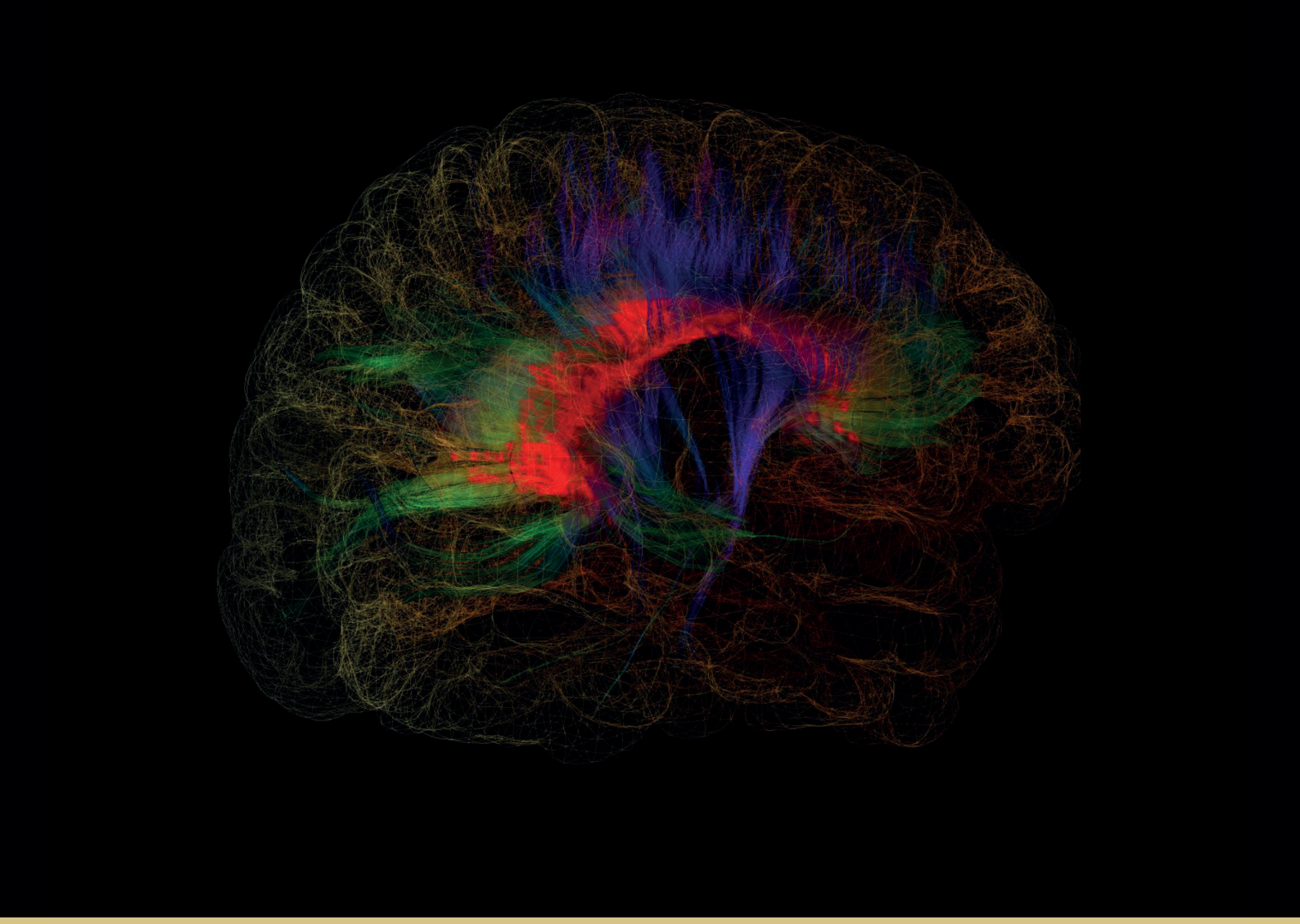

Sónia Raquel Marques Batista

NEURAL CORRELATES OF THEORY OF MIND IMPAIRMENT IN MULTIPLE SCLEROSIS

Tese de Doutoramento do Programa de Doutoramento em Ciências da Saúde - ramo de Medicina, orientada pelo Professor Doutor Luís Cunha, pela Professora Doutora Isabel Santana e pelo Professor Doutor António Freire Gonçalves, apresentada à Faculdade de Medicina da Universidade de Coimbra

2017

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Universidade de Coimbra

On the front cover: Combined image of a MRI Diffusion Tensor Imaging sequence with Mesh brain from Freesurfer software obtained from a patient with Multiple Sclerosis

SÓNIA RAQUEL MARQUES BATISTA

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Tese apresentada à Faculdade de Medicina da Universidade de Coimbra
para candidatura ao grau de Doutor em Ciências da Saúde – ramo de Medicina,
realizada sob a orientação científica do Professor Doutor Luís Cunha,
da Professora Doutora Isabel Santana e do Professor Doutor António Freire Gonçalves

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À minha Mãe, porque é verdadeiramente o meu porto de abrigo, acolhendo-me e incentivando-me sempre com o seu amor incondicional.
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TABLE OF CONTENTS

ABSTRACT

Despite the gathered knowledge about cognitive impairment in multiple sclerosis (MS), little is known concerning the disease impact on social cognition. An essential aspect for social cognition is theory of mind (ToM), defined as the ability to infer other persons' mental states. In the present study, we aimed to explore how ToM is affected in MS, particularly if ToM deficits are independent of the classic cognitive impairment associated with the disease; to examine the relationship of ToM and executive functions (EF) performance; and to identify the underlying neural correlates.

We enrolled consecutively 60 patients with MS and 60 healthy controls (HC) matched for age, gender, and education. All participants underwent ToM testing (Eyes Test and Videos Test); global cognitive assessment with a standard neuropsychological battery for MS (MACFIMS) in order to classify patients as either cognitively impaired or cognitively intact; tests tapping different processes of executive functions; and 3Tesla brain MRI. Using FreeSurfer software, cortical and subcortical grey matter (GM) volumes were calculated. Tract-based spatial statistics (TBSS) were applied for whole-brain voxel-wise analysis of fractional anisotropy (FA) and mean diffusivity (MD) on normal-appearing white matter (NAWM).

We found that patients with MS performed worse on both tasks of ToM compared to HC, i.e. Eyes Test (58.7±13.8% vs. 81.9±10.4%, $p<0.001$) and Videos Test (75.3±9.3% vs. 88.1±7.1%, $p<0.001$). There were no significant differences on Eyes Test and Videos Test performance between MS patients with cognitive impairment (n=34; 56.7%) and those with normal cognitive performance (n=26; 43.3%). Moreover, the group of patients without cognitive impairment presented significantly lower scores on both tasks compared to HC. A hierarchical cluster analysis showed that ToM measures were clustered separately from the EF measures, distinguishing three executive clusters (attention/working memory; inhibitory control/shifting ability; verbal initiative/abstract reasoning) and one ToM cluster. ToM performance in MS was positively correlated with the volume of subcortical structures (amygdala, putamen) and cortical regions (entorhinal cortex, fusiform gyrus, superior temporal gyrus, superior parietal gyrus,

supramarginal gyrus, medial orbitofrontal cortex, anterior and posterior cingulate gyrus). In regression analysis, amygdala volume was the single predictor of ToM performance. Regarding the TBSS analysis, ToM tests were positively correlated with FA and inversely correlated with MD across widespread NAWM tracts of both hemispheres. The largest effects were for the Eyes Test: body and genu of corpus callosum, fornix, tapetum, uncinate fasciculus, and left inferior cerebellar peduncle; for the Videos Test: genu and splenium of corpus callosum, fornix, uncinate fasciculus, left tapetum, and right superior fronto-occipital fasciculus.

Taken together, these results suggest that social cognition is impaired in patients with MS independently of the classic MS-related cognitive impairment and of the EF performance. The social brain network in MS is affected by two different mechanisms: direct damage of the main cortical and subcortical GM nodes, particularly amygdala, and by disconnection between the mentioned nodes caused by injury of the interconnecting white matter tracts.

RESUMO

Apesar de todo o conhecimento reunido sobre o défice cognitivo na esclerose múltipla (EM), pouco se sabe sobre o impacto da doença na cognição social. Um aspecto essencial para a cognição social é a teoria da mente (ToM), definida como a capacidade de inferir os estados mentais das outras pessoas. No presente estudo, tivemos como objectivos explorar o modo como a ToM é afectada na EM, particularmente se os défices na ToM são independentes da clássica disfunção cognitiva associada à doença; examinar a relação entre o desempenho na ToM e funções executivas (FE); e identificar os correlatos neurais subjacentes.

Incluímos de forma consecutiva 60 doentes com EM e 60 controlos saudáveis, emparelhados por idade, género e escolaridade. Todos os participantes foram submetidos a testes de ToM (Teste dos Olhos e Teste dos Vídeos); avaliação cognitiva global com uma bateria neuropsicológica específica para EM (MACFIMS) por forma a classificar os doentes como tendo défice cognitivo ou desempenho cognitivo normal; testes para avaliar vários processos das FE; e RM cerebral 3 Tesla. Com o *software* FreeSurfer calcularam-se os volumes de substância cinzenta corticais e subcorticais. O Mapeamento Estatístico Baseado em Tratos (TBSS) foi usado para uma análise baseada em voxeis da fracção de anisotropia (FA) e difusividade média (MD) da substância branca de aspecto normal de todo o cérebro.

Os doentes com EM apresentaram um desempenho pior em ambas as tarefas de TOM, isto é, Teste dos Olhos (58.7±13.8% vs. 81.9±10.4%, $p<0.001$) e Teste dos Vídeos (75.3±9.3% vs. 88.1±7.1%, $p<0.001$). Não se verificaram diferenças estatisticamente significativas relativamente ao desempenho no Teste dos Olhos e no Teste dos Vídeos entre os doentes com défice cognitivo (n=34; 56.7%) e os doentes sem défice cognitivo (n=26; 43.3%). Além disso, os doentes com défice cognitivo apresentaram pontuações significativamente inferiores em ambos os testes comparativamente aos controlos saudáveis. Uma análise de *clusters* hierárquica revelou que as medidas de ToM se agregam separadamente das medidas de FE, distinguindo-se 3 *clusters* de FE (atenção/memória de trabalho; controlo inibitório/capacidade de alternância; iniciativa

verbal/raciocínio abstracto) e um *cluster* de ToM. O desempenho da ToM nos doentes com EM correlacionou-se positivamente com os volumes de estruturas subcorticais (amígdala, putamen) e de regiões corticais (córtex entorrinal, giro fusiforme, giro temporal superior, giro parietal superior, giro supramarginal, córtex orbitofrontal medial, giro do cíngulo anterior e posterior). Na análise de regressão, o volume da amígdala foi o único preditor do desempenho na ToM. Relativamente à análise TBSS, os testes ToM correlacionaram-se positivamente com a FA e negativamente com a MD de vários tractos de substância branca de aspecto normal em ambos os hemisférios. As associações mais robustas para o Teste dos Olhos foram: corpo e joelho do corpo caloso, fornix, tapetum, fascículo uncinado, e pedúnculo cerebeloso inferior esquerdo; para o Teste dos Vídeos foram: corpo e joelho do corpo caloso, fornix, fascículo uncinado, tapetum esquerdo, e fascículo fronto-occipital superior direito.

Em conjunto, estes resultados sugerem que a cognição social é afectada nos doentes com EM, independentemente do clássico défice cognitivo associado a doença e do desempenho nas FE. A rede neuronal que constitui o cérebro social é afectada na EM por dois mecanismos diferentes: lesão directa dos seus principais nodos de substância cinzenta corticais e subcorticais, principalmente a amígadala, e por desconexão entre os referidos nodos causada pelo dano dos tractos de substância branca que os interligam.

PUBLICATIONS ARISING FROM THIS THESIS

Articles in international peer-reviewed journals:

I. **Batista S**, d'Almeida OC, Afonso A, Freitas S, Macário C, Sousa L, Castelo-Branco M, Santana I, Cunha L. Impairment of social cognition in multiple sclerosis: amygdala atrophy is the main predictor. *Multiple Sclerosis Journal*. 2017 Sep; 23(10):1358-1366

Cover image: Figure 2 from this article has been selected as the front cover image for the September issue (2017) of *Multiple Sclerosis Journal.*

II. **Batista S**, Alves C, d'Almeida OC, Afonso A, Félix-Morais R, Pereira J, Macário C, Sousa L, Castelo-Branco M, Santana I, Cunha L. Disconnection as a mechanism for social cognition impairment in multiple sclerosis. *Neurology*. 2017 Jul 4;89(1):38-45.

Accompanying Editorial: Cotter J, Muhlert N. White matter changes and social cognitive function in MS: When all is no longer in the eyes. *Neurology*. 2017 Jul 4;89(1):16-17

III. **Batista S**, Freitas S, Afonso A, Macário C, Sousa L, Cunha L, Santana I. Theory of mind and executive functions are dissociated in multiple sclerosis. Accepted for publication in the journal *"Archives of Clinical Neuropsychology"*.

PURPOSE STATEMENT

Multiple sclerosis (MS) may cause a wide range of manifestations including motor, sensory, cognitive, and neuropsychiatric symptoms, all of which can occur independently of one another[1].

In the last decades, cognitive dysfunction has been recognized as a common and early manifestation of MS [2]. Nevertheless, the underlying pathologic substrate has not yet been entirely clarified, remaining an active research area. According to the classic paradigm of MS as primarily a white matter (WM) disease, the disruption of WM pathways mediating the transmission of information across distributed brain networks was the first mechanism being hypothesized and recognized [3, 4]. However, the involvement of grey matter (GM) in MS is currently unquestionable [5, 6] and its contribution for cognitive impairment has been clearly demonstrated [7-9].

Conversely, despite the significant impact on quality of life of patients and caregivers [10], social functioning disturbance in MS has been overlooked and little is known regarding its pathophysiology. Recent studies suggest that social cognition is impaired in MS patients, thus contributing for social behavior maladjustments [11-13]. An essential domain of social cognition is "Theory of Mind" (ToM), defined as the ability to infer other persons' mental states, including beliefs, desires, and intentions [14, 15]. Thoroughly studied in autism, ToM is currently recognized as a critical cognitive ability for successful social interactions. Still, how ToM is affected in MS and the respective neural correlates remains poorly understood.

This thesis aims to fill this gap through a case-control study with MS patients and healthy controls, assessed with neuropsychological testing emphasizing ToM and with advanced MRI techniques focusing both GM and WM pathology. We expect that the analysis of the cognitive and MRI-derived measures will contribute to expand current knowledge about the cognitive pattern in MS and, ultimately, to extend the insight into the neural basis of ToM in MS.

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THESIS OUTLINE

This thesis is divided in six parts, which content is summarized below:

Part I is a general introduction to the thesis, covering the fundamental aspects of the clinical and pathologic features of multiple sclerosis, with special emphasis on the related cognitive impairment (Chapter 1), and gathering the current knowledge about social cognition and theory of mind (Chapter 2).

In **Part II**, the main hypothesis and the key research aims addressed in this thesis are summarized.

The **Part III** contains a global overview of the research plan and methodology, including the sample size calculation, participants enrolment, neuropsychological instruments and MRI techniques used.

Part IV of this thesis contains the original research articles published or submitted to international peer-reviewed journals. Chapter I comprises the original paper *"Impairment of social cognition in multiple sclerosis: amygdala atrophy is the main predictor"* that addresses mainly the contribution of grey matter atrophy for theory of mind impairment in multiple sclerosis, while in Chapter 2 we studied the role of normal appearing white matter damage in the manuscript "*Disconnection as a mechanism for social cognition impairment in multiple sclerosis".* In Chapter 3 we examined the relationship of theory of mind and executive functions in the article *"Theory of Mind and executive functions are dissociated in multiple sclerosis".*

Part V includes an integrated conclusion summarising the main results of this thesis.

In **Part VI**, since research gives answers but raises even more questions, an outlook into possible future research directions is presented.

