self (**Fig. 1**).

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The authors report no conflict of interest.

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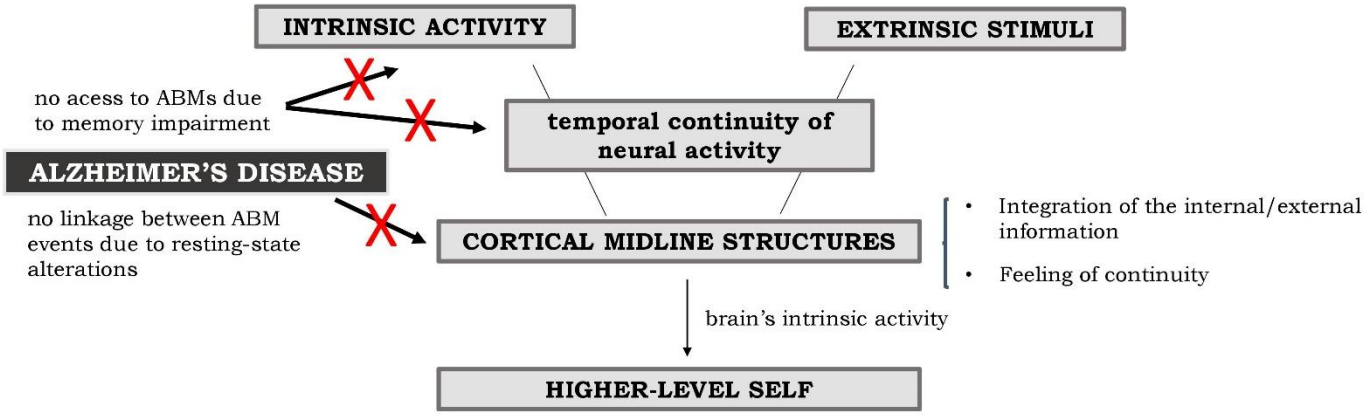

FIGURE LEGEND

Fig 1: The updating of the old/internal by the new/external information is processed by the cortical midline structures (CMSs) during resting periods (brain's intrinsic activity). Resting periods are necessary for personal events processing, and a healthy CMSs unit (i.e., Default Mode Network processing -DMN) is needed for the formation and updating of the self. AD patients besides having no longer access to the autobiographical memories (ABMs) due to memory impairment, present alterations in the CMSs/DMN low frequency fluctuations and functional connectivity (i.e., the brain's intrinsic activity). Thus, they no longer link the different events of the ABM events and no longer experience a temporal continuum, shifting towards the past and presenting self-awareness alterations.

Outros – Capítulo 8

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CNS Neuroscience & Therapeutics

SPECIAL ISSUE ARTICLE

CNS Neuroscience & Therapeutics

Propagation of Pathology through Brain Networks in Neurodegenerative Diseases: From Molecules to Clinical Phenotypes

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SUMMARY

The cellular mechanisms underlying the stereotypical progression of pathology in neurodegenerative diseases are incompletely understood, but increasing evidence indicates that misfolded protein aggregates can spread by a self-perpetuating neuron-to-neuron transmission. Novel neuroimaging techniques can help elucidating how these disorders spread across brain networks. Recent knowledge from structural and functional connectivity studies suggests that the relation between neurodegenerative diseases and distinct brain networks is likely to be a strict consequence of diffuse network dynamics. Diffusion tensor magnetic resonance imaging also showed that measurement of white matter tract involvement can be a valid surrogate to assess the *in vivo* spreading of pathological proteins in these conditions. This review will introduce briefly the main molecular and pathological substrates of the most frequent neurodegenerative diseases and provide a comprehensive overview of neuroimaging findings that support the "network-based neurodegeneration" hypothesis in these disorders. Characterizing network breakdown in neurodegenerative diseases will help anticipate and perhaps prevent the devastating impact of these conditions.

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Introduction

Neurodegenerative diseases have an enormous diversity in clinical phenotypes, affecting distinct cerebral functions. In recent years, however, intense research has been made in the field, arising the knowledge that they also share some common features. One of these commonalities is the accumulation of disease-specific proteins into insoluble aggregates [1,2], such as amyloid β (Aβ) in plaques in Alzheimer disease (AD), tau in neurofibrillary tangles (NFTs) in AD and many cases of frontotemporal lobar degeneration (FTLD), TAR DNA-binding protein 43 (TDP-43) aggregates in amyotrophic lateral sclerosis (ALS) and cases of FTLD, and α-synuclein (α-syn) in Lewy bodies (LB) in Parkinson disease (PD) and Dementia with Lewy bodies (Figure 1). This evidence has allowed the diseases to be recategorized in proteinopathies based on their molecular traits. Second, pathological changes in various neurodegenerative diseases progress with time in a stepwise characteristic anatomical pattern. Neuropathological studies have shown that NFTs in AD [3], LB in PD [4], and, more recently, TDP-43 aggregates in ALS [5] and the behavioral variant of frontotemporal dementia (bvFTD) [6] initiate very early in the disease in a circumscribed area of the brain and then progress in a topographically predicted manner through anatomical connections (Figure 1). Until recently, the causative mechanisms for this networked spread were thought to be passive, including secondary Wallerian degeneration, disconnection, loss of signaling, axonal reaction, and postsynaptic dendrite retraction [1,2]. The latest evidence, however, favors the hypothesis that the stereotypical and topographical patterns of pathological progression in the central nervous system (CNS) of patients with neurodegenerative diseases may be explained by a "prion-like" transsynaptic or transneuronal spreading of misfolded proteins between different brain regions over years [1,2]. Understanding how and where pathological protein propagation is initiated and the characterization of the major factors playing a role in the modulation of intracerebral spreading will lead to the identification of new

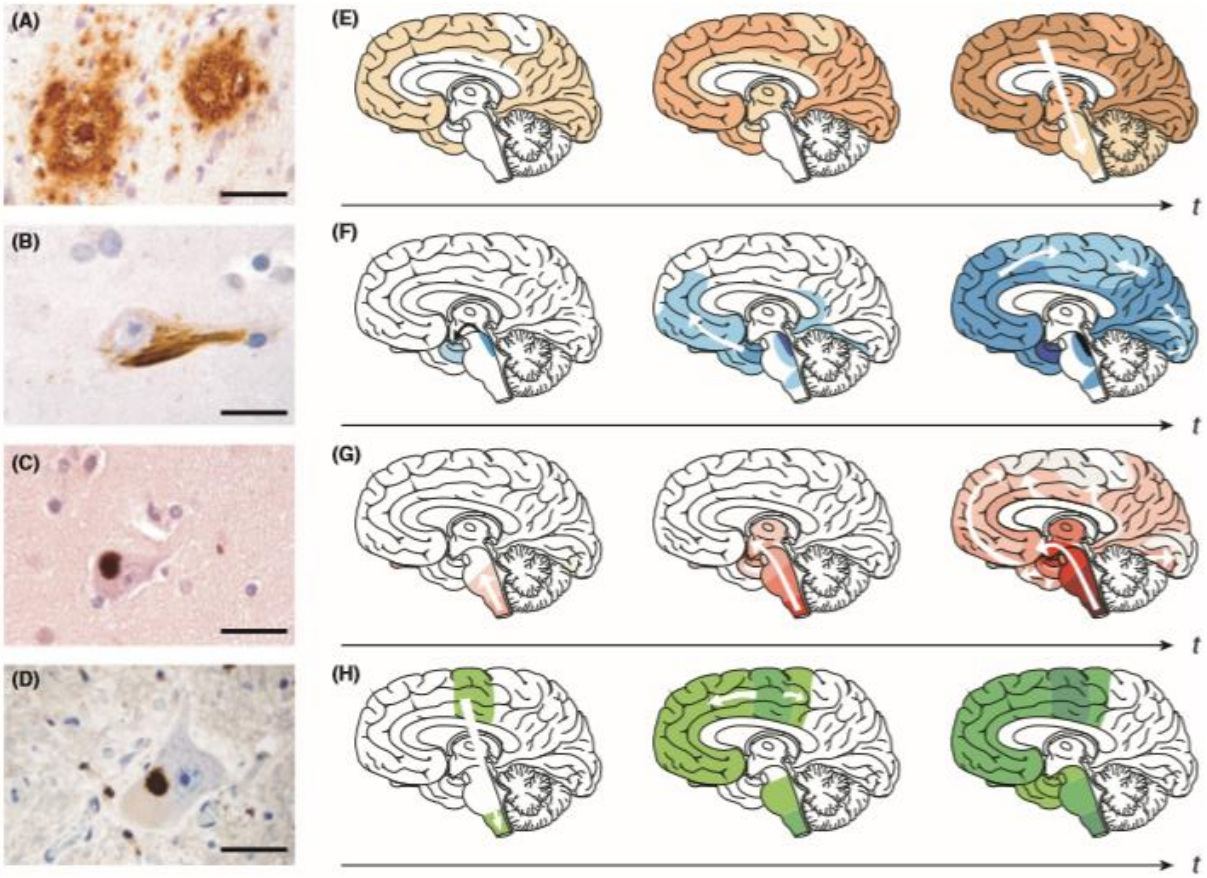

Figure 1 Protein aggregates show "prion-like" self-propagation and spreading in experimental settings, consistent with the progressive appearance of the lesions in the brain of patients with neurodegenerative diseases. (**A**) Aβ deposits in the neocortex of a patient with Alzheimer disease (AD). (**B**) Tau inclusion as a neurofibrillary tangle in a neocortical neuron of a patient with AD. (**C**) α-Synuclein inclusion (Lewy body) in a neocortical neuron from a patient with Parkinson disease (PD)/Lewy body dementia. (**D**) TDP-43 inclusion in a motor neuron of the spinal cord from a patient with amyotrophic lateral sclerosis (ALS). Scale bars are 50 μm in a and 20 μm in **B**–**D**. (**E**–**H**) Characteristic progression of specific proteinaceous lesions in neurodegenerative diseases over time (t, black arrows), inferred from postmortem analyses of brains. Aβ deposits and tau inclusions in brains of patients with AD (**E** and **F**), α-synuclein inclusions in brains of patients with PD (**G**), and TDP-43 inclusions in brains of patients with ALS (**H**). Three stages are shown for each disease, with white arrows indicating the putative spread of the lesions. Reproduced with permission from [2].

therapeutic targets aiming at slowing or stopping the disease progression.