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AVP	Arginine vasopressin
BDI	Beck Depression Inventory
BICAMS	Brief International Cognitive Assessment for Multiple Sclerosis
BRB-N	Brief Repeatable Neuropsychological Battery
BVMTR	Brief Visuospatial Memory Test-Revised
CI	Confidence interval
CIS	Clinically isolated syndrome
CNS	Central nervous system
COWAT	Controlled Oral Word Association Test
CSF	Cerebrospinal fluid
CVLT2	California Verbal Learning Test-2nd edition
DIR	Double inversion recovery
D-KEFS	Delis-Kaplan Executive Function System
DTI	Diffusion tensor imaging
EDSS	Expanded Disability Status Scale
EBV	*Epstein Barr Virus*
EF	Executive functions
FA	Fractional anisotropy
FDR	False discovery rate
FLAIR	Fluid Attenuated Inversion Recovery
fMRI	Functional magnetic resonance imaging
FWE	Family-wise error
GM	Grey matter
HC	Healthy controls
ICV	Intracranial volume

IQR	Interquartil range
JLO	Judgment of Line Orientation test
MACFIMS	Minimal Assessment of Cognitive Function in Multiple Sclerosis
MD	Mean diffusivity
MFIS	Modified Fatigue Impact Scale
MMSE	Mini-Mental State Examination
MoCA	The Montreal Cognitive Assessment
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MS	Multiple sclerosis
MSRV	*Multiple Sclerosis Associated Retro Virus*
MSSS	Global multiple sclerosis severity score
MTI	Magnetization transfer imaging
NAA	N-acetyl aspartate
NAWM	Normal-appearing white matter
NP	Neuropsychological testing
OXT	Oxytocin
PASAT	Paced Auditory Serial Addition Test
PET	Positron emission tomography
PPMS	Primary progressive multiple sclerosis
RAPM	Raven's Advanced Progressive Matrices
RNS	Reactive nitrogen species
ROI	Region-of-interest
ROS	Reactive oxygen species
RRMS	Relapsing-remitting multiple sclerosis
SD	Standard deviation
SDMT	Symbol Digit Modalities Test

SPART	10/36 Spatial Recall Test
SPECT	Single photon emission computed tomography
SPMS	Secondary progressive multiple sclerosis
SRT	Selective Reminding Test
TBSS	Tract-Based Spatial Statistics
TeLPI	Irregular Word Reading Test
TMT	Trail Making Test
ToM	Theory of mind
WCST	Wisconsin Card Sorting Test
WM	White matter

PART I

INTRODUCTION

CHAPTER 1 MULTIPLE SCLEROSIS

1. Definition and impact of the disease

Multiple Sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) that affects approximately 2.3 million people worldwide, representing the most common cause of nontraumatic disability in young adults [1]. The prevalence in Portugal is estimated to be approximately 50/100,000 inhabitants, corresponding to medium-high levels of prevalence [2]. Evidence suggests that the incidence of relapsing remitting MS might be increasing, particularly in women [3-5]. While in the past it was shown that there are two women for every man with a diagnosis of MS, the female to male sex ratio in patients with relapsing remitting MS has changed and women are now affected three times more often than are men [4, 6].

It is a potentially disabling disease that frequently leads to working inability [7, 8], to a reduced quality of life both in patients [9] and caregivers [10], and to a reduction in life expectancy by 7 to 14 years compared with the general population [11-13]. On other perspective, MS is also associated with a substantial socioeconomic burden resulting from direct medical costs and also from high indirect costs related to reduced productivity, as it typically strikes adults during the primary productive time of their life [14, 15].

The cardinal features of the MS lesion, namely focal demyelination, inflammation, and gliosis of white matter (WM), were described and illustrated over 160 years ago by Carswell (1838), Cruveilher (1841), and Charcot (1868, 1880) [16]. In the last decades, advances in basic pathology, neurobiology and neuroradiology redefined the paradigm of the disease beyond a simply focal WM condition to one that is also characterized by grey matter (GM) damage and widespread neurodegeneration [17].

Despite this progress, many questions remain stubbornly elusive, such as: determining the etiology; defining the causal nexus between inflammation and neurodegeneration; understanding the precise mechanisms of tissue injury as well as the subsequent remyelination and repair processes; and clarifying whether or not different disease presentations can truly be classified as a single disease.

2. Clinical features and disease course

MS is recognized by its noteworthy clinical heterogeneity and unpredictable course. The high degree of variability includes the age of onset, initial manifestation, neuroradiological appearance, frequency, symptomatology and sequelae of relapses, and rate of disability progression [18]. Ultimately, this heterogeneity is also reflected in a large variability of response to treatments among patients.

The varied clinical features reflect both the involvement of multifocal areas of CNS and the heterogeneity of the underlying pathological mechanisms. This has been recognized by the seminal paper of Luchinetti et al. [19] which defined four distinct patterns of demyelination found in the MS plaques (described in detail in the section 5.1 of this chapter).

Even though the age of onset in relapsing forms of MS is typically between 20 and 40 years, with a mean age of 29 to 32 years in most studies, it can occur virtually at any age [20, 21]. Cases in childhood have been reported as early as 15 months [22], with recent estimates indicating that about 1% of patients with MS present the first symptoms of the disease before the age of 10 years and approximately 5% before the age of 18 years [23]. At the other extreme, onset of the disease after the age of 50 years has been considered rare, although recent reports suggest that it may be more common than suspected [24, 25].

Theoretically, MS may cause any symptom or sign referable to the CNS. Nevertheless, there are clinical presentations undeniably more typical, including unilateral visual loss due to acute optic

neuritis, diplopia due to an internuclear ophthalmoplegia, cerebellar ataxia, asymmetric limb weakness, facial sensory disturbances or other sensory symptoms with a CNS pattern and urge incontinence [26, 27]. Few of the clinical features are disease-specific, but particularly characteristic are Lhermitte's symptom and the Uhthoff phenomenon [26]. Fatigue is also among the most common symptoms, reported by at least 75% of MS patients at some point during the disease course [28]. Nevertheless, fatigue is still poorly understood and often neglected due to its complexity and subjective nature [29]. Additionally, cognitive impairment (described in detail in section 6 of this chapter) and psychiatric symptoms are now increasingly recognized as frequent symptoms in MS, occurring at any disease stage and with a major impact on quality of life [30].

Despite the aforementioned clinical heterogeneity, efforts to classify patients by general patterns of disease presentation and evolution have allowed to distinguish distinct disease subtypes. In 1996, Lublin and Reingold proposed a formal classification of MS disease subtypes which has become widely accepted [31]. The most common subtype, affecting approximately 85% of patients, is relapsing-remitting MS (RRMS). It is characterized by an initial episode of neurological dysfunction (clinically isolated syndrome), followed by a remission period with clinical recovery and then recurring bouts of relapse and remission at a random frequency [32]. Natural history studies indicate that after 10 to 20 years post diagnosis, nearly 80% of patients develop secondary progressive MS (SPMS) which is characterized by progressive neurological decline with or without occasional relapses. In clinical practice, the line between RRMS and SPMS is not distinct and there is no way to clinically determine a precise moment of transition between these two categories, making the diagnosis of SPMS possible only retrospectively. In one study [33], the mean period of diagnostic uncertainty during the transition from RRMS to SPMS was 4.3 years.

Approximately 15% of patients with MS are diagnosed with primary progressive MS (PPMS), which features progressive cumulative disability from the outset. Finally, the fourth proposed

subtype was progressive relapsing MS (PRMS), which is characterized by disease progression from onset punctuated afterwards by clear acute relapses.

Primary and secondary progressive MS often manifest as a spinal disease, but syndromes that are attributable to dysfunction of optic nerves, cerebrum, or brainstem can also occur [26]. In both these situations, progression starts at around 40 years of age [34]. These observational data suggest that the clinical phenotype and the course of the disease are age dependent and lead to the proposal of an unifying concept of MS in which primary and secondary progression would be basically similar. In this hypothesis, primary progressive forms of MS could be regarded as "amputated" from the usual preceding relapsing-remitting phase [34].

Recently, the Advisory Committee on Clinical Trials of MS and the MS Phenotype Group have proposed an update of the classification of MS clinical course phenotypes (Figure 1) [35]. In this revision, clinically isolated syndrome (CIS) has been added as a new subtype and is now considered to be part of the RRMS disease spectrum. All subtypes of MS should be further subcategorized as either "active" or "non-active", with active MS being defined as the occurrence of clinical relapse or the presence of new T2 or gadolinium-enhancing lesions over a specified period of time, preferably at least one year. Progressive disease, whether PPMS or SPMS, should display an additional subcategory ("with progression" or "without progression") which differentiates between those patients who have shown signs of disability progression over a given time period and those who have remained stable. The former PRMS subtype has been eliminated and should be categorized according to this new classification as PPMS with activity.

The addition of markers of activity and measures of disease progression to this revised classification will be of great value for enhance clinical research and for improve ongoing clinical care, particularly in the case of progressive subtypes [36].

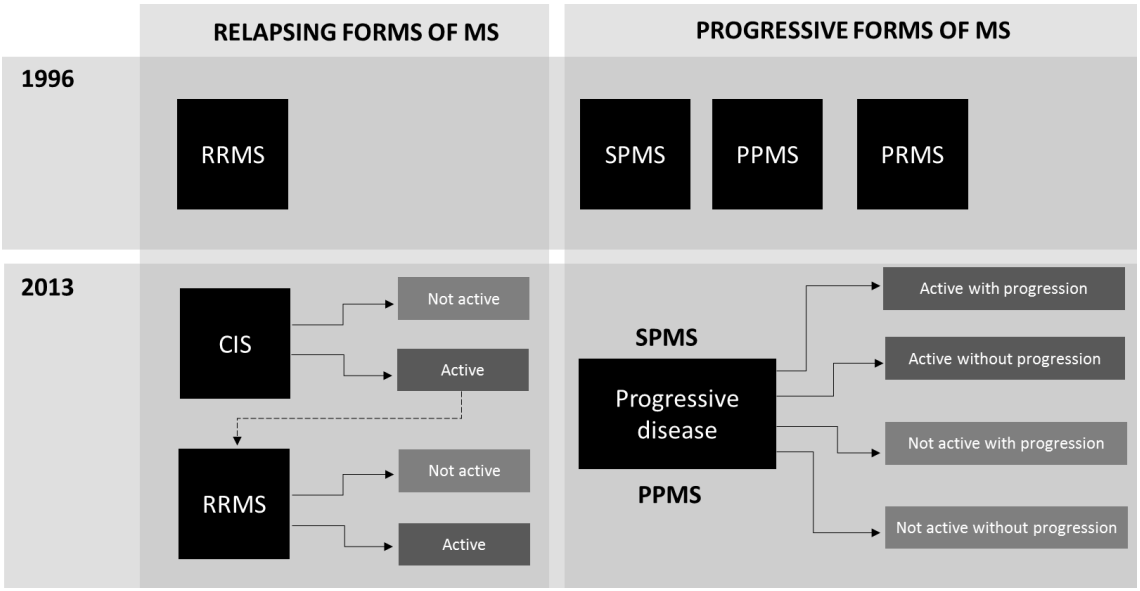

Figure 1. The 1996 vs. 2013 classification of multiple sclerosis clinical course

Abbreviations: MS: multiple sclerosis; SPMS: secondary progressive multiple sclerosis; RRMS: relapsing remitting multiple sclerosis; CIS: clinically isolated syndrome; PPMS: primary progressive multiple sclerosis; PRMS: progressive relapsing multiple sclerosis.

Adapted from Lublin *et al* [35] and Cerqueira [37].

3. The diagnosis of MS

MS diagnostic criteria have evolved over time to include the use of paraclinical markers, especially magnetic resonance imaging (MRI), as supportive evidence of the diagnosis. The ultimate goal is to reach a definite diagnosis earlier in the disease course than a strict reliance on clinical features would allow and, as such, an early treatment.

Despite these advancements, current criteria still rely on the key principles of MS diagnosis proposed by Schumacher et al. [38] in 1965: (I) demonstration of dissemination of CNS lesions in space; (II) demonstration of dissemination of CNS lesions in time and (III) exclusion of alternative etiologies. However, these criteria were based exclusively on clinical history and examination.

In 1985, the Poser criteria arising from the Workshop on the Diagnosis of Multiple Sclerosis supplanted previous criteria [39]. These criteria were the first to incorporate the MRI into the diagnostic process and established the fundamental concept that MRI is able to provide evidence for both dissemination in space and dissemination in time. In fact, MRI was included together with computed tomography, hyperthermia testing, evoked potentials, and urodynamic evaluation in the category of "paraclinical evidence" useful to establish the diagnosis when the clinical examination was not sufficient. Poser separated cerebrospinal fluid (CSF) evidence of intrathecal immunoglobulin synthesis from the other paraclinical assessments to allow the diagnosis of "laboratory-supported" definite or probable MS in circumstances in which the clinical and paraclinical features were insufficient to demonstrate dissemination in space or dissemination in time.

Thereafter, various groups looked for specific MRI characteristics that allowed the diagnosis of MS after a single relapse, i.e. clinically isolated syndrome [40-42]. These MRI features were subsequently used to develop the most recent diagnostic criteria, known as the "McDonald criteria", which were initially published in 2001 [43] and subsequently revised and adapted in 2005 [44], and lastly in 2010 [45].

The 2010 revision to the McDonald Criteria (Table 1) allow that dissemination in space and time can be established by a single scan and consequently permits a more rapid diagnosis of MS, with improved sensitivity while maintaining the specificity of the past versions of the criteria [46]. CSF testing to support the diagnosis of MS is no longer required by the 2010 criteria, but it might be relevant in certain patients, particularly those for whom MRI is not entirely diagnostic or reveals features that are unusual in MS [45]. 1

Since the last update of these criteria, many improvements in MRI technology have occurred and new data on application of MRI to establish dissemination in space and time have become available. Therefore, it is expected that a modification of the current MRI criteria will soon emerge based on the recent expert-opinion consensus of the MAGNIMS Study Group [47].

Table 1: 2010 revision of the McDonald criteria

MS subtype	Dissemination in space	Dissemination in time
Relapsing–remitting	One or more lesions in each of two or more characteristic locations* All symptomatic lesions excluded in brainstem and spinal cord syndromes	One of the following criteria: - New T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of the timing of the baseline scan - Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time
Primary progressive	Two of the following criteria: - Presence of one or more T2 lesions in at least one area characteristic of MS (excluding the spinal cord)* - Presence of two or more T2 lesions in the spinal cord - Evidence of oligoclonal IgG bands and/or increased IgG index in the cerebrospinal fluid	1 year of disease progression (retrospectively or prospectively determined)

*Characteristic areas for MS lesions include the posterior fossa, juxtacortical regions, periventricular regions and spinal cord.
Abbreviation: MS: multiple sclerosis.

Adapted from Polman *et al.* [45]

4. The etiopathogenesis of MS

The exact cause of MS still remains elusive, but it is currently accepted that the disease occurs in genetically susceptible individuals exposed to stochastic environmental factors.

Genetic variants accounts for approximately 30% of the overall disease risk, with more than 100 distinct genetic regions being identified as MS-associated genes by genome-wide association studies [48, 49]. The majority of these genes are involved in immunological pathways, including the *HLA-A*02:01*(protective effect) and *HLA-DRB1*15:01*(risk effect) variants, and the genes encoding the α-chains of the IL-2 and IL-7 receptors, emphasizing the prominent role of the

immune system in disease predisposition [50]. On the other hand, the best-confirmed environmental factors associated with increased disease susceptibility are *Epstein Barr Virus* (EBV) infection, smoking and low levels of Vitamin D [51, 52]. More recently, gut-microbiota and nutritional salt intake have been recognized as potential contributors to disease susceptibility or modulators of the disease course [53-55]. Furthermore, there is also evidence that early childhood and adolescent obesity interacts with genetic and environmental factors to increase MS susceptibility [56, 57].

Notwithstanding the lack of a clear defined etiology, what is known for certain about the pathogenesis of MS is that it is an immune-mediated disease. However, it remains an open question whether MS is triggered in the periphery or in the CNS [50, 58]. The most widely accepted hypothesis for the pathogenesis is that autoreactive T cells are activated first in the periphery and are then transferred to the previously not harmed CNS *(outside-in hypothesis)* [59, 60]. The activation of autoreactive T cells against myelin-sheath and oligodendrocytes components in the peripheral tissues might be a result of direct cross reactivity, molecular mimicry, or bystander activation. After migration to the lymph nodes, these T cells and B cells will invade the CNS by crossing the blood-brain barrier or the blood–CSF barrier at the choroid plexus [50]. Release of inflammatory mediators will open the blood–brain barrier and attract the influx of monocytes and additional lymphocytes, leading to the formation of the inflammatory demyelinating lesion [58].

In an alternative hypothesis, CNS-intrinsic events may trigger the disease onset, with the infiltration of autoreactive lymphocytes occurring as a secondary phenomenon *(inside-out hypothesis)* [59, 61, 62]. These CNS-intrinsic events might include a chronic latent viral infection of CNS, namely by the recently identified *Multiple Sclerosis Associated Retro Virus* (MSRV) [62], or a primary defect of oligodendrocytes causing its spontaneous death [61]. Any of these events would cause the subsequent activation of resident microglia with a secondary recruitment of adaptive and innate immune cells from the periphery in the predisposed patient, possibly further driving neurodegeneration [58].

As the disease progresses, immune cell infiltration of the CNS from the periphery wanes, possibly due to exhaustion of the peripheric immune system from chronic antigen exposure [50]. However, chronic CNS-intrinsic inflammation and neurodegeneration continues. Meningeal tertiary lymphoid-like structures, mainly composed by B cells aggregates, and the CNS-resident innate cells, may contribute to late-stage chronic inflammation in patients with progressive forms of MS [63].

Regarding the neurodegenerative component of the disease, a contemporary view is that MS encompasses an intermingling of inflammation and diffuse chronic neurodegeneration from the onset. Multiple mechanisms are hypothesized to contribute for neurodegeneration, including direct immune attack to axons or neurons; "bystander injury" because demyelination exposes axons to the chronic inflammatory milieu; lack of trophic support and altered expression of ion channels in demyelinated axons; and oxidative stress resulting in mitochondrial injury and subsequent induction of demyelination and neuronal death [64, 65]. Oxidative stress seems to be mainly driven by chronic inflammation, which results in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), but these effects might be amplified by age-dependent iron accumulation in the brain and by mitochondrial gene deletions [64]. These degenerative mechanisms can spread from the initial site of axonal injury towards the neuronal cell body (retrograde degeneration or "neuronal dying back") or towards the distal axon terminal (anterograde degeneration or Wallerian degeneration) and can also affect adjacent presynaptic and postsynaptic neurons respectively, ultimately leading to neuronal apoptosis [50].

After several years of disease, when the patients have reached a threshold level of irreversible tissue damage in CNS and an exhaustion of functional compensation mechanisms, a progressive clinical deterioration develops, heralding the onset of secondary progressive phase of MS [64].

PPMS can be regarded as having the same sequence of pathogenetic events described in SPMS but in which an "amputed" relapsing-remitting phase was not evident clinically because the inflammatory lesions did not cross the clinical threshold [66]. On the other hand, other authors

still argue that PPMS is primarily neurodegenerative in its pathogenesis and therefore may be a distinct disease entity, apart from the MS disease spectrum [67] .

5. The pathology of MS

The classic pathological hallmark of MS is the sclerotic plaque, which represents the end stage of a process involving inflammation, demyelination, remyelination, astrocytosis, and neurodegeneration [26, 68]. These pathological features are all present in both RRMS and SPMS, as well as in PPMS although they vary both quantitatively and qualitatively between these three forms of MS and among patients with the same form [64].

Much progress has been made in knowledge and MS is now recognized as a disease not limited to the sclerotic plaque, affecting the WM diffusively, including the normal-appearing WM (NAWM), and also the GM.

5.1. White matter pathology

Multifocal inflammatory demyelinating lesions of the WM are the classical pathological feature of MS. These lesions can occur anywhere in the brain or spinal cord but tend to be most common in the periventricular regions, the corpus callosum, the optic nerves, the corticomedullary junction, and the subpial section of the brainstem. In the spinal cord, lesions are most often observed in the anterior columns flanking the median fissure, centrally in the dorsal columns, and subpially [65].

The acute lesions, which dominate in the relapsing–remitting stage of MS, consist of perivascular inflammatory infiltrates, dispersion of lymphocytes throughout the tissue, and substantial macrophage and microglial activation [64]. The reference work of Lucchinetti et al. [19] have revealed a profound heterogeneity in immunopathologic mechanisms of demyelination in these active lesions, segregated in four distinct patterns: Pattern I: Macrophage associated

demyelination; Pattern II: Macrophage associated demyelination with local precipitation of immunoglobulins and activated complement (antibody associated demyelination); Pattern III: Demyelination with primary alterations in the most distal oligodendrocyte processes and oligodendrocyte apoptosis (distal dying-back oligodendrogliopathy associated demyelination); Pattern IV: Primary degeneration of oligodendrocytes in the periplaque WM with secondary myelin destruction (primary oligodendrogliopathy). However, there is a lack of consensus regarding whether lesion heterogeneity can exist within the same patient, being stage-dependent [69], or in contrast, is patient-dependent and reflects distinct pathogenic subtypes of MS [70].

Remyelination accounts for the appearance of shadow plaques. It is most active during the acute inflammatory process coinciding with phagocytic removal of myelin debris, but also occurs in the progressive phase [26]. The mature CNS maintains a pool of oligodendrocyte precursors that can migrate in response to semaphorin 3A and 3F to surround the lesions and potentially remyelinate the axons [71]. However, a successful remyelination only occurs in approximately 20% of the cases, with cycles of demyelination and remyelination apparently exhausting the capacity for tissue repair [72].

Finally, the chronic inactive MS plaque is sharply circumscribed, relatively hypocellular, with marked myelin loss, prominent fibrillary astrocytosis, and reduced axonal density [73]. There is no evidence of active myelin-breakdown, and mature oligodendrocytes are markedly diminished or absent [73]. Additionally, scarce chronic inflammatory infiltrates consisting of T-lymphocytes and macrophages may still be present, particularly in the perivascular regions [73].

Axonal injury is also a prominent feature of MS lesions which occurs early in the disease course, particularly in acute lesions and in the borders of chronic active lesions, as reported firstly in the seminal paper of Trapp et al. [74]. This finding is consistent with other studies showing that amyloid precursor protein, a sensitive marker of axon damage, is expressed in many axons in acute lesions and the borders of chronic active lesions [75], and that brain levels of N-acetyl

aspartate (NAA), a putative marker of axon integrity, are significantly reduced in patients with early MS [76].

Besides focal lesions, the NAWM of patients with MS also show diffuse and global changes, including widespread inflammation, microglial activation, astrocytic gliosis, and mild demyelination and axonal loss [77]. The extent and severity of diffuse WM injury increases with disease duration and is most pronounced in the progressive forms of MS. More recently, studies with advanced MRI techniques as proton magnetic resonance spectroscopy (MRS) [78], diffusion tensor imaging (DTI) [79, 80] or magnetization transfer imaging (MTI) [81], allow to reveal the full extent of damage in NAWM not detected by conventional MRI, therefore confirming those histological findings. From a clinical point of view, the NAWM damage significantly correlates with physical disability and cognitive impairment in MS patients, and its evaluation with the abovementioned MRI techniques holds promise as a prognostic marker of disease course and as a tool for monitoring treatment response.

5.2. Grey matter pathology

The involvement of GM in MS was recognized since the early pathological descriptions of the disease in the 19th century [82]. Nevertheless, this finding was somehow neglected afterwards, mostly due to technical difficulties involved in the visualization of GM lesions using classical myelin histochemical staining methods [83].

It was only in the last decades that new tissue processing and immunohistochemistry methods targeting myelin proteins and advanced MRI techniques confirmed that GM damage is a key component of MS pathology. It begins early in the disease course but is clearly more extensive in patients with longer disease duration [84]. In fact, numerous cross-sectional and longitudinal studies revealed that GM damage is a better predictor of physical disability and cognitive impairment than WM damage [85, 86]. Moreover, the damage of GM is global and involves not only the neocortex but also the hippocampus and the subcortical nuclei [83].

The cortex in MS may be involved either as demyelinated focal lesions or as diffuse neuronal loss and atrophy. Regarding the former, although several classifications were proposed to distinguish cortical lesions types, for practical purposes these can be best grouped in 3 subtypes according to their location within the cortex [73, 84, 87, 88]: (I) Subpial lesions, the most common subtype, extending from the pial surface to cortical layer three or four, or to the entire width of the cortex; (II) Intracortical lesions which are small demyelinated lesions confined within the cortex with the sparing of both superficial cortex and adjacent WM; (III) Leukocortical lesions involving both GM and WM at the grey matter–white matter junction with sparing of the superficial cortical layers.

Conventional MRI techniques fail in detecting intracortical and subpial lesions. While recent imaging protocols using double inversion recovery (DIR) sequences and high-field MRI have markedly improved their detection, most cortical lesions are still not visualized by any MRI technique [89, 90].

Cortical lesions are fundamentally different from those seen in the WM, at least in autopsy cases, presenting a smaller amount of inflammation and blood–brain barrier damage, minimal oedema and less disruption of the cytoarchitecture [88, 91]. To further complicate the scenario, the pattern of inflammation that occurs is variable depending on the type of cortical lesion, with leukocortical lesions having higher counts of inflammatory cells than those that are exclusively intracortical or subpial [92, 93]. Additionally, subpial lesions are topographically associated with meningeal inflammatory infiltrates [93-95]. Another general feature of cortical plaques in all patients with MS is the massive activation of cortical microglia [87, 92].

Besides focal demyelination, the normal appearing cerebral cortex of patients with MS is also affected by diffuse neuronal and synaptic loss. The neuronal loss is more severe in MS cases where overlying meningeal inflammatory lymphoid-like follicles are present and specific neuronal subpopulations seem to be particularly vulnerable [96, 97]. Synaptic loss is also a prominent feature of cortical pathology, with one study showing a marked reduction in synaptophysin [98].

MRI studies focused on imaging the normal appearing GM, with MTI [99, 100], DTI [101, 102], MRS [103, 104] and gradient echo MRI [105], confirm the diffuse abnormalities found in histological studies. Moreover, application of automated MRI volumetric methods consistently revealed a decreased GM volume as well as cortical thinning in patients with MS, occurring early in the disease course and across different MS types [106-109].

MRI and histopathological studies have shown that atrophy and demyelinated lesions can also occur in subcortical GM structures such as the thalamus, hippocampus, caudate, putamen, pallidum, claustrum, hypothalamus, amygdala and substantia nigra [83, 110, 111]. These structures are also affected since the early stages of the disease, supporting the concept of "diffuse brain involvement" in MS. Amongst the subcortical GM structures, the thalamus has been the most extensively studied [112, 113], and is currently recognized as being severely affected by MS pathology since the CIS phase. The pathologic features of the MS-related thalamic damage include focal demyelinated lesions, which have less adaptive and innate inflammation when compared with the classic active WM lesions, but also diffuse microglial activation, decreased nonlesional neuronal density and reduction of global thalamic volume[112].
Thalamus is a well-known "relay organ", centrally positioned within the cortical-subcortical neuronal loops which underlie a wide range of neurologic functions including motor, sensory, integrative, and higher cortical functions [114]. As such, damage to the thalamic nuclei and their connections translates into significant clinical manifestations, including cognitive impairment. Due to the particular susceptibility to MS neuropathology from the earliest disease stages and since thalamic volume can be reliably measured with the help of automated MRI volumetric methods, thalamus atrophy is a potential imaging biomarker of prognosis and treatment response [112].

The causes of GM pathology in MS remain unclear and might be different from those leading to WM damage. One of the main hypothesis is that meningeal inflammation releases myelinotoxic agents which then diffuse into the cortex causing demyelination, especially in the case of subpial lesions [86]. Mitochondrial dysfunction is one of the other proposed hypothesis, as neurons with

deficiencies in the respiratory chain have been discovered in the cortex of patients with MS, irrespective of the presence of GM lesions [115]. The combination of a redistribution of sodium channels along demyelinated axons and mitochondrial abnormalities could generate a disbalance in cellular energy, leading to dying back axonopathy, and ultimately neuronal death [86].

6. Cognitive impairment in MS

Cognitive impairment in MS was documented as early as 1877 by Charcot. He observed that some patients might have "marked enfeeblement of the memory; conceptions are formed slowly; the intellectual and emotional faculties are blunted" [82]. Nevertheless, cognitive impairment was forgotten during most of the following century and the main focus was directed towards physical disability.

It was only in the early 1980s that the systematic use of formal neuropsychological testing and the advent of MRI have greatly contributed to improve understanding of the prevalence and pathological correlates of cognitive disorders in MS. Since then, there has been an outpouring of research on MS-related cognitive impairment which is now recognized as a common and early manifestation of MS. More recently, the search for effective strategies for managing cognitive dysfunction has led to trials with pharmacologic agents and cognitive rehabilitation.

6.1. Prevalence and impact

The first population-based study of cognitive impairment in MS patients was performed by Rao et al. who identified a prevalence rate of 43% [116]. Afterwards, multiple studies have been performed with prevalence rates ranging from 40% to 70% depending on the research setting (clinic-based or community-based studies) and the diagnostic methodology [30]. Both cross-sectional studies and longitudinal studies indicate that cognitive impairment is the strongest predictor of unemployment among MS patients [117-119]. Moreover, cognitive impairment in

MS is associated with higher rates of divorce, reduction of several dimensions of quality of life and limitation of the patients adherence to treatment [119-121].

Cognitive impairment affects patients across all MS subtypes, occurring since the early phases of the disease. CIS patients show cognitive impairment in a pattern similar to RRMS, with studies reporting prevalence rates from 20% to 50% [122, 123]. The cognitive deficits tend to worsen as the disease progresses, thus the more severe levels of cognitive impairment are usually found in the secondary progressive phase of the disease [124]. Regarding PPMS, the few studies assessing cognitive functions estimate a frequency of cognitive impairment ranging from 7% to 50% [125-127]. Most of these studies rely on single-center small cohorts, which may account for the widely varying frequency of cognitive impairment and inconsistent results when comparing PPMS with other MS subtypes. While cognitive performance of patients with progressive forms of MS has been consistently shown to be poorer than those with RRMS, studies comparing SPMS with PPMS have yielded contradictory results [128-130]. The majority of studies have reported a more severe impairment in patients with SPMS compared to patients with PPMS [131, 132] but others found similar cognitive deficits in both subtypes [133, 134].

Even in the so-called "benign MS", "in which the patient remains fully functional in all neurological systems 15 years after disease onset"[31], cognitive impairment was found to occur in up to 45% of the patients, with a significant impact on work and social life [135-138]. Therefore, maintenance of a normal cognitive profile has been recently suggested for inclusion as an additional criterion in the definition of benign MS [136].

Cognitive impairment also occurs in pediatric MS, with a prevalence rate of approximately 30% [139-141]. Furthermore, around 30% of the children had an Intelligence Quotient (IQ) in the lower range (<90) and 8% had an IQ score less than 70 [139]. The main neuropsychological difference between children and adults with MS occurs in the language domain. In pediatric MS, an involvement of language is frequently seen in contrast to adult-onset MS that rarely has language problems [139, 141, 142], probably because children are still developing linguistic skills.

Children with MS may be more susceptible for cognitive dysfunction, possibly due to the disruption of myelinogenesis and neural networks in the developing CNS [142]. Moreover, difficulties on coping with chronic illness and school absences occurring during key formative periods may have negative consequences on children's academic attainment.