In parallel to the molecular and pathological advances, the idea that the pathological substrates of neurodegenerative diseases spread along discrete brain networks has also been increasingly strengthened by neuroimaging studies [7]. It has been observed, indeed, that neurodegenerative diseases spatially affect patterns that reflect the healthy brain's network architecture [8]. In this review, we will introduce briefly the main molecular and pathological substrates of the most frequent neurodegenerative diseases. Then, we will provide a comprehensive overview of neuroimaging findings that support the "network-based neurodegeneration" hypothesis in patients with AD, bvFTD, ALS, and PD, bringing studies that range from the large-scale brain networks alterations to the microscopic abnormalities of structural pathways.

Clinical Phenotypes, Molecules, and Pathology of Neurodegenerative Diseases

Alzheimer Disease

Alzheimer disease is the most common form of dementia. Typically, AD is characterized by an insidious onset of cognitive decline, starting with deficits in episodic memory. As the disease progresses, other deficits such as aphasia, apraxia, agnosia, visuospatial difficulties, and executive dysfunction arise gradually [9]. The patient becomes increasingly dependent on others. Psychiatric and behavioral problems such as mood disorders, psychosis, agitation, and sleep disorders occur more frequently in the advanced phase of the disease. The term mild cognitive impairment (MCI) identifies those individuals who have subjective

memory and/or cognitive symptoms accompanied by objective evidence of isolated memory and/or other cognitive impairment and whose activities of daily living are considered to be generally normal [10]. Progression to clinically diagnosable dementia occurs at a higher rate from MCI than from normal (typically 10–15% per year—compared to rates of ~1% with normal aging), but is clearly not the invariable clinical outcome at follow-up [10].

Besides the typical neuropsychological profile of AD presenting with early memory deficits, there is evidence from clinicopathological studies that patients with AD may present with different cognitive profiles. Atypical presentations are more often seen in patients with early-onset AD (EOAD) (arbitrarily defined as before the age of 65). EOAD is often characterized by atypical manifestations with greater impairment in attention, executive, language, and visuospatial functions at the time of presentation. Furthermore, AD can present as relatively focal clinical syndromes, more frequently associated with early age-of-onset, that is, as posterior cortical atrophy (PCA) and logopenic variant (lv) of primary progressive aphasia (PPA) [11]. PCA presents with visual and visuospatial impairment with less prominent memory loss [12,13]. Over time, patients with PCA can develop visual agnosia, topographical difficulty, optic ataxia, simultanagnosia, ocular apraxia (Balint syndrome), alexia, acalculia, right–left confusion, and agraphia (Gerstmann syndrome), and later a more generalized dementia. Patients with lvPPA present with language deficits, characterized by slow rate of speech, with long word-finding pauses [14]. Grammar and articulation are usually preserved in lvPPA, although phonological paraphasias could be present. Repetition and comprehension are impaired for sentences but preserved for single words, and naming is moderately affected [14].

Two abnormal protein aggregates characterize AD pathology: neuritic plaques and NFTs [15]. Neuritic plaques are extracellular deposits and consist of a dense central core of Aβ fibrils with inflammatory cells and dystrophic neurites in its periphery. Aβ peptide is a normal proteolytic product of the Aβ precursor protein (APP) [16]. Due to the ability of the protease γ-secretase to cleave APP at multiple sites, Aβ peptides are 39–43 amino acid residues in length, but $A\beta_{40}$ and $A\beta_{42}$ are the predominant species *in vivo*. In contrast, plaques in AD are composed primarily of $A\beta_{42}$ and $A\beta_{43}$, which are more hydrophobic and aggregation-prone than the slightly shorter and more polar (but very abundant) $A\beta_{40}$. The second major proteinopathy in AD is aggregated tau, which consists of intraneuronal polymers primarily composed of hyperphosphorylated tau in the form of NFTs [15]. Tau is a natively unfolded cytoplasmic protein that normally helps microtubule stabilization [17]. If hyperphosphorylated, tau becomes prone to aggregation. In AD, the pattern of tau pathology is highly regular, whereas Aβ plaque pathology is much more varied. NFTs follow a stereotypic topographical progression scheme as described by Braak and Braak [3], first appearing in the entorhinal cortex and closely related areas, then progressing to the hippocampus, to paralimbic and adjacent medial-basal temporal cortex, to association cortex, and last to primary sensorimotor and visual cortical areas.

The initiating event in the molecular cascade that eventually leads to clinical and pathological AD has been controversial for decades. The amyloid cascade hypothesis, which posits that Aβ production and aggregation in the brain are the prime pathogenic drivers, leading to tau hyperphosphorylation and other histological and clinical features of AD, has dominated research for the past 20 years [18]. The amyloid cascade hypothesis was reinforced by the identification of gene defects in *APP*, *PSEN1*, and *PSEN2* in patients with an early-onset, inherited form of the disease [19]. The APP gene on chromosome 21 encodes the APP, from which Aβ is liberated after stepwise, amyloidogenic, proteolytic processing. The genes *PSEN1* and *PSEN2* encode presenilin 1 and presenilin 2, which are part of the γ-secretase complex, the enzyme that carries out the second cleavage in APP processing. An alternative position is that tau hyperphosphorylation and Aβ accumulation are independent interacting pathophysiological processes [20–22]. According to this second hypothesis, it is tau-related neurodegeneration that is ultimately responsible for clinical symptoms [23].

Frontotemporal Lobar Degeneration

Frontotemporal lobar degeneration is the umbrella term encompassing a group of progressive proteinopathies, which are heterogeneous with regard to etiology and neuropathology, but share atrophy of the frontal and/or temporal cortex as a morphological feature and the deposition of abnormal, ubiquitinated protein inclusions in the cytoplasm and nucleus of neuronal and glial cells as major pathological constituent [24]. FTLD includes three clinical syndromes and three major underlying neuropathological subtypes. The clinical syndromes, which are distinguished by the early and predominant symptoms, are as follows: a bvFTD; a language disorder (nonfluent and semantic PPA variants); and a motor disorder such as ALS, corticobasal syndrome, and progressive supranuclear palsy (PSP) syndrome [25]. This review focused on evidence for the "network-based neurodegeneration" hypothesis in bvFTD and ALS. bvFTD is characterized by a prominent change in personality and social behavior, with apathy and/or disinhibition, emotional blunting, stereotyped or ritualized behaviors, loss of empathy, alterations in appetite and food preference with limited or no insight [26]. ALS, the most common form of motor neuron disease, is a relatively rare progressive degenerative condition affecting the lower motor neurons within the spinal cord and the brainstem, accompanied by degeneration of the upper motor neurons in the motor cortex [27]. Up to 50% of patients with ALS have also cognitive and/or behavioral changes, ranging from an overt FTD to mild executive and/or nonexecutive cognitive impairment and behavioral deficits [28]. The neuropathological subtypes are characterized by an abnormal accumulation of proteins [29]: microtubule-associated protein tau (MAPT), TDP-43, and fused in sarcoma protein (FUS). FTLD-tau, FTLD-TDP, and FTLD-FUS represent 45%, 50%, and 5% of all FTLD cases, respectively, at postmortem examination.

Frontotemporal lobar degeneration-tau cases include those with the neuropathology of Pick disease, PSP, corticobasal degeneration (CBD), and cases of familial FTLD caused by mutations in the MAPT gene. FTLD-tau subtypes are characterized by specific inclusions: Pick bodies in Pick disease, tufted astrocytes and numerous NFTs in subcortical nuclei in PSP, and astrocytic plaques and abundant thread pathology in CBD [29]. In addition, the biochemical form of tau that accumulates in the inclusions varies among the different subtypes, with Pick bodies composed primarily of tau isoforms with three microtubule-binding domains

(3-repeat), while the inclusions of PSP and CBD contain 4-repeat tau [29].

In 2006, the majority of cases with tau-negative inclusions that stained positive for ubiquitin in FTLD were found to contain TDP-43 protein, as did the majority of sporadic and familial ALS cases [30]. TDP-43 is a highly conserved and widely expressed RNA-binding protein that is a member of the heterogeneous nuclear ribonucleoprotein family of proteins [31]. It is predominantly found in the nucleus, but shuttles between there and the cytoplasm, where it is present only at low levels. Pathological modifications of TDP-43 in the disease state include a redistribution from the nucleus to the cytoplasm in cells with inclusions, hyperphosphorylation, ubiquitination, and N-terminal truncation [31]. Dominantly inherited genetic mutations within the gene that encodes TDP-43 (TAR DNA-binding protein, *TARDBP*) are linked with ALS and FTLD-TDP phenotypes [24]. Different patterns of FTLD-TDP are now recognized, based on the cortical distribution and relative abundance of cytoplasmic inclusions compared to neurites, with each having fairly specific clinical and genetic correlations [29].

Most of the remaining tau-/TDP-negative FTLD subtypes are characterized by cytoplasmic inclusions that are immunoreactive for FUS [32]. FUS is a 526 amino acid protein identified as a fusion oncogene causing human myxoid liposarcomas. When in the nucleus, FUS is thought to be involved in regulation of transcription and pre-mRNA splicing. Cytoplasmic FUS in neurons appears to have a role in mRNA transport, where it can potentially facilitate local protein synthesis at synapse.

Recent pathological studies based upon the distribution patterns of phosphorylated TDP-43 indicate that the disease progression in ALS and bvFTD cases with FTLD-TDP pathology progresses in a sequential regional pattern possibly through axonal pathways [5,6]. ALS and FTLD-TDP bvFTD are characterized by four neuropathological stages. In ALS [5], initial lesions (stage 1) develop in the frontal and sensorimotor cortex, brainstem motor nuclei, and in spinal cord α-motor neurons, with beginning involvement of the prefrontal cortex, brainstem reticular formation, precerebellar nuclei, and red nucleus in stage 2; in stage 3, pathology progresses in the prefrontal and postcentral cortices, and striatum, followed by changes in anteromedial portions of the temporal lobe, including the hippocampal formation, during stage 4. FTLD-TDP bvFTD cases with the lowest burden of pathology (pattern 1) are characterized by widespread phosphorylated TDP-43 lesions in the orbitofrontal cortex and amygdala [6]. With increasing burden of pathology (bvFTD pattern 2), TDP-43 lesions emerged in the middle frontal and anterior cingulate gyrus as well as in anteromedial temporal lobe areas, the superior and medial temporal gyri, striatum, red nucleus, thalamus, and precerebellar nuclei. More advanced bvFTD cases show a third pattern (3) with involvement of the motor cortex, bulbar somatomotor neurons, and the spinal cord anterior horn, whereas cases with the highest burden of pathology (pattern 4) are characterized by TDP-43 lesions in the visual cortex.

Parkinson Disease

Parkinson disease, the most common neurodegenerative movement disorder, is characterized clinically by four cardinal motor symptoms: rigidity, tremor, bradykinesia, and postural instability [33]. Symptoms develop slowly and gradually progress over years. Superimposed on the classic motor symptoms, autonomic and sensory dysfunction, sleep disturbances, cognitive impairments and dementia are also common features in PD [34,35].

The pathological hallmark of PD is the presence of intraneuronal proteinaceous intracytoplasmic inclusions called LB. One of the main protein components of the LB is α-syn [36]. α-syn is a 14-kDa natively unfolded protein, consisting of 140 amino acids, that binds lipids through its amino-terminal repeat region. It is localized in the presynaptic terminals, nucleus, cytosol, and in some cellular membranes, such as the mitochondria-associated membrane in the endoplasmic reticulum. Although the exact function of α-syn remains unknown, substantial evidence suggests that α-syn function is related to its capacity to interact directly with membrane phospholipids, particularly highly curved membranes such as vesicles [37]. In particular, α-syn seems to play a role in the vesicle trafficking during the neurotransmission release. In PD, this protein leaves its binding sites within synaptic boutons and, together with other components such as phosphorylated neurofilaments and ubiquitin, gradually adopts insoluble oligomeric and/or fibrillary conformations [38]. α-syn pathological species are toxic *in vivo* by several mechanisms including the disruption of normal α-syn function in neurotransmission release and vesicular transport, and impairing mitochondrial structure and the efficiency of some protein-degradation mechanism [39].

In 2003, Braak et al. [4] performed several longitudinal analyses to evaluate the neuroanatomical changes in the brain of patients with PD and proposed a model in which the disease stages are correlated with the regional distribution of LB in the CNS. According to the Braak's model, LB formation starts early in the disease (even before the motor symptoms emerge) and LB originate in the olfactory bulb and in the brainstem, specifically at the dorsal motor nucleus of the vagus nerve. In parallel to disease progression, LB are detected in other brain regions and appear to propagate through brain structures, in a stereotypic pattern, to reach the other regions including the midbrain and, at later stages, the cerebral cortex.

The "Prion-Like" Transmission of Pathogenic Proteins in Neurodegenerative Diseases

Prion diseases are a unique group of neurodegenerative disorders in which the conformationally altered prion protein PrP^{Sc} constitutes the infectious agent that corrupts normal cellular PrP through "seeded" fibrillization [40]. Although not being infectious, that is, transmissible between people, a rapidly growing body of literature has provided compelling evidence that a "prion-like" self-propagating mechanism may be applicable to a wide range of disease-associated proteins, including Aβ, tau, TDP-43, and α-syn [1,2]. The self-propagation of aggregates of Aβ was predicted decades ago [1,2]. More recently, the ability of tau to propagate transsynaptically through well-established brain anatomical pathways has been reported, including AD and FTLD cases with argyrophilic grain pathology [17]. Experimental support for the existence of a cell-to-cell transfer of α-syn inclusions has come from the seminal research showing that misfolded intraneuronal α-syn can transfer to neighboring cells both in culture and in the

brains of patients with PD who had received fetal mesencephalic nerve cell transplants 11–16 years earlier revealing the presence of LB in the grafts [41,42]. Then, several *in vitro* and *in vivo* studies suggested that α-syn can undergo a toxic template conformational change, spread from cell to cell and from region to region, and initiate the formation of LB-like aggregates, contributing to the PD pathogenesis [41,42]. Whereas a cell-to-cell transmission of TDP-43 has not been demonstrated conclusively, a recently discovered C-terminal prion-like domain has been implicated in the aggregation of TDP-43 in cultured cells from diseased brains [31,43]. In addition, a notable feature shared by nearly all neurons involved in ALS is that they receive strong afferents from neocortical pyramidal cells, supporting a neuron-to-neuron propagation through corticofugal connections [5].

It seems likely that prion-like aggregates are able to travel within the neuron to reach potential site for interneuronal transfer, to be released from the originating cell and taken up by neighboring cells, where they penetrate the cytoplasm and nucleate further aggregation [1,2]. Both tau and α-syn aggregates can move anterogradely as well as retrogradely within a neuron, possibly by axonal transport. Among the potential mechanisms of the cell-to-cell spreading of proteins, endocytosis or receptor-mediated endocytosis, transfer through exosomes or even by nanotubes that directly connect the cytoplasm of two cells, has been reported [1,2]. Regardless of the mechanism of transmission between cells and the consequent ability of self-amplification, what triggers the initial conversion of normally produced proteins into abnormal aggregates remains unknown.

Functional and Structural Connectivity-Based Findings in Neurodegenerative Diseases

Functional and Structural Connectivity-Based Imaging Techniques

Resting-state fMRI constitutes an advanced technique that measures the spontaneous low-frequency (<0.001–0.001 Hz) fluctuations of the blood oxygen level-dependent signal while the individual rests in the scanner without performing any task. Resting-state fMRI allows to examine brain connectivity between functionally linked brain regions with no bias toward specific motor, visual and cognitive functions [44]. Spatially distributed maps of temporal synchronization can be detected that characterize resting-state networks [45]. Resting-state fMRI assessment has been focused primarily on a characteristic set of brain regions, including the posterior cingulate and precuneus, inferolateral parietal cortex, medial temporal lobe, and medial prefrontal cortex, which is deactivated during a broad range of cognitive tasks and is believed to support a default mode activity of the human brain (i.e., default mode network [DMN]) [46]. Analysis of resting-state fMRI data has more recently suggested the existence of other networks which are thought to subserve cognition, such as the salience, executive, frontoparietal, and associative visual networks [45].

Information on the microstructural integrity of the white matter (WM) pathways connecting the different structures of the human brain can be obtained *in vivo* using diffusion tensor (DT) MRI [47]. DT MRI characterizes the three-dimensional diffusion of water as a function of spatial location [47]. The two most common DT MRI measures are mean diffusivity (MD) and fractional anisotropy (FA). MD is a measure of the magnitude of diffusion and is rotationally invariant. FA describes the degree of anisotropy of the diffusion tensor. The diffusion of water within the tissues will be altered by changes in the tissue microstructure and organization due to many pathologic processes of the CNS, including demyelination, axonal damage, edema, and ischemia [48].