6.2. Neuropsychological profile

The pattern of MS-related cognitive impairment is well characterized and is typically confined to specific cognitive domains rather than presenting as a global impairment. Overt dementia is rare [143] and usually the general intelligence is normal or shows only slight decrements [116, 144].

The cognitive domains frequently affected in MS are information processing speed, working memory, attention, verbal and visuospatial episodic memory, and executive functioning. Conversely, language and semantic memory are rarely involved [145, 146].

A reduced speed of information processing is the most common cognitive deficit in MS [147, 148]. The slowness in processing speed is independent of stimulus modality, as documented in studies examining performance on both visual and auditory processing speed tests [149]. The reduced speed of information processing is usually associated with impairment on working memory (i.e. the ability to maintain and manipulate information in the brain for a short time period), both contributing for a major disruption of information processing efficiency [143]. Therefore, patients with MS perform cognitive tasks slowly and have significant difficulties when dealing with new information: characteristically they recall less on a first learning attempt and have slower improvement in subsequent trials of learning than normal controls [150-152]. Moreover, attention deficits also contributes for the impairment on learning new information. Typically, basic attention tasks (e.g. attention span) are unaffected in patients with MS whereas more complex aspects of attention, as sustained attention or divided attention, are impaired [146]. Additional factors that may underlie the difficulties on learning new information may be executive dysfunction and perceptual deficits [143].

Memory failure is a very common complaint reported by MS patients. However, the basic processes of memory that are affected in MS are still a matter of debate [153]. Usually, semantic knowledge and information storage are preserved in MS. It is the recall of newly learned information that is compromised, which may be related to an impaired information-acquisition process or with a primary retrieval deficit [153-155]. Additionally, memory failures may be also related to deficits in other cognitive domains, such as information processing speed and executive functions, which affect encoding processes [156].

Executive functions are also affected in patients with MS, including abstract and conceptual reasoning, problem solving, verbal fluency, planning, and organization [145, 157]. These deficits may interfere with other cognitive and motor tasks and have a negative impact on daily living activities [145].

6.3. Neuropsychological testing

A comprehensive neuropsychological evaluation is time-consuming, expensive, and requires well-trained professionals, thus hindering its applicability in all patients with MS. Furthermore, the MS-related fatigue can also be a limitation for administering extensive assessment batteries. Therefore, it is of paramount importance that clinicians might have at their disposal brief and well-validated screening instruments to identify those patients who need a comprehensive assessment. Moreover, the development of brief batteries that cover the cognitive domains most often impaired in MS contributed to the implementation of neuropsychological evaluation in clinical routine practice.

Regarding possible screening instruments, many studies support the high validity, discriminative ability and test-retest reliability of the Symbol Digit Modalities Test (SDMT), a measure of visual information processing speed [158]. However, a potential limitation is that it will miss those MS patients who are impaired in other domains frequently affected in MS patients [30, 158]. Other alternatives studied were the Mini-Mental State Examination (MMSE)[159] but it was found to

be insensitive for detection of MS-related cognitive impairment (sensitivity levels ranging from 28% to 36%) [160]. Conversely, the few published studies analyzing the use of The Montreal Cognitive Assessment (MoCA) in MS suggest it may be usefulness as a brief screening instrument for the detection of cognitive impairment in MS patients [161]. Freitas et al. [162] found that the cut-off point for identifying cognitive impairment in MS patients was a MoCA total score below 26 points. Additionally, the authors proposed a EM-MoCA-Subscore composed by the scores of executive functions, visuospatial, short-term memory and temporal/spatial orientation domains, which can reduce the administration time for cognitive screening in clinical setting. The maximum score of the EM-MoCA-Subscore was 19 and the cut-off for cognitive impairment was below 17.

Among the brief batteries, the Brief Repeatable Neuropsychological Battery (BRB-N), developed and validated by Rao et al. [163], can be administered in 1 hour or less (Table 2). The BRB-N was found to have a sensitivity of 71% and specificity of 94% in discriminating cognitively impaired from cognitively intact MS patients [164]. However, this battery does not have a measure of visual/spatial ability or executive functions which are frequently affected in MS patients.

In 2002, a panel of specialists with expertise in the field of MS-related cognitive impairment developed a battery for a minimal neuropsychological testing of the main cognitive domains affected in MS patients [165]. This battery, the Minimal Assessment of Cognitive Function in MS (MACFIMS), is similar to the BRB-N but with additional tests covering visual/spatial ability and executive functions, with an administration time of 90 minutes (Table 2). It was also recommended that premorbid cognitive ability, fatigue and depression should be measured since these factors can affect cognitive performance. Impairment on one test was defined as worse than 1.5 standard deviations below the mean and cognitive impairment was defined as impairment on at least two domains in the MACFIMS battery.

More recently, a shorter battery named Brief International Cognitive Assessment for Multiple Sclerosis (BiCAMS) which can be completed in 15 minutes and comprises the evaluation of information processing speed, visual/spatial memory and verbal memory has been proposed

[166] (Table 2). Its validation is currently under development in different languages and countries, including in Portugal.

Table 2. Neuropsychometric batteries commonly used in multiple sclerosis.

Domain	BRB-N	MACFIMS	BiCAMS
Processing speed (auditory) and working memory	PASAT	PASAT	
Processing speed (visual)	SDMT	SDMT	SDMT
Verbal memory (learning and recall)	SRT	CVLT2	CLVT2*
Visual/spatial memory (learning and recall)	SPART	BVMTR	BVMTR*
Verbal fluency	COWAT	COWAT	
Spatial processing		JLO	
Executive function		D-KEFS	

*For the BiCAMS, only the immediate recall portion of the CVLT2 and BVMTR are included, while both the BRB-N and MACFIMS include both the immediate and delayed recall test.
Abbreviations: BVMTR = Brief Visuospatial Memory Test-Revised; COWAT = Controlled Oral Word Association Test; CVLT2 = California Verbal Learning Test-2nd edition; D-KEFS = Delis-Kaplan Executive Function System; JLO = Judgment of Line Orientation test; PASAT = Paced Auditory Serial Addition Test; SDMT = Symbol Digit Modalities Test; SPART = 10/36 Spatial Recall Test; SRT = Selective Reminding Test.
Adapted from DeLuca *et al.[30]*

6.4. Neuroimaging correlates

Over the last decades, there has been a valuable contribution of both conventional and new techniques of MRI to further elucidate the association between brain structure and function, as well as the effect of MS pathology on cognitive functioning. The relationship is complex and seems to involve the contribution of several pathologic mechanisms, including not only WM focal lesions but also diffuse abnormalities in WM that appears to be normal on the conventional MRI (NAWM), and involvement of both cortical and subcortical GM as well.

6.4.1. Structural MRI

In cross-sectional studies, cognitive impairment in MS has only a mild to moderate correlation with brain WM lesion load on T2 and T1 MRI sequences [167]. On the other hand, MRI studies have consistently found that measures of brain atrophy correlate better with cognitive impairment than those classic indices of WM lesion load. Whole brain atrophy has been found to be a good predictor of cognitive impairment in MS patients but it seems to be supplanted by the atrophy of GM, either cortical GM or subcortical GM atrophy [30].

Cortical atrophy has been shown to have a robust correlation with the performance on various cognitive tasks and with the severity of cognitive impairment in patients with MS [168]. Importantly, in longitudinal studies cortical volume decrease was confirmed to be a reliable parameter discriminating between patients with cognitive decline and those with a stable or improving cognitive performance [169]. Additionally, cortical lesions identified by the new sequence DIR, have been found to contribute for cognitive impairment independently of the cortical volume [170].

Regarding the role of subcortical GM pathology for MS-related cognitive impairment, the first clue was the finding that the width of the third ventricle has a strong association with cognitive performance [171, 172]. It was speculated that this robust relationship may have been due to thalamic atrophy, as the thalamus border the third ventricle [172]. This hypothesis was proven to be accurate afterwards and thalamus atrophy is currently recognized as a strong predictor of cognitive impairment in MS, particularly of reduced information processing speed [173]. The atrophy of the thalamus has been demonstrated in CIS patients and pediatric MS patients, representing one of the earlier markers of GM pathology [174, 175]. More recently, the contribution of pathology in other subcortical GM structures has been acknowledged. Atrophy and T2 hypointensities of basal ganglia were both found to be more frequent in patients with MS cognitively impaired [111, 176, 177].

There are also studies suggesting the role of hippocampal atrophy in MS-related cognitive impairment. Hippocampal volumes were reported to be lower in RRMS and SPMS when compared with healthy controls, more evident on the CA1 regions in RRMS and globally in SPMS. The lower hippocampal volumes were associated with worst performance on memory and information processing speed tasks [178].

Besides the relevant role of GM pathology for cognitive impairment in MS, the disruption of the WM pathways mediating the transmission of information across distributed brain networks is also a potential contributory mechanism. However, studies with conventional MRI have failed to identify a robust correlation with the burden of visible WM lesions in the brain. The use of advanced MRI techniques as MTI, DTI and MRS, allowed to confirm that occult diffuse damage of NAWM is significantly associated with cognitive dysfunction in MS [179-185].

6.4.2. Functional MRI

The application of functional imaging to study brain function has provided valuable insight into the pathologic basis of cognitive impairment in MS. As a matter of fact, cognitive performance of MS patients does not simply depend on the extent of brain tissue destruction, but also on the effectiveness of reparative mechanisms and neural plasticity, including cortical reorganization. This may explain the suboptimal correlation between structural MRI and neuropsychological findings.

Initial studies using single photon emission computed tomography (SPECT), positron emission tomography (PET), and perfusion MRI, have shown a correlation between cognitive impairment and reduced cerebral blood flow and metabolism in specific brain areas [186-189].

More recently, studies with functional MRI (fMRI) have consistently demonstrated functional cortical changes in MS patients. Overall, in fMRI studies using different cognitive tasks, MS patients with normal cognitive performance or with only mild cognitive impairment exhibited

increased activation of the key regions normally involved in the performance of the task plus a recruitment of several additional brain areas, compared with healthy controls. Conversely, in severely impaired patients, the brain activation pattern was comparable to that of the healthy controls [190-192]. A plausible interpretation of these fMRI changes is that it may reflect an adaptive cortical reorganization resulting from neural plasticity which may allow MS patients to have normal cognitive performance despite brain pathology. On the other hand, the failure or exhaustion of the capacity to activate compensatory strategies may lead to severe cognitive impairment in MS [193].

In addition to identifying potentially compensatory changes, fMRI has also shown a number of maladaptive modifications in patients with MS. In a recent longitudinal fMRI study, using an information processing speed task (SDMT), patients with MS (but not healthy controls) exhibited an increment of activation in parietal regions over time, which correlated with worst performance on the SDMT [194]. This finding suggests that the over-recruitment of this cortical region is detrimental for the information processing speed performance.

Finally, investigating the brain 'at rest' may provide additional information about the pathologic mechanisms underlying cognitive impairment in MS. Among the resting state brain networks, the default-mode network is particularly relevant because it is deactivated during performance of cognitive tasks. Activity of the default-mode network has been shown to be impaired in MS, particularly in patients with progressive MS and in those with cognitive impairment [195, 196].

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CHAPTER 2 SOCIAL COGNITION AND THEORY OF MIND

"I feel afraid in this world. It's as if you're on a journey to a very strange country. You don't know the language and everything is different. They do make gestures, but I don't understand a single one. They do exchange glances, but I don't understand a single one of those either."

Dominique Dumortier. From another planet: autism from within (2004).

1. Definition of social cognition and theory of mind

The human species is highly social, constantly building and maintaining diverse relationships throughout the lifespan. The success of human interaction depends upon the ability to identify cognitive and emotional states in others, or in other words, depends on normal social cognitive skills [1]. Despite the challenges posed by the normal variability of sociability and behaviours between people, there has been an increasing understood of human social behaviour through the lens of neuroscience leading to the emergence of social cognition as a demarcated module of cognition and as an active field of research. Following pioneering work in autism [2], social cognitive impairment has been reported in a range of psychiatric, developmental, and neurodegenerative disorders [3-5].

Numerous attempts to define social cognition have been made. Broadly, social cognition refers to the processing of social information in the brain by which people understand themselves and other people [6]. Rather than being a unitary process, social cognition is a collection of multifaceted processes that recruit multiple brain structures. The basic cognitive processes includes information processing of social stimulus (the self, other people or the interaction of the two) and about the norms and procedures of the social world. These processes are likely to occur at both automatic and controlled levels of processing and will also be influenced by motivational biases [6].

The core domains of social cognition are emotion recognition and theory of mind (ToM). Collectively these drive interpersonal skills such as empathy, and have important implications for social functioning [7].

Emotion recognition refers to an individual's ability to identify and discriminate between the emotional states of others [8]. One of the most influential studies of emotions which compared the way emotional expressions were categorised and posed across different cultures concluded that there are six basic emotional categories: happiness, sadness, disgust, anger, surprise and fear [9]. Recognition of more complex emotions such as jealousy, pride, embarrassment and guilt have been viewed as somewhat different compared to basic emotions that are easily recognisable from viewing a person's face. Recognition of more complex emotions may also imply awareness of another person's attitude to oneself or awareness of oneself in relation to other people [8].

The term 'Theory of Mind' was originally introduced by primatologists Premack and Woodruff [10] to account for their findings that chimpanzees could solve tests which involved inferring the mental states of other chimpanzees. ToM, also referred to as "mind reading", was defined as the ability to make inferences about the mental states of others and to appreciate how these mental states might differ from our own [11]. This concept was then adopted by child psychologists to refer to the development of mental perspective-taking in infants and young children [12]. ToM ability is established early in life, by age 3–5 years independently of the culture, and is thought to comprise distinguishable but overlapping cognitive and affective components [7]. Affective ToM requires an understanding of others' emotions, affective states or feelings whereas cognitive ToM requires an understanding of others' cognitive states, beliefs, thoughts or intentions [1]. By allowing us to understand others' cognitive states and emotions, ToM has an important role in prosocial behaviour, contributing to explain and predict behavior of others.

2. The neuroanatomy of social cognition and theory of mind: the social brain

The concept that there is a social brain in humans specialized for social interactions was firstly proposed by Brothers in her seminal review [13]. The function of the social brain is to make predictions during social interactions about people's actions on the basis of their mental states [14]. The better we can predict what someone is going to do next, the more successful our interactions with that person will be. The largely automatic process by which we "read" the mental states of others is called mentalizing and is based on the assumption that we have a ToM [11, 15].

Social brain refers to the large-scale brain networks composed by a broad array of brain regions, highly interconnected, which have been consistently associated with social cognition performance in functional neuroimaging studies or whose structural damage has been linked to social cognitive deficits [14] [16]. Although there is some variability across review papers, there is a relatively high consensus on the importance of the amygdala, the prefrontal cortex (especially the medial prefrontal cortex and the orbitofrontal cortex), the anterior cingulate cortex, the superior temporal gyrus and the temporo-parietal junction (Figure 1) [14, 16].

Specific roles for the various components of the social brain are beginning to emerge (Figure 1). The amygdala has been proposed to be the main hub of the networks involved in social abilities [17]. It has been recognized that the amygdala´s role extends beyond the well-established contribution for processing basic emotions and reward learning to a broader role in making social judgments [18, 19]. A growing body of evidence suggests that the amygdala anchors three partially distinct corticolimbic networks with dissociable social functions [17]: (1) a network supporting "perception", anchored on the ventrolateral sector of the amygdala and including fusiform gyrus, superior temporal gyrus, entorhinal cortex, lateralorbitofrontalcortex and posterior cingulate gyrus, which are involved in the detection and decoding of expressive aspects of faces and bodies; (2) a network supporting "affiliation", important for the processes associated with motivating prosocial or affiliative behaviors, centered on the medial region of amygdala and

including medial orbitofrontal cortex, rostral anterior cingulate gyrus, ventromedial striatum, dorsomedial temporal pole, and medial temporal lobe; (3) a network supporting "aversion", important for the processes enabling avoidant behaviours, such as avoiding an untrustworthy-appearing stranger, anchored by nuclei within the rostrodorsal sector of the amygdala and including insula, caudal anterior cingulate cortex, ventrolateral striatum, hypothalamus, autonomic and dopaminergic nuclei of the brainstem.