Alzheimer Disease

Neurodegeneration in AD leads to a marked reduction of brain tissue. Indeed, typical late-onset, amnestic AD is characterized by global atrophy on MRI. The medial temporal lobes, especially the hippocampus and entorhinal cortex, are among the earliest sites of structural damage [49]. Other severely affected regions include the posterior part of the cingulate gyrus, precuneus, and splenium of the corpus callosum on the medial surface, and the parietal, posterior superior temporal, and frontal regions on the lateral cerebral surfaces [49].

Interestingly (yet probably not coincidently), there is a remarkable overlap between the pattern of Aβ pathology and atrophy in AD and the DMN [50]. A decreased DMN connectivity has been described in patients with AD [51,52] as well as in patients with amnestic MCI [51,53–55] and in healthy elderly subjects harboring amyloid plaques (as measured by amyloid imaging) [56,57] or carrying the apolipoprotein E4 allele [58]. In addition, altered connectivity among the DMN nodes do occur regardless cortical damage [59], suggesting that functional deficits within the network may precede structural damage. As the disease progresses, DMN connectivity continues to decline as shown by cross-sectional studies across successive disease stages [60] and a few longitudinal studies [61].

Other brain networks are inevitably affected with AD progression. However, the sequence of involvement of functional systems outside the DMN is not well known. Resting-state fMRI studies demonstrated aberrant functional connectivity in the executive network and the salience network in patients with AD, along with loss of anticorrelation between the DMN and the executive network along the AD continuum [51,62].

Another compelling evidence supporting the notion that neurodegenerative diseases spread along networks comes from recent studies in patients with atypical AD forms, such as PCA and lvPPA. Recent studies combining structural MRI from patients with resting-state fMRI data from healthy subjects highlighted that the DMN is affected in all AD forms. In addition, there is a good anatomical correspondence between the patterns of atrophy in patients (i.e., of the visual network in PCA and language network in lvPPA), distinct brain functional networks in healthy subjects, and symptoms for each AD variant (Figure 2). Therefore, these recent multimodal analyses seem to suggest that atypical AD forms may reflect a different dissemination of pathology through specific interconnected neural networks relative to typical, late-onset AD [63,64].

White matter tracts that connect regions of the DMN, such as the cingulum (linking the medial temporal lobe with the posterior

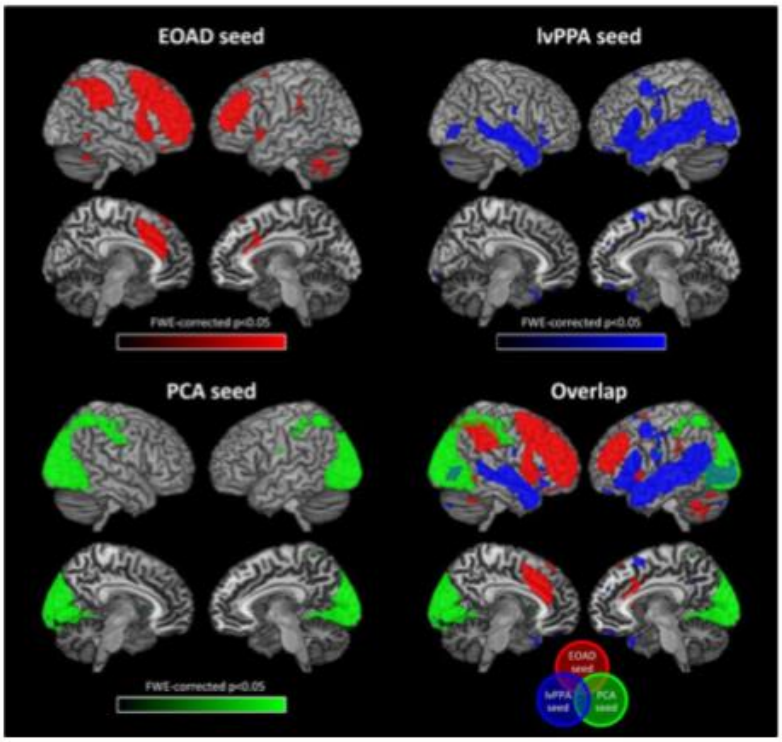

Figure 2 Resting-state functional connectivity network maps in healthy individuals produced by seeding three regions that were specifically atrophied in Alzheimer disease (AD) variants, that is, early-onset AD (EOAD), posterior cortical atrophy (PCA), and logopenic variant of primary progressive aphasia (lvPPA). Figure shows statistical *P* maps after correction for multiple comparisons ($P < 0.05$ family-wise-error corrected for multiple comparisons). Reproduced with permission from [64].

cingulate cortex and the medial frontal regions) and the corpus callosum, are widely affected in patients with AD [65,66]. Damage to these WM regions correlates with cognitive impairment and disease progression in patients with AD [67] and may be related to secondary degeneration. Nevertheless, another major finding of DT MRI studies in AD is that WM damage is more severe and widely distributed than expected on the basis of cortical atrophy. In addition, in MCI and healthy subjects, WM damage can be detected even before the development of cortical atrophy and overt dementia [68,69]. To date, the causes of WM degeneration in AD are still unknown. However, converging data support the notion that WM damage has a central role in how the disease strikes and progresses. Here again, DT MRI findings may reflect the dissemination of pathology from early damaged to yet unaffected cortical regions in AD, thus supporting pathological transmission of $A\beta$ and tau aggregates from neuron to neuron along WM connections [1,2]. In keeping with this hypothesis, a DT MRI study of patients with AD and MCI suggested that microglia activation, which produces neurotoxic and oligodendrotoxic oligomers in the presence of $A\beta$ in excess, can contribute to disease spreading to neighboring and connected areas through WM tracts [70]. In addition, one study investigating the patterns of WM damage in atypical AD variants suggested that the disease has targeted specific peripheral networks (memory, visual, language) at onset in different AD forms and then converged to medial and dorsal frontoparietal regions [71]. The spread of pathology in AD would occur through the corpus callosum and the main long-range WM fibers between the posterior and anterior brain regions [71]. Together with functional connectivity studies, DT MRI findings suggest that clinical heterogeneity of AD may be related to the fact that pathology starts from different medial temporal or lateral neocortical hubs and then eventually progresses along the same WM network to converge to a similar pattern of involvement matching the key hubs of the DMN. Longitudinal studies are needed to confirm such a model clarifying *in vivo* the direction of the pathology spreading through brain networks in AD.

Frontotemporal Lobar Degeneration

Behavioral Variant FTD

In bvFTD, early atrophy occurs in orbitofrontal/subgenual, medial frontal cortex (including anterior cingulate cortex), frontoinsula, anterior temporal lobe, and basal ganglia [72]. In bvFTD, atrophy maps strongly resemble a resting-state fMRI network called salience network [73]. This network is activated in tasks requiring attentional selection, task switching, and self-regulation of behavior, that is, events where we determine which inputs are salient for processing [74]. Within this network, two key nodes have been identified: the frontoinsula, an afferent hub which integrates

inputs coming from other networks with the interoceptive ones; and the anterior cingulate cortex, an efferent hub, which detects information from the previous hub and mobilizes visceroautonomic, emotional, cognitive, and behavioral responses [75]. Patients with bvFTD have reduced connectivity in the salience network when compared either with controls or with patients with AD [76–78]. In patients with bvFTD, the functional disconnectivity between these key nodes has been correlated with clinical severity, apathy, and disinhibition scores [76,78]. In addition, measures of salience network connectivity involving the left insula predict behavioral changes in patients with bvFTD [79].

White matter tracts connecting the key regions of the salience network are also altered [80–82], such as the uncinate fasciculus and genu of the corpus callosum. However, studies have shown that WM alterations may also go beyond the regions of cortical atrophy in a more distributed manner [80–82]. Indeed, with the disease progression, WM abnormalities involve the posterior temporal and parietal regions, reflecting distal propagation of the pathology [83]. It is worth noting that presymptomatic FTLD gene carriers present the same functional network alterations observed in patients with bvFTD without cortical atrophy but with considerable WM abnormalities in frontotemporal regions [84]. These results suggest that WM alterations might precede cortical tissue loss and that DT MRI metrics can be a marker of pathology spreading through WM tracts in FTLD cases.

Amyotrophic Lateral Sclerosis

MRI observations revealed cross-sectional brain atrophy in the motor and/or premotor cortices of patients with ALS [85]. Several resting-state fMRI studies of ALS reported significantly decreased functional connectivity within the sensorimotor network [86–89] in keeping with the structural damage. However, other studies have identified regions of increased functional connectivity in the somatosensory system [89–92]. Two scenarios have been described to explain increased connectivity patterns. First, increased functional connectivity might compensate for structural damage and exhaust with increasing burden of pathology [91,93]. Second, the high level of functional connectivity in ALS might be related to pathogenic loss of local inhibitory circuitry [94]. Indeed, increased functional connectivity was found over a large area spanning sensorimotor, premotor, prefrontal, and thalamic regions overlapping areas abutting WM tracts showing loss of integrity at DT MRI [92,95].

Diffusion tensor MRI studies of patients with ALS have consistently reported the involvement of the corticospinal tract and middle-posterior parts of the corpus callosum, correlating with disease severity and rate of disease progression [85]. Although diagnosed and classified on the basis of motor system involvement only, the growing body of evidence demonstrating a frontotemporal syndrome is undeniable. In keeping with pathological and clinical data, an altered (both decreased and increased) functional connectivity of brain networks associated with cognition and behavior was found in ALS, even in the absence of overt dementia [86,88,93]. Patients with ALS also show abnormalities in extramotor WM regions, especially in frontotemporal areas, in relation to the occurrence of cognitive impairment or ALS-FTD [96–99].

A recent study used DT MRI tractography to assess the pathways that are prone to be involved in ALS according to the different pTDP-43 stages [5], and revealed significant WM tract abnormalities in patients relative to controls in a sequential progression [100] (Figure 3), that is, the corticospinal tract (stage 1), the corticorubral and corticopontine tracts (stage 2), the corticostriatal pathway (stage 3), and the proximal portion of the perforant path (stage 4). These results mirror the proposed neuropathological propagation pattern of ALS [5], supporting *in vivo* the evidence of the progressive expansion of WM damage from the motor to the extramotor networks.

Parkinson Disease

Although conventional structural MRI remains normal in PD until the late stage, advanced techniques have shown abnormalities in the substantia nigra and the cortex [101]. Several studies assessed the resting-state fMRI pattern of the corticostriatal–thalamic–cortical circuits in patients with mild to moderate PD, most of which report reduced functional connectivity in some regions and decreased functional connectivity in others relative to healthy controls [102–105]. A levodopa-induced spatial remapping of the cortico-striatal connectivity has been detected in chronically treated patients with PD [103,104], suggesting that the clinical improvement associated with dopaminergic treatment could be related to the dopaminergic modulation of resting-state functional connectivity. A modulation of thalamocortical functional connectivity by levodopa administration has been demonstrated to occur also in drug-naïve PD cases [106–108].

Diffusion tensor MRI studies of patients with cognitively normal, early, idiopathic PD showed subtle WM alterations along the nigrostriatal projections, in the frontal regions, including premotor areas, and corpus callosum [109–112]. In early PD, diffusion changes precede atrophy that is detectable with conventional MRI, specifically within voxels containing the olfactory tracts [113]. WM damage is emerging as an important pathological substrate of cognitive deficits in patients with PD [114–118]. A large study of idiopathic nondemented PD cases at different disease stages showed that WM damage spreads predominantly to frontal and parietal regions with increasing PD severity and in association with the degree of cognitive impairment [115]. DT MRI studies exploring WM tract abnormalities in patients with PD-MCI showed a more severe involvement of the corpus callosum, cingulum, and major association WM tracts relative to those patients with no cognitive deficits [114,116,118].

Graph Theory and Network Properties in Neurodegenerative Diseases

Network-based analysis of brain structural and functional connections has provided a novel instrument to study the human brain in healthy and diseased individuals [119]. Using the theoretical framework of networks and graphs, the brain can be represented as a set of nodes (i.e., brain regions) joined by pairs by lines (i.e., structural or functional connectivity) [119]. Graph analysis has revealed important features of brain organization, such as an efficient "small-world" architecture (which combines a high level of segregation with a high level of global efficiency) and distributed,

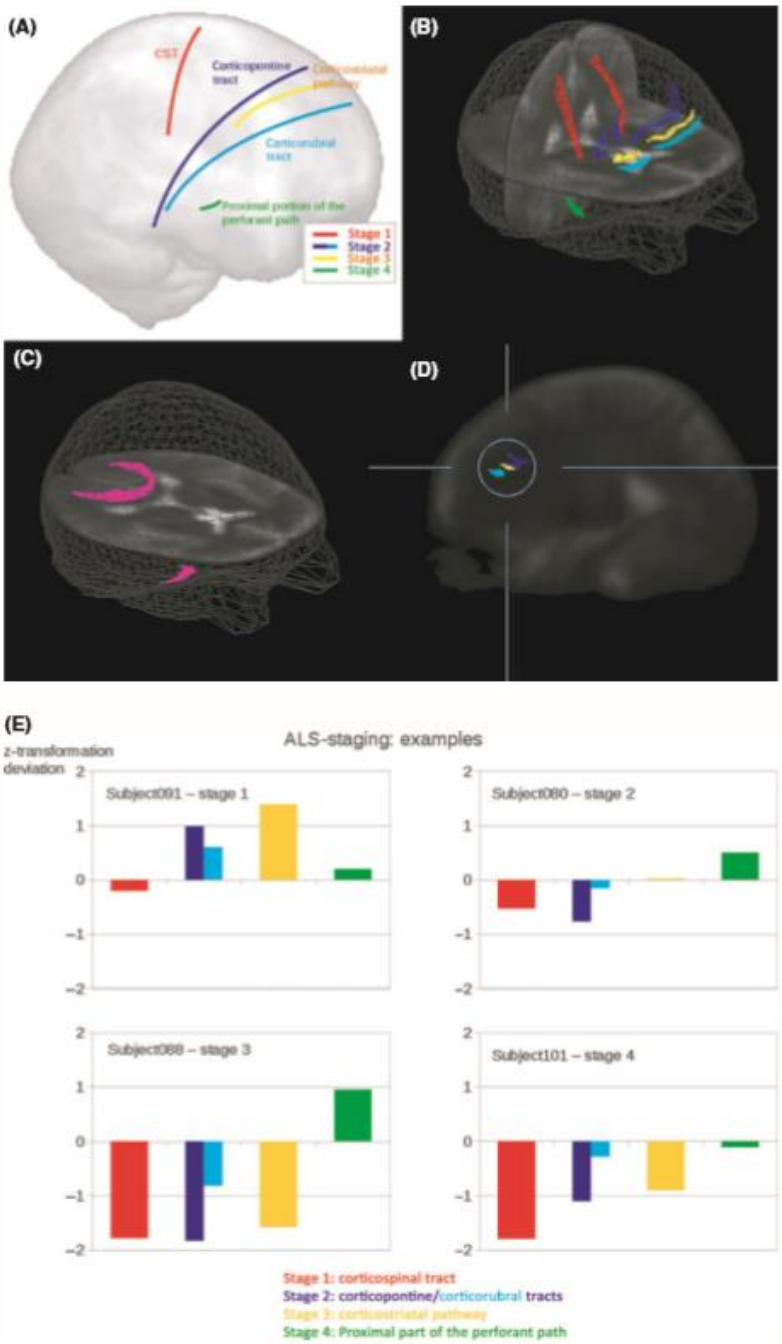

Figure 3 *In vivo* imaging of the disease stages in amyotrophic lateral sclerosis (ALS) using diffusion tensor tractography. (**A**) Schematic representation of the white matter tracts analyzed. (**B**) Three-dimensional images of the corticospinal tract (CST, red) corresponding to ALS stage 1 [5], corticopontine tract (dark blue) and corticorubral tract (light blue) corresponding to ALS stage 2 [5], corticostriatal pathway (yellow) corresponding to ALS stage 3 [5], and proximal portion of the perforant path (green) corresponding to ALS stage 4 [5]. (**C**) Reference paths (magenta) show starting points in the corpus callosum (area V) and starting points in the optic tract. (**D**) Sagittal slice for the illustration of the differences between the corticopontine tract (dark blue), corticorubral tract (light blue), and corticostriatal pathway (yellow). (**E**) Individual examples for the categorization of patients with ALS into ALS stages based upon deviations of *z*-transformed fractional anisotropy values from controls' values for different ALS stages. Modified with permission from [100].

highly connected network regions, called "hubs" [119]. In a small-world network, a high clustering coefficient indicates that nodes tend to form dense regional cliques, implying high efficiency in local information transfer/processing [119]. Path length and global efficiency are measures of network integration, which is the ability to combine specialized information rapidly from distributed brain regions [119]. Distinct modifications of brain network topology have been identified during development and normal aging, whereas disrupted functional and structural network properties have been associated with several neurological and psychiatric conditions, including dementia, ALS, multiple sclerosis, and schizophrenia [119].

Many studies used graph theoretical analysis in AD using both structural and functional MRI [120,121], pointing to a loss of highly connected areas in these patients [122]. A correlation between the site of $A\beta$ deposition in patients with AD and the location of major hubs as defined by graph theoretical analysis of functional connectivity in healthy adults has been demonstrated [50]. These regions include the posterior cingulate cortex/precuneus, the inferior parietal lobule, and the medial frontal cortex, implying that the hubs are preferentially affected in the progression of AD. Although studies showed considerable variability in reported group differences of most graph properties, the average characteristic path length has been most consistently reported to be increased in AD, as a result of loss of connectivity, while the clustering coefficient is likely to be less affected by AD pathology [122]. The global architecture of MCI networks was found to be intermediate between patients with AD and normal elderly controls [122]. Additionally, compared with controls, patients with MCI retained their hub regions in the frontal lobe but lost those in the temporal lobe [123]. Increased interregional correlations within the local brain lobes and disrupted long-distance interregional correlations in MCI and AD were also detected [122]. In patients with AD and MCI, altered graph theory patterns were associated with cognitive deficits [124,125].