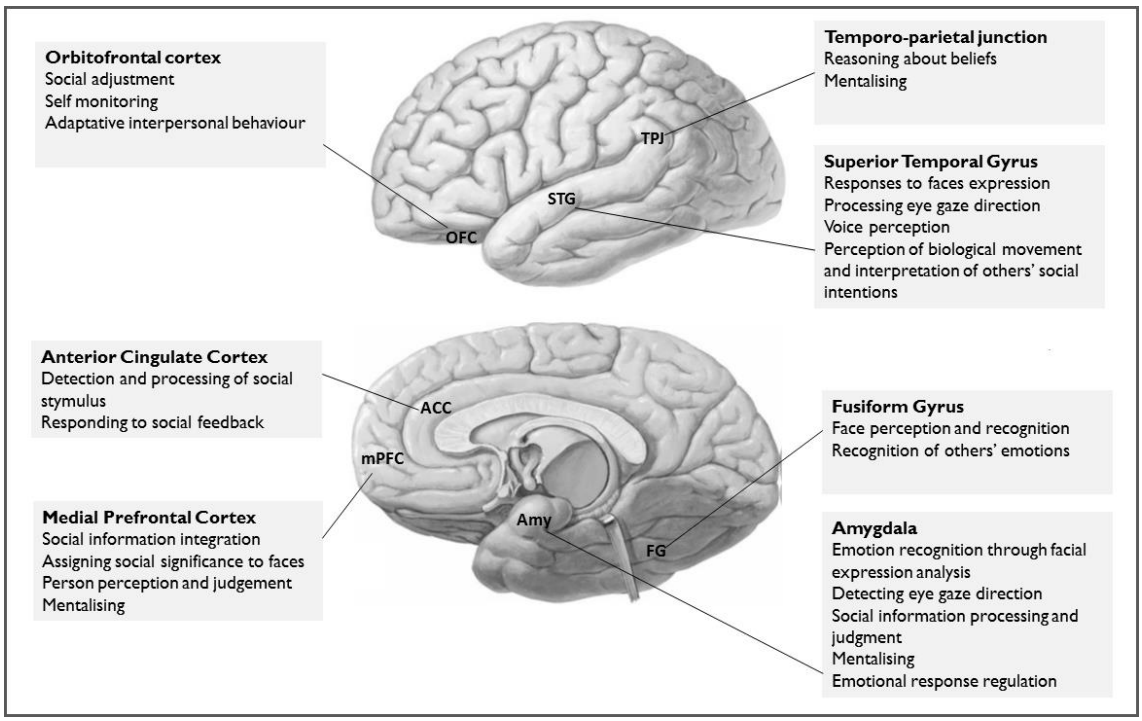

Figure 1. The main nodes of the social brain network.
Adapted and modified from the Atlas of Neuroanatomy and Neurophysiology: Selections from the Netter Collection of Medical Illustrations [20]

Additionally, a "mirror neuron" system has been found in the brain of monkeys and humans which is thought to play a role on the social cognitive strategy that allows understanding other person's goals and intentions by simulating their behaviours [21, 22]. The brain's mirror system is not tied to any particular brain region and is composed by functionally specialized neurons,

known as mirror neurons, present in different cortical areas. The location of the activation will depend upon what is being observed [14]. For example, the ones in the parietofrontal network are visuomotor neurons, which discharge both when performing and observing a goal-directed action. The ones in the insula and anterior cingulate mediate the understanding of other people's emotions and are activated when disgust or pain are experienced, and when one sees others experiencing these emotions.

Although amygdala-based and "mirror neuron" networks of the social brain generally underlie distinct social cognitive roles, a recent study suggests that they can perform similar functions to adaptively compensate for the other's injury [23].

3. The neurochemistry of social cognition

For social neuroscience, the neuropeptides oxytocin (OXT) and arginine vasopressin (AVP) have proven to be crucial molecules and are currently recognized as the "social neuropeptides". These peptides are highly conserved mediators throughout the mammalian evolution with key roles in the regulation of social cognition and behaviour [24].

OXT and AVP are synthesized by magnocellular neurons in the paraventricular and supraoptic nuclei of the hypothalamus, and are transported along the axonal projections to the posterior lobe of the hypophysis, where they are stored in secretory vesicles and released into peripheral circulation [24]. Additionally, there is also dendritic release of OXT and AVP into the extracellular space, causing local effects and also in distant regions of the brain by diffusion [24]. Furthermore, OXT and AVP are produced by parvocellular neurons in the paraventricular nucleus which project directly to other regions in the brain, including the amygdala, hippocampus, striatum, suprachiasmatic nucleus, bed nucleus of stria terminalis and brainstem, where they act as neuromodulators or neurotransmitters [24].

Most placebo-controlled studies investigating the effects of OTX and AVP in human behaviour have used intranasal administration of the neuropeptides, as this provides a direct pathway to the brain [25, 26]. The endpoint was the analysis of the behavioural response, and in some studies, included an additional functional neuroimaging protocol to measure brain structures activation after the neuropeptides administration. Other approaches, such as a correlational analysis between peripheral levels of OXT and AVP and behaviour are controversial because their peripheral levels may not be related to the neuropeptides levels and functions in the brain [27]. A potential alternative method, although invasive and therefore less feasible, is to measure neuropeptides in CSF which might better reflect their availability in the brain [24, 25].

OXT has been most widely studied and the results are consistent with the view that it enhances prosocial behaviour, by improving decoding of subtle social cues such as facial expressions. In one study, intranasal OXT administration improved the performance of healthy men, compared to placebo, in the test "Reading the Mind in the Eyes" [28]. In this test, photos depicting the eye region are shown to participants and they are asked to indicate what the person in the photo was thinking or feeling [29]. There are some studies suggesting that improved facial emotion recognition after OXT administration might be due in part to increased gazing time on the eye region, compared to other parts of the face, when observing neutral and emotional facial expressions [30, 31]. Additionally, OXT may increase the motivation to engage in social interactions by promoting cooperative and trusting behaviours [24]. Most studies support the hypothesis that OXT specifically promotes trusting behaviour even after a betrayal of trust [32], generosity [33] and in-group trust and cooperation [34, 35].

Regarding AVP, the few published findings suggest that AVP might have opposite effects to OXT, at least in males. A study showed enhanced negative emotional response to ambiguous social cues after intranasal AVP administration [36]. Interestingly, the effects of intranasal AVP on social perception revealed gender differences: in men AVP decreased the perception of friendliness to unfamiliar faces, while in women increased it [37]. Similarly, studies in animals and humans have

shown that, in contrast to the stress-reducing effects of OXT, AVP promotes the activation of the hypothalamic–pituitary–adrenal axis, specifically under conditions of social stress [38, 39].

Neuroimaging studies revealed that amygdala is the main target region of OXT, underlying its prosocial effect. In a recent study using high-resolution functional MRI of different amygdala subregions, participants who received intranasal OXT showed an increased probability of fixating the eye region even if they had been instructed to fixate the mouth [31]. This behaviour was accompanied by increased activation of right posterior amygdala [31]. There was also an interaction between the emotional content of the showed faces and the effect of OXT administration on an anatomically distinct subregions of the amygdala [31].

Conversely, less is known about the effect of AVP administration on brain structures as neuroimaging studies are scarce. Whereas the effect of OXT seems to target mainly the amygdala, there is some evidence that AVP has a predominantly cortical effect, particularly on the cingulate cortex, a crucial regulatory region of the limbic system, and on the left temporoparietal junction, a key node of the ToM network [40, 41].

There is increasing evidence that OXT and AVP systems are perturbed in disorders of social cognition, including autism spectrum disorders, Williams syndrome (a neurodevelopmental syndrome associated with hypersocial behavior, due to a deletion on chromosome 7), frontotemporal dementia, schizophrenia and social anxiety disorder [42-45]. Therefore, taking into consideration the gathered evidence about OXT and AVP effects on social behaviour, patients with severe deficits in social cognition may benefit with administration of OXT or OXT receptor agonists, particularly in synergistic combination with psychotherapy [24]. Similarly, selective antagonists of AVP receptor might be useful for the treatment of stress-related disorders and disorders that are characterized by interpersonal violence [24]. Several clinical trials are currently being carried out to investigate the therapeutic value of OXT and AVD for social cognition deficits.

4. Assessment of Theory of Mind in neurological diseases

Social cognition impairment is a relevant feature in many neurodevelopmental, psychiatric and neurological disorders. However, in the early stages of some neurological diseases, such as Alzheimer disease, Parkinson disease and MS, social cognitive disturbances might be relatively subtle and difficult to detect without a formal evaluation [1]. Social cognitive deficits affect negatively the interpersonal relationships and may interfere with doctor-patient communication and adherence to treatment strategies [1]. Therefore, the importance of a structured social cognitive assessment is now formally recognized in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition [46] which introduced social cognition as one of six core components of neurocognitive function that should be assessed in the diagnosis of major neurocognitive disorder. However, at present, such evaluations are mostly designed for research and validated clinical tools with normative data are lacking.

As social cognitive intervention is becoming an increasingly important field of research, gathering of normative data will be essential. Promising inroads include the development of targeted training programmes which have been associated with improvements in some functional domains and with changes in the neural systems underpinning social cognitive processes [47, 48]. Moreover, there is also active research on the potential benefits of pharmacotherapy. Peripheral administration of exogenous oxytocin has already been shown to augment social cognitive skills in schizophrenia [49], and might also be helpful in other diseases.

For the purpose of the current thesis, we present below the main tests available for ToM assessment (Table 1), excluding the other social cognition subdomains.

Table 1. Theory of mind tests

Measure	Experimental task	Population
False-belief Tasks [50]	Participants are told a story that involves two characters, Sally and Anne. The task measures whether a participant can understand that Sally holds a belief that is different to their own, and which is contrary to reality (a false belief)	Developed for children, variants now available for adults
The Awareness of Social Inference Test [51]	Questions focus on the ability to detect sarcasm in a social interaction	Age ≥13 years
Strange Stories Test [52]	Participants are asked to demonstrate their understanding of a written story in which a character's behaviour can be best understood by attributing to them a specific underlying mental state	Children and adults
Faux-Pas Test [53]	Participants are read a story that contains a faux pas and subsequently asked questions that focus on their ability to detect the faux pas, and to understand beliefs, intentions and inappropriateness	Separate child and adult versions available
Reading the Mind in The Eyes Test [54]	Participants are shown photographs of the eye regions of people's faces, and asked to select one of four alternatives describing what the person in the photograph is thinking or feeling	Separate child and adult versions available
Video-based Tests (Movie for the Assessment of Social Cognition [55]; Sullivan and Ruffman Videos Test [56]; Reading the Mind in Films task [57])	These tests require participants to attribute mental states to movie characters in a naturalistic setting	Separate child and adult versions available

5. Recent research into Theory of Mind impairment in Multiple Sclerosis

Despite the accumulated knowledge over the last decades about cognitive impairment in MS, there is a relative lack of research about the MS impact on social cognition, particularly on ToM. Social cognition may influence employment, but also relationships with spouses, caregivers, friends, and family members, and is therefore particularly relevant to patients with a chronic disabling disease as MS, for whom peer support is one of the main determinants of quality of life [58].

The first study assessing ToM abilities in patients with MS was conducted in 2009 by Henry et al. [45], who applied both a test for basic recognition of emotional facial expressions and the Reading the Mind in the Eyes task for ToM assessing (27 patients with MS and 30 controls). The authors found that patients with MS had greater difficulty detecting subtle differences in mental states from pictures of eyes. Although no overall group differences in facial affect recognition were observed, specific difficulties with recognition of anger and fear were reported in the MS group. These findings were replicated in subsequent studies using the same ToM task and video-based ToM tests [59-61]. Recently, two independent meta-analysis [7, 62] reported that adult patients with MS performed significantly worse than healthy controls on ToM tasks, namely on the Reading the Mind in the Eyes test and on ToM video-based tasks. On the other hand, there was no statistically significant difference between patients with MS and the control group on Faux Pas recognition tasks.

There is also one study reporting that ToM performance is impaired in patients with pediatric-onset MS, for whom the consequences of these early deficits could be most problematic as social cognitive skills are likely to still be developing and therefore may have long term implications for social adjustment [63].

More recently, there have been some studies examining the cognitive and affective components of ToM separately in MS. Roca et al. [64] found deficits in the cognitive ToM in the presence of

a preserved affective ToM, while Raimo et al. [65] reported that both components were affected in MS patients.

The relationship of ToM deficits with the classic cognitive impairment in MS has been explored in previous studies but this remains a matter to be clarified. Ouellet et al. [66] reported that only MS patients with cognitive impairment have significant deficits on ToM tasks whereas MS patients without cognitive impairment did not differ from healthy controls. Conversely, other studies found that ToM deficits in MS appear independently of the well-known cognitive deficits [60, 67].

Some contradictory evidence about the relationship between ToM and executive functions (EF) has also been reported. While some studies [64, 66, 67] found that ToM performance was not significantly correlated with EF, others [59, 61, 65, 68] reported that deficits in ToM tasks were mostly correlated with EF deficits. The main limitations of the aforementioned studies were the reduced sample sized, the heterogeneity of the patient cohort, and the use of a statistical approach based on binomial correlation analyses or analysis of variance (ANOVA), which do not test directly the dependency of variables with each other.

Surprisingly, none of the studies examining both ToM and depression reported associations between depressive symptoms and task performance. Similarly, fatigue was unrelated to performance on ToM tasks [7].

Regarding the neural basis of ToM impairment in MS, to date there is only one study that investigated the MRI correlates of ToM through the measurement of white-matter (WM) lesion load and brain cortical thickness [69]. This study revealed that performance in ToM tasks correlated with cortical thinning of fusiform face area, frontal eye field, right entorhinal cortex and left temporal pole. Additionally, ToM performance in MS was correlated with both total and regional T1-lesion load of association fiber tracts interconnecting cortical regions related to visual and emotion processing (genu and splenium of corpus callosum, right inferior longitudinal

fasciculus, right inferior fronto-occipital fasciculus, uncinate fasciculus). However, this study focused on the contribution of WM lesions and cortical thinning and did not investigated the influence of relevant subcortical grey matter (GM) structures known to be involved in ToM, particularly the amygdala, as well as the contribution of normal appearing WM damage. Both topics were undertaken as challenging aims of this thesis.

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PART II

HYPOTHESIS AND AIMS

HYPOTHESIS

The following hypotheses are tested in this thesis:

- Theory of mind (ToM) is impaired in patients with multiple sclerosis (MS) independently of the classic cognitive dysfunction associated with the disease.
- Performance on ToM and executive functions is dissociated in MS.
- Pathological involvement of grey matter (GM) and white matter (WM) both contributes to ToM impairment in MS.

AIMS

1) To evaluate the performance of patients with MS on ToM tasks as compared with healthy controls matched for age, sex and education level.
2) To explore if ToM impairment in MS patients occurs independently of the classic cognitive dysfunction associated with the disease, by analyzing ToM performance in patients classified as either cognitively impaired or cognitively intact.
3) To examine the relationship of ToM and executive functions in MS patients.
4) To analyze the influence of other clinical-demographical variables on ToM performance, such as age, sex, education level, disease duration, severity of physical disability, depression and fatigue.
5) To determine the contribution of GM and WM pathology for ToM impairment in MS patients using advanced MRI techniques to evaluate cortical and subcortical GM volumes and normal appearing WM damage.

PART III

RESEARCH PLAN AND METHODS

1. Study Design

Case-Control study

2. Sample size calculation

Previous published studies in MS with the variables of interest (ToM tests performance) reported effect sizes from medium (d=0.5-0.6) to large (d=0.8) for comparison between groups.