Graph theoretical analysis was recently applied to resting-state fMRI data from patients with bvFTD [126]. Global and local functional networks were altered in patients with bvFTD relative to normal subjects as indicated by reduced mean network clustering coefficient, and global efficiency and increased path length [126]. Altered brain regions were located in structures that are closely associated with neuropathological changes in bvFTD, such as the frontotemporal lobes and subcortical regions [126] (Figure 4).

Graph theoretical approach showed that overall functional organization of the motor network was unchanged in patients with ALS compared to healthy controls; however, the level of functional connectedness was correlated with disease progression rate, that is, stronger interconnected motor networks show a more progressive disease course [90]. The effects of ALS on structural brain topology were assessed using DT MRI and graph theoretical analysis [127,128]. While the organization of the global brain network was intact in ALS, an impaired subnetwork of regions with reduced WM connectivity was detected [127] centered on primary motor regions, including secondary motor regions (frontal cortex and pallidum) as well as high-order hub regions (posterior cingulate cortex and precuneus). A more recent study investigating the overlap between structural and functional connectivity abnormalities in patients with ALS showed coherent loss of structural and functional connections in the motor network [129].

Only two studies so far have investigated brain networks using graph analysis in patients with PD [130,131], suggesting a decreased global and nodal functional efficiency relative to healthy controls. In addition, one study indicated that the topological properties of brain functional networks are severely impaired in PD patients with cognitive deficits [130]. Patients with PD-MCI had connectivity reductions predominantly affecting long-range connections as well as increased local interconnectedness manifested as higher measures of clustering coefficient and small-worldness [130]. This latter measure also correlated negatively with cognitive performance in visuospatial and memory functions. Furthermore, normal hubs displayed reduced centrality and degree in these patients [130].

Recent graph theoretical MRI analyses tested various models of how neurodegenerative diseases spread across networks [128,132,133]. Combining atrophy patterns of patients with five different neurodegenerative diseases with resting-state fMRI data from healthy subjects, a first study revealed that, within each targeted network, neurodegenerative process spreads primarily between neurons according to the functional proximity of specific brain regions acting as critical hub-like "epicenters," rather than various alternative candidate mechanisms [133] (Figure 5). A second study modeled network diffusion based on brain structural connectivity networks obtained from DT MRI data of healthy subjects and derived robust spatial eigenmodes that correspond closely to known patterns of atrophy in patients with AD and bvFTD [132]. A longitudinal study of patients with ALS demonstrated no progressive impairment of the initially affected connections of the motor system, but a propagating loss of brain connections over time to frontal and parietal regions [128]. Therefore, all these sophisticated analyses best fit a transneuronal spread model of network-based vulnerability from initial disease epicenters to directly connected neighboring nodes in patients with different neurodegenerative diseases.

Conclusions

Neurodegenerative diseases feature characteristic patterns of early neuronal and regional vulnerability, with resulting neurological first symptoms. In turn, a common finding among neurodegenerative disease is that they show typical progressions of regional degeneration with associated downstream clinical disturbances. The cellular mechanisms underlying such a stereotypical progression of pathology in neurodegenerative diseases are incompletely understood, but increasing evidence indicates that misfolded protein aggregates can spread by a self-perpetuating process that leads to amplification, templating, and neuron-to-neuron transmission of these pathologies. Novel neuroimaging techniques can help elucidating how these disorders spread across brain networks. Recent knowledge from structural and functional connectivity studies suggests that the relation between neurodegenerative diseases and separate brain networks is likely to be a strict consequence of diffuse network dynamics. Furthermore, in the majority of these conditions, measurement of WM tract involve-

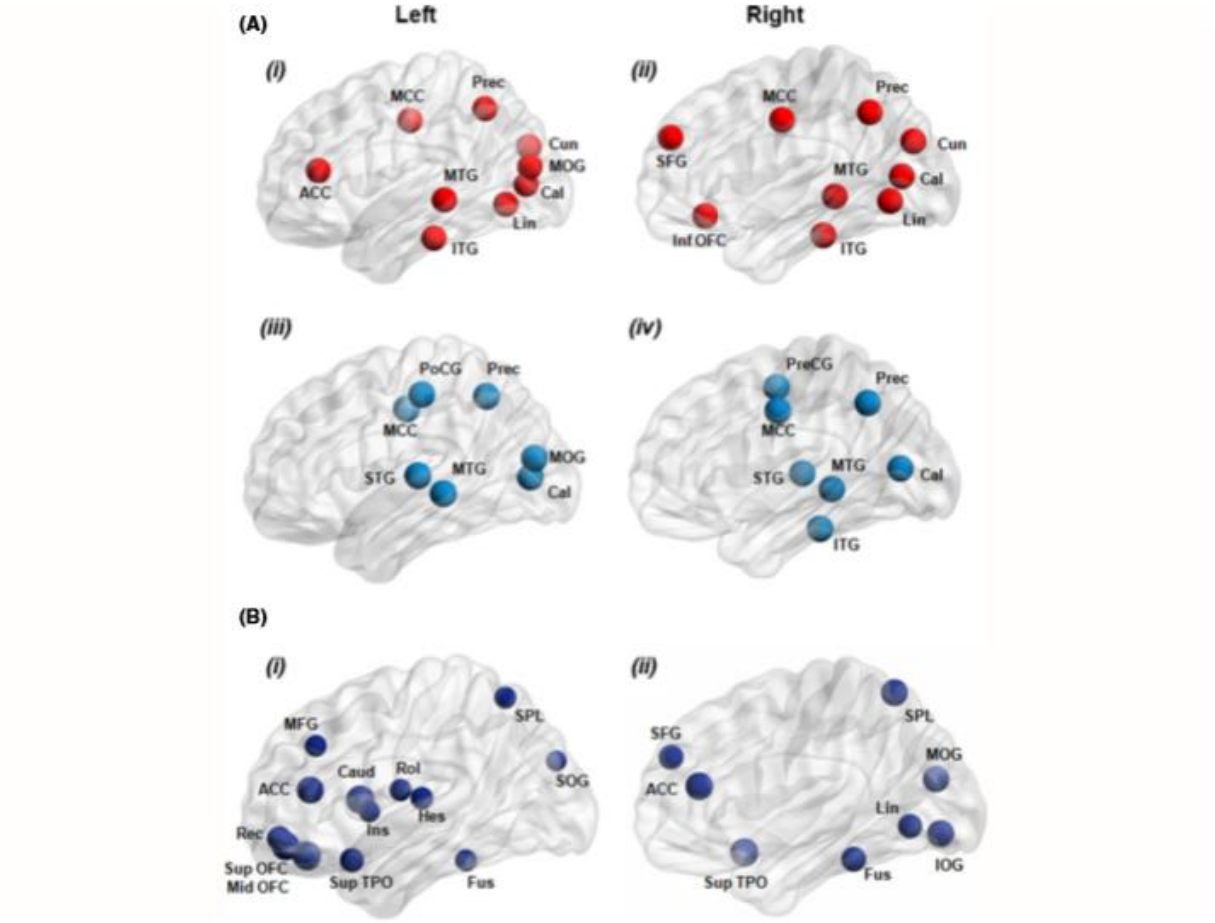

Figure 4 (**A**) Cortical hubs of brain functional networks in healthy controls (i, ii) and patients with the behavioral variant of frontotemporal dementia (bvFTD) (iii, iv). (**B**) Regions showing decreased integrated nodal degree (i, ii) in patients with bvFTD compared to healthy controls. Node size is proportional to the difference in the value of the integrated nodal parameters between the two groups. ACC, anterior cingulate cortex; Cal, calcarine cortex; Caud, caudate nucleus; Cun, cuneus; Fus, fusiform gyrus; Hes, Heschl gyrus; Ins, insula; IOG, inferior occipital gyrus; ITG, inferior temporal gyrus; Lin, lingual gyrus; MCC, middle cingulate cortex; MFG, middle frontal gyrus; MOG, middle occipital gyrus; MTG, middle temporal gyrus; OFC, orbitofrontal cortex; Prec, precuneus; PoCG, postcentral gyrus; PreCG, precentral gyrus; Rec, gyrus rectus; Rol, rolandic operculum; SFG, superior frontal gyrus; SOG, superior occipital gyrus; SPL, superior parietal lobule; STG, superior temporal gyrus; TPO, temporal pole. Reproduced with permission from [126].

ment seems to be a valid surrogate to assess the *in vivo* spreading of pathological proteins. Therefore, characterizing network breakdown in neurodegenerative diseases will help anticipate and perhaps prevent the devastating impact of these disorders. However, the reviewed literature also arises several burning questions. First, the direction of pathology spreading in each neurodegenerative disease is still not completely understood. Longitudinal analyses of multimodal imaging datasets, involving subjects in the preclinical phase of the diseases, are currently being acquired to allow for more explicit testing of the hypothesis of predictable disease spread. In addition, new analyses techniques that relate those changes to underlying pathology, for example, tau imaging, will shed new light on how neurodegenerative diseases develop and spread. Finally, limited information is available about how selective vulnerability works and how pathological proteins interact with disease-susceptible networks in these patients.