Our sample size estimates was therefore based on finding medium effects (d=0.5). A between-group comparison with a 0.05 one-sided alpha significance level and 80% power would require n=51 per group. We inflated the numbers for up to 60 per group in order to account for potential technique failure in the MRI analysis.

3. Methodology

3.1. Participants and Enrollment:

We enrolled consecutively 60 MS patients evaluated in our department and 60 healthy volunteers who served as healthy controls (HC), matched for age, sex and education level. All participants provided written informed consent to participate in the study, which was approved by the Ethical Committee of Faculty of Medicine of University of Coimbra (CE-027/2011).

3.1.1. Inclusion criteria for MS patients:

- Age 18-55 years;
- Meeting McDonald Criteria for MS diagnosis, as defined by the consultant neurologist;
- Relapsing-remitting or secondary-progressive subtype (patients with primary progressive MS were excluded to reduce sample heterogeneity since it is still much debated if this subtype is part of the MS disease spectrum or a separate disease entity; additionally it is well-known that some cases currently classified as primary progressive

MS may represent as yet unidentified disorders, including demyelinating, metabolic or genetic diseases);

- Portuguese language skills adequate for cognitive testing.

3.1.2. Inclusion criteria for HC:

- Age 18-55 years;
- Portuguese language skills adequate for cognitive testing.

3.1.3. Exclusion criteria for MS patients and HC:

- a significant visual, auditory or language impairment that would negatively affect their ability to satisfactorily complete tests or understand test instructions;
- conditions that would preclude MRI;
- current or past history of other neurological disease or systemic disease;
- history of psychiatric illness, with the exception of stable mild to moderate depressive symptoms;
- history of head injury resulting in loss of consciousness;
- current or prior use of antipsychotic medication;
- starting or stopping antidepressants in the previous 2 months;
- current or past history of alcohol, drug or substance abuse;
- Strictly for MS patients: a relapse or steroid treatment within 8 weeks preceding evaluation.

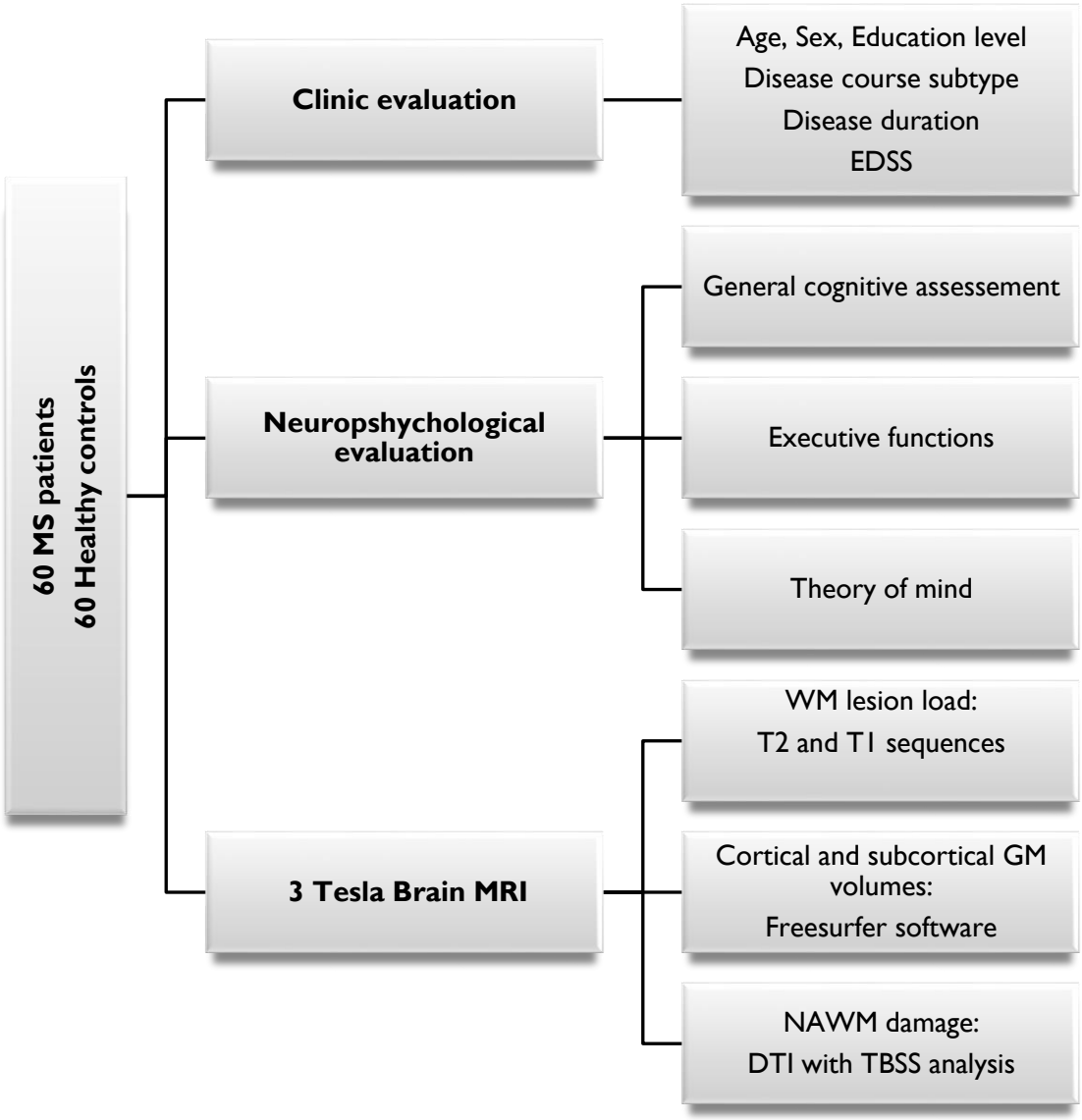

Figure 1. Flowchart with the study protocol including neuroimaging and neuropsychological assessments.

Abbreviations: MS = Multiple Sclerosis; EDSS = Expanded Disability Status Scale; MRI = Magnetic Resonance Imaging; WM = White matter; GM = Grey Matter; NAWM = Normal-appearing white matter; DTI = Diffusion Tensor Imaging; TBSS = Tract-Based Spatial Statistics.

3.2. Demographic and Clinical data:

Demographic data of all subjects (age, sex, education level) and clinical data of MS patients, namely disease course subtype, disease duration and current disease-modifying treatment was collected. Neurological disability was evaluated using Expanded Disability Status Scale (EDSS).

3.3. Neuropsychological evaluation:

The same battery of tests was administered to all subjects in equal fixed order. For the purposes of the current study, it comprised: (1) a general cognitive assessment with tests focusing cognitive domains commonly affected in MS in order to classify patients as either cognitively impaired or cognitively intact; (2) tests tapping different processes of executive functions; (3) tasks measuring ToM.

General cognitive assessment and **executive functions testing** were performed by a neuropsychologist (AA) blinded to the performance in ToM tests and MRI findings. Tests were chosen according to the recommendations of a consensus panel and included: the Irregular Word Reading Test (TeLPI) for estimation of premorbid intelligence level; the Rao adaptation of Symbol Digit Modalities Test-oral version, as a measure of visual information-processing speed; the Rao adaptation of Paced Auditory Serial Addition Test, 3-second, as a measure of auditory information-processing speed and working memory; the Brief Visuospatial Memory Test- Revised for the evaluation of visuospatial learning and memory; the California Verbal Learning Test for the assessment of verbal episodic learning and memory; the Judgment of Line Orientation Test to assess spatial perception; Controlled Oral Word Association Test to measure verbal fluency; Wisconsin Card Sorting Test to measure executive functions; Beck Depression Inventory and Modified Fatigue Impact Scale to evaluate depression and fatigue respectively, as they represent covariables known to influence cognition.

Impairment for a single test was defined as a z score <-1.5. Cognitive impairment was defined as a defect on two or more test measures.

Regarding executive functions testing, in addition to the Controlled Oral Word Association Test and the Wisconsin Card Sorting Test included in the battery used for general cognitive assessment, the administered tests were: Stroop Test; Adults Raven's Progressive Matrices; Proverbs Interpretation Test. These tests are all described in the Chapter 3 of the Part IV.

The **ToM tests** were performed by the same investigator (SB) blinded to the performance in other neuropsychological tests and MRI findings. The selected tests were Baron-Cohen´s Adult Eyes test and Videos Test (Sullivan and Ruffman), described with detail in the publications section (Part IV).

3.4. MRI Acquisition and Analysis:

All participants were examined on a 3Tesla Siemens TIM Trio scanner. The following analysis was performed: (1) conventional assessment of lesion load with standard T1, T2 and FLAIR images; (2) cortical and deep grey matter volumes using FreeSurfer software, calculating both regional and global volumes; (3) white matter damage was examined and quantified at tract level in normal-appearing white matter (NAWM) by Diffusion Tensor Imaging (DTI) with Tract-Based Spatial Statistics (TBSS).

PART IV

PUBLICATIONS

CHAPTER 1

Impairment of social cognition in multiple sclerosis: amygdala atrophy is the main predictor

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ABSTRACT

Background: Patients with multiple sclerosis (MS) frequently reveal social behaviour disturbance. Nevertheless, little is known regarding the impact of MS on social cognition, particularly theory of mind (ToM), and its neural basis.

Objectives: To explore how ToM is affected in MS and its neural correlates.

Methods: Enrolled 60 consecutive MS patients and 60 healthy controls (HC) matched on age, sex, and education. Participants underwent ToM testing (Eyes Test, Videos Test) and 3Tesla brain MRI. Using Freesurfer software, cortical and subcortical grey matter (GM) volumes were calculated.

Results: MS patients performed worse on Eyes Test (58.7±13.8% vs. 81.9±10.4%, $p<0.001$) and Videos Test (75.3±9.3% vs. 88.1±7.1%, $p<0.001$). Eyes Test performance in MS was positively correlated with the volume of subcortical structures (amygdala, putamen) and cortical regions (entorhinal cortex, fusiform gyrus, superior temporal gyrus, superior parietal gyrus, supramarginal gyrus, medial orbitofrontal cortex, anterior and posterior cingulate gyrus). In regression analysis, amygdala volume was the single predictor of performance (R2 change=0.064, $p=0.031$) and a mediation analysis indicated that it contributes for the differences observed between MS and HC.

Conclusion: Patients with MS have impairment on social cognition. Amygdala atrophy was the main predictor probably due to its central position within the "social brain" network.

KEYWORDS

Multiple Sclerosis, Social Cognition, Theory of Mind, Social Brain, Amygdala

INTRODUCTION

In the last decades, research on cognitive impairment associated with multiple sclerosis (MS) has burgeoned. However, the impact on social cognition has received little attention. Yet, patients with MS not uncommonly reveal an inappropriate social behavior ranging from mild difficulties understanding subtle social cues and reading the social context to significant impairment of interpersonal interaction [1].

An essential aspect for social cognition is theory of mind (ToM), defined as the ability to infer other persons' mental states, including beliefs, desires, and intentions, hence explaining and predicting behavior [2]. Studies to date focusing ToM in MS have demonstrated deficits in cognitive inferences about complex mental states or emotions of others in ToM tasks [3-7]. Nevertheless, it remains unclear if ToM impairment in MS is secondary to the well-known classic cognitive deficits or whether they may occur independently.

The neural basis of ToM has been widely investigated in healthy subjects and in several diseases characterized by impairment in social cognition, particularly in high-functioning autism and in schizophrenia. In MS, the neural basis of ToM deficits remains to be determined. In the present study, we aimed to fill this gap using an MRI whole-brain analysis of cortical and subcortical grey matter (GM) volumes.

PARTICIPANTS AND METHODS

Participants

We enrolled 60 consecutive patients with MS regularly followed in our department. Patient´s eligibility for the present study included the following inclusion criteria: age between 18 to 55 years old, definite diagnosis of MS according to the McDonald criteria 2010 [8], and relapsing-remitting or secondary-progressive subtypes. A group of 60 healthy volunteers, matched for age,

sex and educational level with MS patients was recruited from the community and served as healthy controls (HC).

Exclusion criteria for all participants were history of neurological (other than MS in patient group) or systemic disease; history of psychiatric illness, with the exception of stable mild to moderate depressive symptoms; history of head injury resulting in loss of consciousness; a significant visual, auditory or language impairment that would negatively affect their ability to satisfactorily complete tests or understand test instructions; current or prior use of antipsychotic medication; alcohol, drug or substance abuse; starting or stopping antidepressants in the previous 2 months; conditions that would preclude MRI and, for MS patients, a relapse or steroid treatment within 8 weeks preceding evaluation.

This study was approved by the local ethics committee and all participants gave written informed consent prior to participation.

Clinical assessment

A full medical history and detailed neurologic examination were obtained for all patients. The following data were collected: age, sex, handedness, years of education, disease duration, and current disease-modifying treatment. Physical disability was evaluated using the Expanded Disability Status Scale (EDSS) [9]. For HC, medical history was obtained by an interview preceding assessment.

Theory of Mind testing

The tests selected to assess ToM were performed by the same investigator (SB) blinded to the performance in neuropsychological testing and MRI findings. These tests were administered in a fixed order and included:

1. Revised Mind in the Eyes test

The Revised Mind in the Eyes test (referred to as "Eyes test") is the most commonly used ToM task in adult research. It consists of 36 black-and-white photographs of the eye region [10]. These are surrounded by four words, one target and three foils, describing com plex mental states. Participants were asked to select which words best described the thoughts or feelings of the person in the photograph.

The response options were presented on the screen throughout the test in order to minimise the working memory load. Correct answers were rated 1, incorrect answers were rated 0. The total raw score ranges from 0 to 36 with higher scores indicating better performance.

2. ToM Videos test

This ToM task has been used recently in adult research in an attempt to mirror the real demands of live social situations. It consists of 26 silent colour video clips adapted from Sullivan and Ruffman [11], that showed characters interacting. Participants were instructed to choose the word that best described the thoughts or feelings of the person in the video. For 3 seconds before the clip appeared on the screen, two words were presented on the screen and remained as the clip played to reduce working memory load.

Correct answers were rated 1, incorrect answers were rated 0. The total raw score ranges from 0 to 26 with higher scores indicating better performance.

General Cognitive Assessment - Neuropsychological Testing

Neuropsychological testing (NP) was performed by a neuropsychologist (AA) blinded to the performance in ToM tests and MRI findings. Tests were chosen according to the

recommendations of a consensus panel [12], and were administered in a fixed order. The battery included: the Rao adaptation of Symbol Digit Modalities Test- oral version, as a measure of visual information-processing speed [13, 14]; the Rao adaptation of Paced Auditory Serial Addition Test, 3-second, as a measure of auditory information-processing speed and working memory [14, 15]; the Brief Visuospatial Memory Test- Revised for the evaluation of visuospatial learning and memory [16]; the California Verbal Learning Test for the assessment of verbal episodic learning and memory [17]; the Judgment of Line Orientation Test to assess spatial perception [18]; Controlled Oral Word Association Test to measure verbal fluency [18]; and Wisconsin Card Sorting Test to measure executive functions [19, 20].

Impairment for a single test was defined as a z score <-1.5. Cognitive impairment was defined as a defect on two or more test measures, based on the same general procedure described by Benedict et al [21].

Additionally, premorbid intelligence was estimated using the Irregular Word Reading Test (TeLPI) [22]. The Beck Depression Inventory (BDI),[23] and the Modified Fatigue Impact Scale (MFIS) [24, 25], were used to determine the influence of depression and fatigue, respectively.

MRI Acquisition and Analysis

All participants were examined on a 3 Tesla Siemens Magnetom TrioTim scanner (Erlangen, Germany) using a 12-channel birdcage head coil. Two high-resolution T1-weighted (T1w) three-dimensional Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE sequence) acquisitions were obtained for each participant as well as a sagittal 3D Fluid Attenuated Inversion Recovery (FLAIR sequence). Scans were prescribed in an axial-oblique orientation, parallel to the sub-callosal line.