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Conflict of Interest

F. Agosta serves on the editorial board of the Journal of Neurology; has received speaker honoraria from Biogen Idec and EXCEMED—Excellence in Medical Education; and receives research supports from the Italian Ministry of Health, and AriSLA (Fondazione Italiana di Ricerca per la SLA). M. Weiler reports no disclosures. M. Filippi is Editor-in-Chief of the Journal of Neurology; serves on scientific advisory boards for Teva Pharmaceutical Industries; has received compensation for consulting services and/or speaking activities from Bayer Schering Pharma, Biogen Idec,

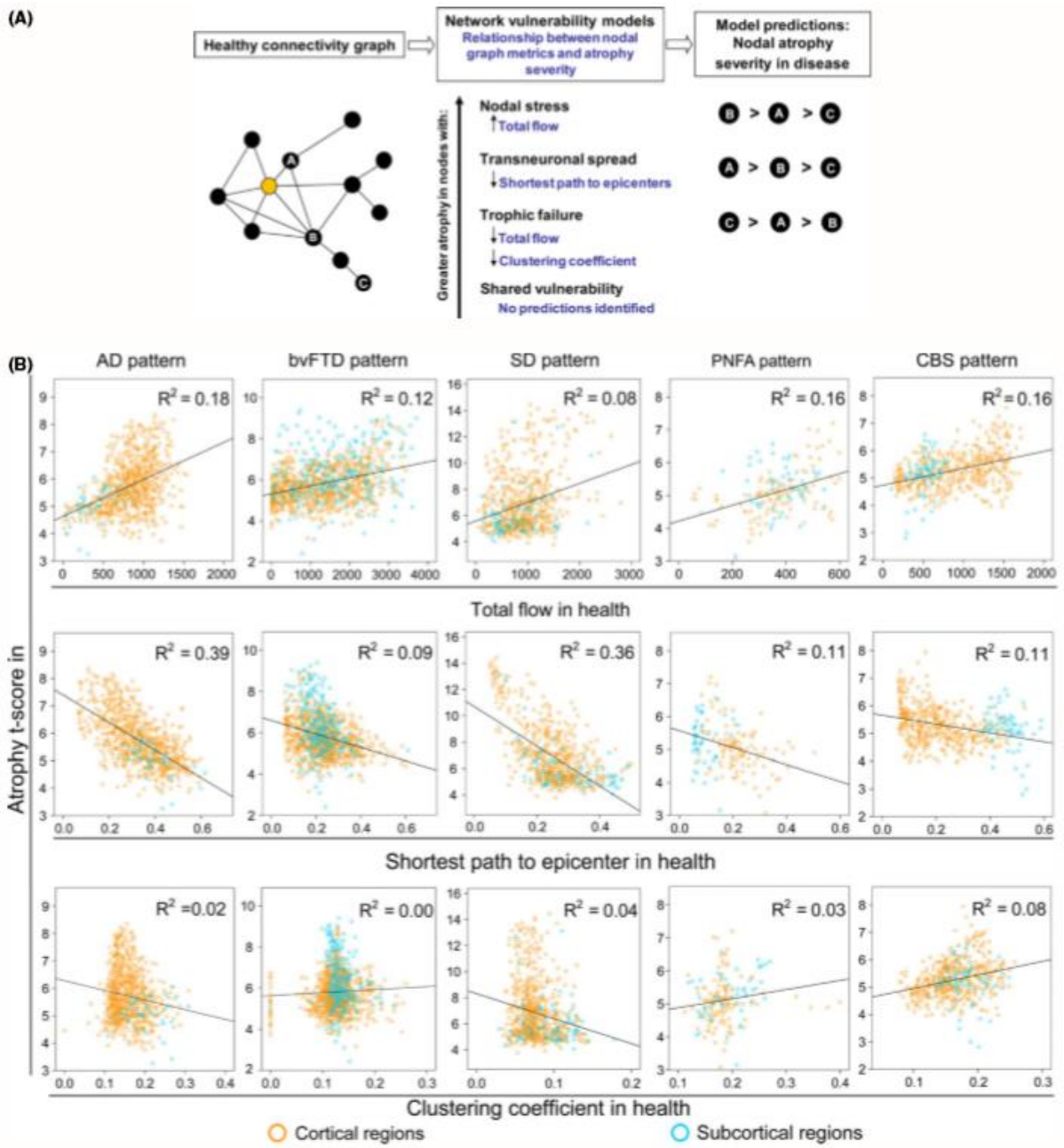

Figure 5 (**A**) Predictions made by network-based degeneration models: effects of healthy intrinsic connectivity graph metrics on atrophy severity in neurodegenerative diseases. A simplified healthy connectivity graph is shown (far left) for illustration purposes only; circles represent nodes (brain regions), lines represent edges (a connection between two nodes), and edge lengths represent the connectivity strength between nodes, with shorter edges representing stronger connections. The orange node represents an epicenter. Three nodes, labeled as "A", "B", and "C", feature contrasting graph theoretical properties to illustrate predictions made by the network-based vulnerability models (far right). Listed in the center column are the relationships predicted by each model. For example, the transneuronal spread model predicts that nodes with shorter (↓) paths to the epicenter in health will be associated with greater (↑) atrophy severity in disease. (**B**) Regions with high total connectional flow (row 1) and shorter functional paths to the epicenters (row 2) showed significantly greater disease vulnerability ($P < 0.05$ family-wise-error corrected for multiple comparisons) in Alzheimer disease (AD), behavioral variant of frontotemporal dementia (bvFTD), semantic dementia (SD), progressive supranuclear palsy (PNFA), and corticobasal degeneration (CBS), whereas inconsistent weaker or nonsignificant relationships were observed between clustering coefficient and atrophy (row 3). Cortical regions = blue circles; subcortical regions = orange circles. Modified with permission from [133].

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DISCUSSÃO GERAL E CONCLUSÕES

Nas últimas décadas, o imageamento por RM proporcionou uma análise *in vivo* e com grande detalhamento de diversos aspectos cerebrais, abrindo novos horizontes tanto para a medicina diagnóstica quanto para a pesquisa em neurociência. A RMf, especificamente, nos permitiu visualizar regiões cerebrais que estão funcionalmente conectadas entre si, seja durante desempenho de uma função cognitiva ou mesmo na manutenção de um estado mental. Ainda, a RMf em repouso teve papel fundamental no campo da pesquisa na DA, visto que esses pacientes muitas vezes encontram dificuldades em realizar as tarefas requeridas durante o experimento designado. Esse método tem papel relevante em pesquisa clínica na área da DA tanto na análise das disfunções de redes como na busca por biomarcadores de imagem.

A organização cerebral das funções mentais está relacionada à conectividade dessas redes neurofuncionais. Danos nessas redes podem, por sua vez, causar problemas cognitivos e/ou psiquiátricos, surgindo daí a importância desse trabalho. Uma correta caracterização do padrão de funcionamento das redes neurofuncionais na DA, devido aos motivos acima citados, se torna cada vez mais relevante. Assim, ferramentas como a RMf e o DTI conquistaram grande espaço no sentido de avaliar a conectividade e integridade de redes neurofuncionais, permitindo avanços significativos não apenas no entendimento da conexão dessas redes, mas também na maneira como essas redes estão alteradas nas doenças.

Esse processo de desconexão de redes, sobretudo funcional, pode ocorrer de forma precoce no espectro da DA, contribuindo para as alterações cognitivas típicas da doença, como amnésia episódica, por exemplo. Nesse contexto, a DMN é uma das principais candidatas a mostrar disfunção na DA, já que dela fazem parte regiões que processam esse tipo de memória. Ainda, são nas estruturas cerebrais que compõem a DMN que se encontram as alterações patológicas características da doença, como depósitos de βA e ENFs. Estudos recentes mostraram que redes neurofuncionais específicas (e por sua vez, vulneráveis) podem ser um caminho na propagação dos processos neurodegenerativos, em geral secundários a proteinopatias.

Assim, nesse trabalho, visamos caracterizar as alterações de conectividade funcional e estrutural ocorrentes na DA. Tivemos como principal objetivo desta Tese avaliar, especificamente, a integridade dessas redes na DA - enfatizando sobretudo na DMN, e suas relações com a cognição. Além disso, perguntas paralelas foram surgindo ao longo desses anos, e estudamos também outros aspectos anatômicos na DA, como alterações em tálamo, corpo caloso e a progressão temporal da degeneração de substâncias branca e cinzenta no cérebro inteiro.