Cortical surface reconstruction and volumetric segmentation were performed using a semi-automatic pipeline through FreeSurfer (version 5.3.0, *http://surfer.nmr.mgh.harvard.edu/*) in a Linux

(CentOS 6) platform. The main procedure was followed as described elsewhere [26, 27]. The cortical labeling of the brain was based on the Desikan-Killiany atlas. Several checkpoints were made to visually inspect for processing inaccuracies regarding the skull-stripping quality, white-matter (WM), GM and pial surfaces segmentation and subcortical labelling. Whenever necessary manual edits were made and the subsequent steps re-executed. Of the 60 MS participants included in the study, 3 datasets were excluded due to bad quality data and poor subcortical segmentation and cortical reconstructions. Volumes were corrected for the estimated total intracranial volume (eTIV) as a measure of the relationship between the intracranial volume (ICV) and the spatial linear transformation to the MNI305 space. Thus, to obtain normalized volumes, subcortical GM, cortical GM and WM volumes were divided by eTIV and then multiplied by 100 to produce percentage volume fractions. A preliminary analysis was conducted to check for any lateral hemispheric difference regarding ToM performance. As we didn´t find significant differences, left and right hemispheric volumes were averaged in order to reduce the number of variables to the smallest possible set. A fully automated method, based on a lesion growth algorithm [28], was used to assess the T2-hyperintense white matter lesion load from FLAIR images. This algorithm is implemented in the Lesion Segmentation Toolbox *(http://www.applied-statistics.de/lst, version 1.2.3)* for SPM. Once lesion probability maps have been calculated, binary lesion masks were visually inspected and, if necessary, manually corrected using MRIcron software *(www.mricro.com/mricron)*. T1-weighted lesion volumes were automatically estimated for each participant's using Freesurfer.

Statistical Analysis

Group comparisons were performed using the *t* test for unpaired samples, the nonparametric Mann-Whitney test, and a Chi-Square Test, when appropriate. Effect sizes for group comparisons were calculated according to Cohen´s *d* formula [29].

Correlations between ToM tests, demographic, clinical and MRI variables were examined using Pearson's coefficients, Spearman's rank order coefficient or partial correlation when appropriate. Forward stepwise linear regression models (entrance criterion $p<0.05$ and exit criterion $p=0.10$) were generated in order to determine the strongest GM volumes predictors of Eyes Test and Videos Test performance. In each case, age and gender were entered as covariates and retained in block 1, and the significant GM volumes were entered in block 2 using the forward stepwise technique. Finally, each model was then repeated with T1 and T2 lesion volumes included in block 1.

Lastly, the hypothesis that differences in GM volumes (retained in regression analysis) between MS patients and HC explained the variance in social cognition between groups was tested using mediator analysis performed in the PROCESS macro for SPSS (www.afhyes.com). Age and sex were used as covariates in the mediation model. The direct and indirect effects were calculated and significance was determined using bootstrapping (k=5000) with 95% confidence intervals (CI).

Analyses were conducted using SPSS for Windows version 20.0 (SPSS Inc, Chicago, Ill). All tests were performed two-tailed and statistical threshold was set at $p< 0.05$ corrected through false discovery rate (FDR) for multiple testing.

RESULTS

Sample characteristics

MS patients and HC did not differ significantly in age, sex, education level, handedness or intelligence quotient estimate (Table 1).

Regarding the MS population, the disease course was relapsing–remitting in 50 patients (83.3%) and secondary progressive in 10 patients (16.7%). The mean disease duration was 10.6 ± 6.6

years and the median EDSS score was 2.0 (IQR 1.5). All patients were under treatment with disease-modifying drugs.

Table 1. Sociodemographic data of multiple sclerosis patients and healthy controls

	MS (n =60)	HC (n=60)	P value
Age, mean years ±SD	37.2± 7.5	36.1 ± 9.4	0.475
Education, mean years ±SD	13.2± 4.0	14.0 ± 3.9	0.258
Female, n (%)	40 (66.7)	40 (66.7)	1.0
Right handedness, n (%)	56 (93.3)	58 (96.7)	0.539
IQ estimate, mean±SD	113.5 ± 11.0	116.6 ± 8.3	0.081

Abbreviations: MS= patients with MS; HC= healthy controls; IQ= Intelligence quotient

Theory of Mind performance in MS

Patients with MS had significantly lower scores on both tasks of ToM compared to HC, i.e. Eyes Test (58.7± 13.8% vs. 81.9± 10.4%, p<0.001FDR-corrected) and Videos Test (75.3±9.3% vs. 88.1±7.1%, p<0.001 FDR-corrected) (Figure 1).

In order to explore whether ToM performance was associated with cognitive impairment within the MS group, patients were classified as either cognitively impaired (n=34; 56.7%) or cognitively intact (n=26; 43.3%) based on the NP testing performance. There were no significant differences on Eyes Test (56.9± 14.5% vs. 61.0± 12.7%, p=0.251) and the Videos Test (74.5± 11.2% vs. 76.3± 5.9%, p=0.431) between MS patients with cognitive impairment and those with normal cognitive performance, respectively. Moreover, even the group of patients without cognitive impairment presented significantly lower scores on ToM tasks compared to HC (Figure 1).

Performance on ToM tests was not associated with age, sex, years of education, disease duration, EDSS, depression and fatigue indexes, i.e. BDI and MFIS scores respectively ($p>0.05$ FDR-corrected).

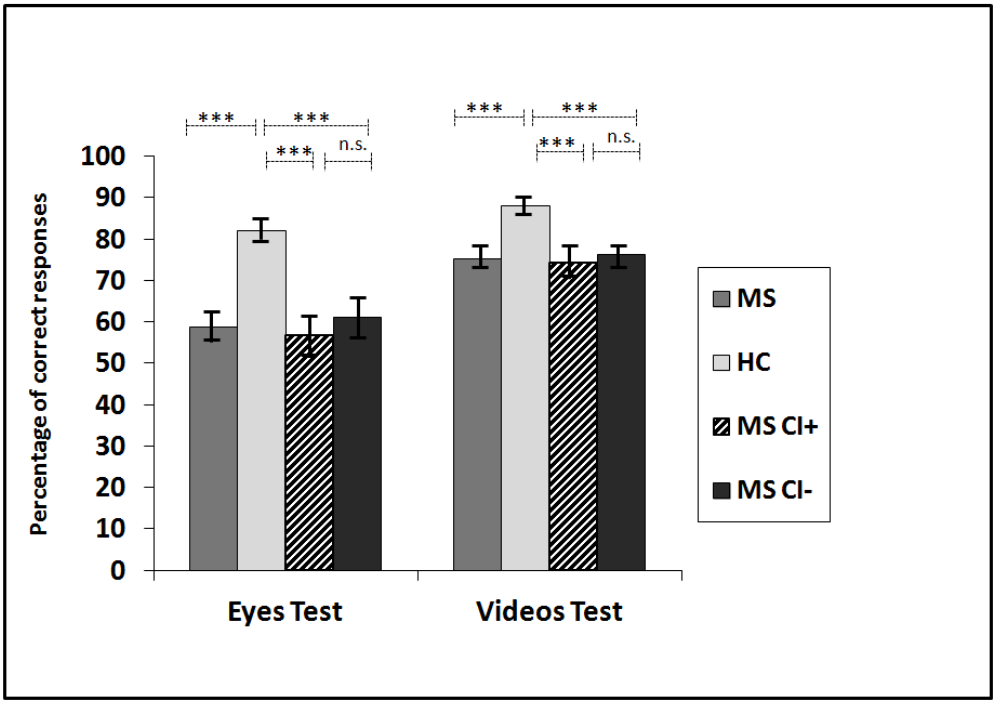

Figure 1. Results of Theory of Mind tests in patients with multiple sclerosis and healthy controls

Patients with MS had lower scores on both tasks of ToM compared to HC, even the subgroup of patients without cognitive impairment. There were no significant differences on both tests of ToM between the two subgroups of MS patients based on cognitive performance.

Abbreviations: HC= Healthy controls; MS=patients with Multiple Sclerosis; MS CI+= MS patients with cognitive impairment; MS CI- = MS patients without cognitive impairment; n.s.= non-significant

Statistical test conducted: chi-square test

*** $p<0.001$ FDR-corrected

Error bars indicate 95% confidence intervals.

MRI correlates of Theory of Mind performance in MS

1. Lesion Load and Global Brain Volumes

Compared with HC, MS patients presented a significant reduction of total WM volume, total GM volume, cortical GM volume, and subcortical GM volume, with the largest effect for subcortical GM (Cohen´s *d*= 1.26, p<0.001) (Table 2).

The Eyes Test and Videos Test performance were both negatively correlated with T2 lesion volume (r=-0.505, p<0.001; -0.362, p=0.005, respectively) and with T1 lesion volume (r=-0.484, p<0.001; -0.314, p=0.017, respectively).

Conversely, global brain volumes did not reveal any significant correlation with ToM tasks (p>0.05 FDR-corrected).

Table 2. MRI data in patients with multiple sclerosis and healthy controls

	MS (n =57)	HC (n =60)	P value*	Cohen´s *d*
Total T1 Lesion volume, mL	12.9±5.3	NA	NA	NA
Total T2 Lesion volume, mL	16.8±15.8	NA	NA	NA
White Matter volume	28.0 ± 2.6	29.8 ± 1.6	<0.001	0.83
Total Grey Matter volume	37.6 ± 3.2	39.8 ± 2.6	<0.001	0.75
Cortical volume	28.0± 2.4	29.4 ±2.1	0.002	0.62
Subcortical Grey Matter volume	3.4± 0.4	3.8 ± 0.2	<0.001	1.26
Amygdala volume	0.10± 0.01	0.11 ± 0.01	<0.001	0.70
Putamen volume	0.31± 0.06	0.36± 0.04	<0.001	0.98
Fusiform gyrus volume	0.58± 0.06	0.62± 0.06	0.002	0.64

Table 2. MRI data in patients with multiple sclerosis and healthy controls

	MS (n =57)	HC (n =60)	P value*	Cohen´s *d*
Entorhinal cortex volume	0.12± 0.02	0.13± 0.02	<0.001	0.68
Superior temporal gyrus volume	0.88±0.09	0.92± 0.08	0.008	0.52
Superior parietal gyrus volume	0.68±0.08	0.71±0.07	0.035	0.40
Supramarginal gyrus volume	0.57± 0.07	0.60± 0.06	0.006	0.55
Medial orbitofrontal cortex volume	0.25± 0.03	0.26± 0.03	0.076	0.33
Anterior cingulate volume	0.27± 0.03	0.28± 0.03	0.044	0.38
Posterior cingulate volume	0.18± 0.02	0.19± 0.02	0.018	0.46

Volumes were normalized except for T1 and T2 lesion volumes and are reported as volume fractions (in percentage) of estimated total intracranial volume. Only the regional grey matter volumes that were significantly correlated with Theory of Mind performance are displayed.

Data are given as mean ± SD unless otherwise indicated.

* p values are corrected for multiple comparisons by False Discovery Rate method

2. Regional GM volumes

We explored the entire cortex and subcortical GM structures to find the areas correlated with ToM performance, controlling for age and sex (Supplementary table 1, please see at the end of this chapter).

The Eyes Test performance in MS patients showed a significant correlation with the volume of two subcortical structures: amygdala (r=0.518, p<0.001 FDR-corrected) and putamen (r=0.329, p=0.014 FDR-corrected) (Table 3, Figure 2). Regarding the cortical volumes, Eyes Test correlated with the following regions: fusiform gyrus, entorhinal cortex, superior temporal gyrus,

superior parietal gyrus, supramarginal gyrus, medial orbitofrontal cortex, anterior and posterior cingulate gyrus (Table 3, Figure 2).

Table 3. Regional GM volumes with significant correlation with Eyes test in multiple sclerosis (controlled for age and sex)

	Eyes Test	**p value***
Amygdala	0.518	<0.001
Putamen	0.329	0.043
Fusiform gyrus	0.384	0.041
Entorhinal cortex	0.417	0.031
Superior temporal gyrus	0.372	0.039
Superior parietal gyrus	0.336	0.031
Supramarginal gyrus	0.330	0.048
Medial orbitofrontal cortex	0.339	0.043
Anterior cingulate gyrus	0.359	0.031
Posterior cingulate gyrus	0.364	0.037

Only the significant correlations are displayed in the table.

All correlations are partial correlation coefficients controlled for age and sex.

* p values are corrected for multiple testing by False Discovery Rate method

Abbreviations: GM= Grey matter; ToM= Theory of Mind

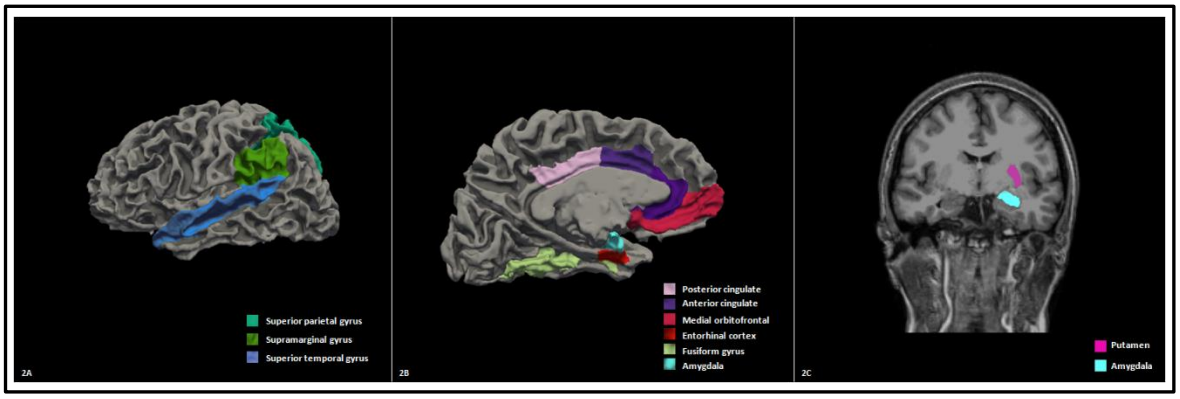

Figure 2. Cortical and subcortical areas correlated with Theory of Mind performance in multiple sclerosis.
Representative images of 3 Tesla brain MRI, T1-weighted sequences, with brain segmentation using Freesurfer software. Cortical areas correlated with Eyes Test performance are shown in the lateral (2A) and medial (2B) views of inflated cortical surface of the left hemisphere. Color-coded labels of anatomical areas are based on the DKT40 classifier atlas. The subcortical structures, left amygdala and left putamen, are identified on a coronal slice (2C).

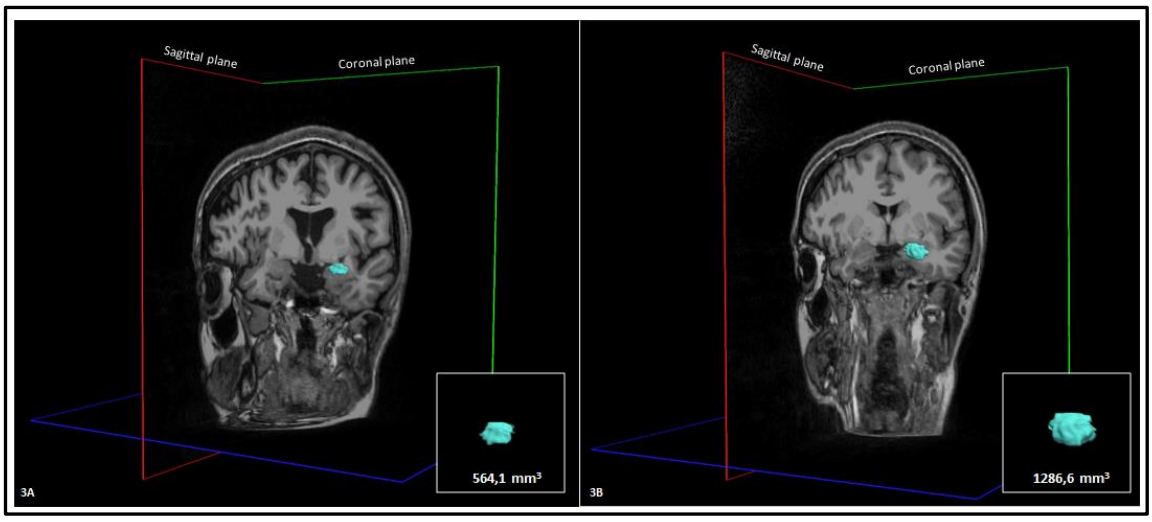

Figure 3. Tridimensional representation of amygdala segmentation in a patient with multiple sclerosis and in a healthy control subject.
Representative images of 3 Tesla brain MRI, 3D T1-weighted sequences, resulting from an intersection of coronal and sagittal planes, with left amygdala (light blue) segmented using Freesurfer software. Note the volume reduction of the amygdala in a 34-year old female patient with multiple sclerosis (3A) comparatively to an age and sex-matched healthy control (3B).