No ARTIGO 1, por exemplo, mostramos que sob o ponto de vista estrutural as alterações causadas pela doença não se restringem a regiões corticais, mas afetam também áreas subcorticais. É o caso do tálamo, região responsável não apenas pelo envio de informações sensoriais ao córtex, mas também representa uma das mais importantes vias de comunicação entre regiões corticais (Sherman e Guillery, 2002). Danos nessa estrutura podem, por sua vez, estar relacionados com o déficit cognitivo dos pacientes, conforme demostramos nesse trabalho. No ARTIGO 2, trouxemos resultados mais detalhados dos principais achados envolvendo diversas técnicas de RM estrutural, mais uma vez demostrando que, apesar de a atrofia nas estruturas mediais temporais consistirem um processo inexorável na DA, a avaliação de outras áreas cerebrais é de extrema importância na prática clínica e pesquisa. O papel da neuroimagem no campo da DA tem se tornado, dessa maneira, cada vez mais importante para um correto diagnóstico da doença, e novos achados estão cada vez mais sendo candidatos a biomarcadores na área da pesquisa.

Em outro trabalho com carácter mais exploratório (ARTIGO 6), mostramos que, além das esperadas regiões corticais (temporal medial e parietal), danos estruturais também podem ser vistos nos tratos de substância branca desses pacientes. Dentre esses tratos, destacam-se o corpo caloso, fascículo do cíngulo, tratos nas regiões temporo-occipital, parietal, frontal, radiação talâmica e cerebelo. À medida em que a doença progride, as regiões corticais frontais, temporais e parietais apresentam maior atrofia do que os estágios iniciais da doença; enquanto que danos na substância branca foram observados nos tratos que ligam regiões afetadas inicialmente pela doença com regiões afetadas mais tardiamente. Interessantemente, nossos dados sugerem

que danos na substância branca podem ocorrer de maneira independente ao dano cortical. Além disso, pacientes com níveis liquóricos de tau total apresentam danos mais severos no fascículo do cíngulo, um dos principais feixes que interligam as regiões da DMN.

O ARTIGO 8, nesse contexto, traz um apanhado de resultados moleculares e de imagem que reforçam a hipótese de uma degeneração de redes neurofuncionais específicas em doenças neurodegenerativas. Mesmo não sendo o único, um dos mecanismos de propagação dos danos abordado nesse texto consiste nas propriedades 'prionoides' das proteínas patológicas características de cada doença. No caso da DA, especificamente, essa propagação ocorreria ao longo dos tratos da DMN, visto que suas regiões são as que possuem maiores alterações moleculares - fazendo os tratos dessa rede especificamente vulneráveis aos danos causados por suas proteínas alteradas. Com isso em mente, no ARTIGO 4 tivemos como objetivo isolar apenas os tratos da DMN para avaliar o quão íntegro eles se apresentam na doença, e o quanto essa integridade pode afetar o processamento cognitivo dos pacientes. Pudemos observar que pacientes com DA apresentam alterações microestruturais no fascículo do cíngulo (e também na sua porção parahipocampal), que contribuem para os déficits cognitivos. O declínio cognitivo observado nos nossos pacientes com CCLa entretanto, deve-se provavelmente a outros fatores que não a conectividade estrutural dos tratos da DMN, visto que não encontramos alterações nesse grupo de sujeitos.

No ARTIGO 5, investigamos não apenas a conectividade funcional das regiões da DMN, como também a média das amplitudes de baixa frequência (ALFF) do sinal BOLD dessas regiões. Apesar das bases fisiológicas dessa métrica não estarem bem definidas ainda, encontramos resultados interessantes nesse trabalho. Sujeitos com CCLa, por exemplo, possuem ALFF reduzido na região temporal da DMN em relação a idosos saudáveis e a pacientes com DA; porém, não apresentam desconexão entre as regiões da DMN. Pacientes com DA, por vez, possuem ALFF reduzido no giro do cíngulo posterior em relação a idosos saudáveis e sujeitos com CCLa, e desconexão dessa região específica com quase todas as demais regiões da DMN analisadas.

Devemos interpretar a amplitude do sinal BOLD cautelosamente, mas nossos achados sugerem que essa amplitude possui alguma associação com a conectividade funcional - visto que o ALFF do giro do cíngulo posterior possui relação com as alterações na conectividade funcional envolvendo essa região. Além disso, mesmo que alteradas tanto em pacientes com DA e sujeitos com CCLa em diversas áreas da DMN, essas amplitudes não possuem relação com o déficit cognitivo apresentado pelos dois grupos. Alterações na conectividade funcional dessas regiões, em contrapartida, possuem. Nossos resultados vão ao encontro da proposta da DA como 'síndrome de desconexão' (Delbeuck, Van der Linden e Collette, 2003; Vallet *et al.*, 2013) – onde os déficits cognitivos característicos da DA não seriam causa somente de danos em regiões cerebrais específicas ou mesmo de um único sistema neural, mas sim de um problema na interação entre diversos desses sistemas.

As alterações em conectividade funcional na DA, entretanto, não se limitam à DMN. No ARTIGO 3, mostramos que outras redes neurofuncionais, como a de Controle Executivo e de Linguagem também estão afetadas. Interessantemente, a rede de Controle Executivo foi a única que se mostrou hiperconectada em relação ao grupo controle, e esses resultados podem ser interpretados na luz de um 'mecanismo neural compensatório'. Para tal, assumimos que em pacientes com DA desenvolvam-se mecanismos de recrutamento de recursos neurais adicionais para manutenção de um funcionamento cognitivo mais próximo do normal. Em termos de correlatos neuropsicológicos, apenas a conectividade da DMN mostrou relação com os testes propostos – nesse caso, com a performance em testes de memória episódica. A relação da DMN com esse domínio cognitivo, tanto do ponto de vista de sua desativação (Pihlajamaki e Sperling, 2009) quanto da sua conectividade em estudos de RMf de repouso (Dunn *et al.*, 2014) já foi demonstrada em outros trabalhos. No ARTIGO 7, por vez, tivemos como objetivo explorar outras hipóteses envolvendo as regiões corticais mediais. Nele, abordamos a questão da alteração do *self* nos pacientes com DA, e sugerimos uma relação com a perda do sentimento de continuidade temporal. O processamento desse sentimento de continuidade, por vez, provavelmente ocorra durante momentos

mentais ditos ‘passivos’, onde regiões corticais mediais – áreas envolvendo a DMN, desempenham um papel chave.

O imageamento na DA possui um importante papel no auxílio do diagnóstico da doença, sobretudo sob o ponto de vista estrutural e identificação de regiões atróficas. Entretanto, a identificação de alterações em redes neurofuncionais tem se tornado mais fácil nos últimos anos, principalmente com o uso de abordagens que analisem as conectividades estrutural e funcional (como o DTI e o sinal BOLD, por exemplo). Por meio dessas técnicas, pudemos demonstrar que os efeitos da doença vão além dos danos estruturais regionais, mas caracterizam-se também por desconexões entre regiões cerebrais, caracterizando a DA como uma ‘redepatia’. Dessa maneira, esse trabalho mostrou que, além do conhecido padrão de atrofia presente nos pacientes com DA, a doença também se caracteriza por alterações em redes neurofuncionais, mesmo nos seus estágios mais leves. A DMN, pelas razões anteriormente citadas, é a rede neurofuncional de maior interesse no campo da pesquisa na DA e tem se tornado uma forte candidata a biomarcador da doença, aos poucos conquistando seu espaço também na prática clínica.

Importante ressaltar que tanto alterações na conectividade funcional quando estrutural da DMN possuem relação com o déficit cognitivo apresentado pelos pacientes. Além disso, alterações nessa rede podem também estar ligadas ao sentimento de auto-continuidade temporal. A perda desse sentimento, por vez, está ligada às alterações nos aspectos cognitivos considerados de ‘alto nível’, como aqueles que compõem o *self* narrativo. Nos pacientes com DA, isso pode ser exemplificado pela perda na identidade, déficit no reconhecimento de características pessoais, perda de conhecimento da própria história, entre outros aspectos comumente visualizados na prática clínica e por entes próximos.

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Campinas, 14 de março de 2012.

Venho, por meio deste, informar a mudança de título e projeto da aluna Marina Weiler, RA 123444, matriculada no curso de Mestrado no Departamento de Neurologia da Faculdade de Ciências Médicas da UNICAMP.

Título do novo projeto: Avaliação por ressonância magnética das conectividades funcional e estrutural das redes neurofuncionais na demência da doença de Alzheimer leve e comprometimento cognitivo leve amnéstico.

Pesquisadora: Marina Weiler

Orientador: Prof. Dr. Marcio Luiz Figueredo Balthazar

O projeto referido acima está vinculado a um projeto de pesquisa existente sob o número de protocolo 122/2009, onde todos os dados já estão coletados e devidamente autorizados pelos sujeitos de pesquisa - sob os devidos termos de consentimento - e aprovação prévia pelo Comitê de Ética em Pesquisa da UNICAMP.

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II – PARECER DO CEP.

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP tomou ciência e aprova o adendo que inclui o projeto intitulado **"AVALIAÇÃO DA CONECTIVIDADE ANATÔMICA NAS REDES FUNCIONAIS DEFAULT MODE E SALIENCE NA DOENÇA DE ALZHEIMER"**, com a finalidade de mestrado da aluna Marina Weiler, referente ao protocolo de pesquisa supracitado.

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