Compared with HC, the volumes of these subcortical structures and cortical areas associated with performance in Eyes Test were significantly reduced in patients with MS ($p<0.05$ FDR-corrected) except for medial orbitofrontal cortex (Table 2, Figure 3).

The Videos Test did not correlate significantly with any cortical region or subcortical structures for a statistical threshold at p< 0.05 FDR-corrected and therefore was not analyzed further on regression models (Supplementary table 1).

The regression models predicting Eyes Test performance in MS patients included only the GM regions that showed statistically significant correlation with Eyes Test performance (Table 4). In the first tier, controlling for age and sex, only amygdala volume was retained as predictor (R^2 change= 0.260, p<0.001). In the second tier, after controlling for total T2 and T1 lesion volumes, amygdala was still retained as the single predictor (R^2 change= 0.064, p=0.031).

Finally, in order to test if differences in amygdala volumes found between MS patients and HC contributed to explain the variation in Eyes Test performance, we conducted a mediation analysis with age and sex as covariates. We found that amygdala volume significantly mediates the variation of performance in Eyes Test observed between MS patients and HC ($Coefficient_{ab}$= -1.2, CI [-2.7, -0.3], ratio of the total effect = 0.14)

Table 4. Linear Regression Models predicting Eyes Test performance in Multiple Sclerosis

	Step 1 R^2	**Variables Retained After Step 1 (Standardized Beta; R^2 change)**	**Final Model R^2**	**P value***
EYES TEST				
Controlling for age and sex	0.032	AMYGDALA (0.517; 0.260)	0.292	<0.001
Controlling for age, sex, T2 lesion volume, T1 lesion volume	0. 269	AMYGDALA (0.358; 0.064)	0.333	0.031

* p value for R^2 change

DISCUSSION

The results obtained in the present study confirm that patients with MS have significant social cognition impairment and reinforce the need to expand current perspectives concerning the cognitive profile in MS. Compared to HC, patients with MS revealed worse performance both in the Eyes Test and in the Videos Test indicating that they have difficulties on decoding other persons' mental states either from static or dynamic visual stimuli.

It is noteworthy that ToM deficits appear to be not simply a consequence of the well-known MS-related cognitive impairment but may occur independently. This finding is conceivable taking into account the multifocal nature of MS lesions, which may disrupt specific neural networks involved in social cognition while preserving those responsible for the classic cognitive domains. Nevertheless, previous studies had yield conflicting results with one study reporting ToM deficits only in MS patients cognitively impaired [4], while other found ToM impairment even in patients without substantial neuropsychological deficits [5]. Moreover, we cannot exclude that ToM deficits may be associated with impairment of specific cognitive domains, for example the executive functions or attention.

In an attempt to explore the neural correlates of ToM impairment in MS, we analyzed the contribution of conventional MRI measures of lesion load and cortical and subcortical GM volumes. In this study we found that ToM impairment, as measured by Eyes Test, was related to a multifocal pattern of atrophy involving cortical regions and subcortical structures. More importantly, we demonstrate that amygdala was the main predictor of Eyes Test performance in MS and that it mediates the differences in the performance observed between MS patients and HC, supporting the hypothesis that amygdala might be a central structure of the complex network that composes the "social brain".

In line with these findings, a growing body of evidence suggests that amygdala is the main hub of large-scale brain networks involved in social abilities [30]. The amygdala shares anatomical

connections with almost every other brain regions implicated in social cognition, thus placing it in a pivotal position to decode the multimodal sources of stimuli and to associate those with its emotional and social significance [30]. An important line of evidence comes from investigations in autism. Structural and functional imaging studies of people with autism, as well as post-mortem investigations, have revealed abnormalities in the amygdala complex [31]. Moreover, these findings are consistent with the behaviors observed in amygdala-damaged patients which include less eye-contact, insensitivity to personal boundaries and deficits in social avoidance judgments [32]. Interestingly, both structural and functional MRI studies have indicated that amygdala is important in processing information concerning eyes regions, and in particularly making accurate judgments about the direction of gaze from the eyes [33]. Thus, it seems reasonable to propose that amygdala atrophy significantly contributes to the reported impairment on social cognition in MS patients.

Although the remaining correlates identified in our study are not as robust, we cannot fail to notice that they have been implicated as neural nodes of brain networks that make up the "social brain" [30]. Functionally they can be divided in two distinct groups of networks, although some of the circuits are probably shared: amygdala-based networks (fusiform gyrus, entorhinal cortex, superior temporal gyrus, medial orbitofrontal cortex, anterior and posterior cingulate gyrus, putamen) and mirror-neuron network (supramarginal gyrus, superior parietal gyrus).

Ultimately, in addition to the role of cortical and subcortical GM pathology demonstrated in our study, ToM impairment in MS might also be related to the WM damage. As a matter of fact, we found that both T1 and T2 lesion volumes were significantly correlated with ToM tasks. The disruption of the WM pathways mediating the transmission of information across distributed brain networks is a well-recognized mechanism underlying cognitive dysfunction in MS [7, 34].

In this study, the Videos Test did not correlate significantly with any cortical region or subcortical structures. A possible explanation could be the low spread of data in a modest sample size resulting in insufficient statistical power to detect relationships. These results are in line with the

accepted knowledge that Eyes test is one of the most challenging socio-cognitive tests requiring more cognitive effort to decode complex emotions or thoughts, in opposition to the Videos Test that uses additional cues to aid in the recognition of emotions [11, 35]. On the other hand, the Videos Test task involves not only the information processing concerning eyes regions, but also body posture, facial expression and the surrounding social context. Therefore, it may be hypothesized that Videos Test performance may rely on a more diffuse and vicariant brain network involving cortical and subcortical areas, hindering the possibility of capture it with a pure volumetric approach of GM regions. Additional tests measuring other dimensions of ToM, and functional MRI may contribute to further elucidate this question.

There are some limitations of our study to be considered. First, ToM was assessed with tests that rely exclusively on visual stimuli and in real life other sources are important cues, particularly the voice intonation. Second, the investigator that conducted the ToM tests was not blinded to the disease group status. Third, the accuracy and reliability of automated segmentation algorithms for GM, particularly subcortical GM, is still debatable. Therefore, these results should be confirmed using other methods of segmentation. Finally, we did not assess other structural and functional MRI variables that may have accounted for variance in ToM performance.

In conclusion, this study reinforces the presence of ToM impairment in MS and furthers the knowledge about its neural correlates. We conclude that amygdala atrophy is the main predictor of ToM impairment in MS supporting the hypothesis that amygdala might be a central structure of the complex "social brain" network, where the social relevant information converges and is integrated, modulating other neural nodes involved in social cognition.

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COMPETING INTERESTS

S. Batista has received honoraria for serving on scientific advisory boards of Biogen and Novartis Pharma, and for speaking in scientific meetings of Teva, Merck Serono, Genzyme, Biogen, and Novartis Pharma.

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O. C. d'Almeida, A. Afonso, Sandra Freitas, M. Castelo-Branco, I. Santana and L. Cunha have nothing to disclose.

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Supplementary Table 1. Correlation between ToM tests and the entire cortical and subcortical GM areas in multiple sclerosis (controlled for age and sex)

	Eyes Test	p value*	Videos Test	p value*
Amygdala	0.518	<0.001	0.217	0.316
Putamen	0.329	0.043	0.304	0.744
Thalamus	0.201	0.244	0.233	0.381
Caudate	0.187	0.279	0.275	0.326
Pallidum	0.059	0.670	0.158	0.432
Accumbens	0.230	0.202	0.268	0.298
Anterior cingulate gyrus	0.359	0.031	0.161	0.439
Posterior cingulate gyrus	0.364	0.037	0.250	0.336
Isthmus cingulate gyrus	0.240	0.184	0.109	0.491
Lateral orbitofrontal cortex	0.112	0.497	-0.197	0.310
Medial orbitofrontal cortex	0.339	0.043	0.145	0.410
Dorsolateral prefrontal cortex	0.167	0.288	0.127	0.461
Precentral gyrus	0.184	0.276	0.149	0.412
Paracentral gyrus	0.245	0.183	0.233	0.300
Superior parietal gyrus	0.336	0.031	0.233	0.337
Inferior parietal gyrus	0.338	0.051	0.302	0.386
Postcentral gyrus	0.181	0.355	0.065	0.703
Supramarginal gyrus	0.330	0.048	0.190	0.318

Supplementary Table 1. Correlation between ToM tests and the entire cortical and subcortical GM areas in multiple sclerosis (controlled for age and sex)

	Eyes Test	p value*	Videos Test	p value*
Precuneus gyrus	0.224	0.209	0.138	0.426
Superior temporal gyrus	0.372	0.039	0.226	0.301
Middle temporal gyrus	0.216	0.219	0.200	0.369
Inferior temporal gyrus	0.179	0.256	0.0570	0.724
Transverse temporal gyrus	0.096	0.538	0.120	0.472
Entorhinal cortex	0.417	0.031	0.160	0.398
Parahippocampal gyrus	0.183	0.267	0.150	0.426
Fusiform gyrus	0.384	0.041	0.284	0.372
Lateral occipital gyrus	0.092	0.541	0.004	0.976
Cuneus gyrus	0.206	0.239	0.199	0.348
Pericalcarine gyrus	0.090	0.529	0.049	0.746
Lingual gyrus	0.107	0.502	0.109	0.508
Insula	0.162	0.293	0.199	0.323

All correlations are partial correlation coefficients controlled for age and sex.

* p values are corrected for multiple testing by False Discovery Rate method

Abbreviations: GM= Grey matter; ToM= Theory of Mind

CHAPTER 2

Disconnection as a mechanism for social cognition impairment in multiple sclerosis

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ABSTRACT

Objective: To assess the contribution of microstructural normal appearing white matter (NAWM) damage for social cognition impairment, specifically in the theory of mind (ToM) domain, in multiple sclerosis (MS).

Methods: We enrolled consecutively 60 MS patients and 60 healthy controls (HC) matched on age, sex, and education level. All participants underwent ToM testing (Eyes Test, Videos Test) and 3Tesla brain MRI including conventional and diffusion tensor imaging sequences. Tract-based spatial statistics (TBSS) were applied for whole-brain voxel-wise analysis of fractional anisotropy (FA) and mean diffusivity (MD) on NAWM.

Results: MS patients performed significantly worse on both tasks of ToM compared to HC, i.e. Eyes Test (58.7±13.8 vs. 81.9±10.4, $p<0.001$, Hedges g:-1.886) and Videos Test (75.3±9.3 vs. 88.1±7.1, $p<0.001$, Hedges g:-1.537). Performance on ToM tests was correlated with higher values of FA and lower values of MD across widespread white matter tracts. The largest effects (≥90% of significant voxels) were for the Eyes Test: body and genu of corpus callosum, fornix, tapetum, uncinate fasciculus, and left inferior cerebellar peduncle; for the Videos Test: genu and splenium of corpus callosum, fornix, uncinate fasciculus, left tapetum, and right superior fronto-occipital fasciculus.

Conclusion: These results indicate that a diffuse pattern of NAWM damage in MS patients contributes for social cognition impairment in the ToM domain, probably due to a mechanism of disconnection within the social brain network. Grey matter pathology is also expected to have an important role; thus further research is required to clarify the neural basis of social cognition impairment in MS.

KEYWORDS

Multiple Sclerosis, MRI, All Neuropsychology/Behavior, Neuropsychological assessment, Social cognition

INTRODUCTION

Social functioning deficits significantly interfere with everyday life activities in multiple sclerosis (MS) patients [1]. Nevertheless, this issue only recently started to receive some attention, with growing interest regarding the underlying causes. Social cognition impairment has been identified as a potential contributor with recent studies reporting that MS patients have deficits in different dimensions, including theory of mind (ToM) [2-10].

ToM, an essential domain of social cognition, is the ability to infer other persons' mental states, including beliefs, desires, and intentions, hence explaining and predicting behaviour [11]. The complexity and multidimensionality of these cognitive processes requires the coordinated functioning of a widely distributed neural network, often referred to collectively as the "social brain", which includes superior temporal gyrus, temporal poles, amygdala, temporo-parietal junction, cingulate cortex and pre-frontal cortex as the main nodes [12-14].

In MS, grey matter (GM) pathology involving these core regions is expected to have an important role in the pathophysiology of social cognition impairment, similarly to what has been demonstrated in classic cognitive impairment [15]. On the other hand, it is reasonable that disruption of connectivity within the social brain network, due to damage of critical white matter (WM) tracts, also contributes. However, even though WM injury is the cardinal pathological process of MS, this association has not been examined before.

We hypothesize that diffuse microstructural damage in normal-appearing WM (NAWM) contributes for social cognition impairment in MS. To test this hypothesis, we used tract-based spatial statistics (TBSS) to map damaged WM tracts which predict performance of social cognition in MS patients.

PARTICIPANTS AND METHODS

Participants

We enrolled consecutively 60 patients with MS regularly followed in our department and 60 healthy volunteers recruited from the community who served as healthy controls (HC), matched on age, sex and educational level (Table 1).

Patient´s inclusion criteria for the present study were: age between 18 to 55 years old, definite diagnosis of MS according to the 2010 McDonald criteria [16], and relapsing-remitting or secondary-progressive subtypes. Exclusion criteria for all participants were history of neurological (other than MS in patient group) or systemic disease; history of psychiatric illness, with the exception of stable mild to moderate depressive symptoms; history of head injury resulting in loss of consciousness; a significant visual, auditory or language impairment that would negatively affect their ability to complete tests or understand test instructions; current/prior use of antipsychotic medication; alcohol, drug or substance abuse; starting/stopping antidepressants in the previous 2 months; and, for MS patients, a relapse or steroid treatment within 8 weeks preceding evaluation.

As a result of the matching procedure, MS patients and HC did not differ significantly in age, sex or education level (Table 1). Regarding the MS population, the disease course was relapsing–remitting in 50 patients (83.3%) and secondary progressive in 10 patients (16.7%). The mean disease duration was 10.6±6.6 years and the median Expanded Disability Status Scale (EDSS) score was 2.0 (IQR 1.5). All patients were under treatment with disease-modifying drugs.

Table 1. Demographic and clinical characteristics of MS patients and HC

	MS patients (n =60)	**HC (n=60)**	**P value**
Age, mean years ±SD	37.2± 7.5	36.1 ± 9.4	0.475
Education, mean years ±SD	13.2± 4.0	14.0 ± 3.9	0.258
Female, n (%)	40 (66.7)	40 (66.7)	1.0
Right handedness, n (%)	56 (93.3)	58 (96.7)	0.539
IQ estimate, mean±SD	113.5 ± 11.0	116.6 ± 8.3	0.081
Disease course, n (%)			
Relapsing-remitting	50 (83.3)	NA	NA
Secondary progressive	10 (16.7)		
Disease duration, mean years±SD	10.6 ± 6.6	NA	NA
EDSS, median (IQR)	2.0 (1.5)	NA	NA
Total T1 Lesion volume, mean ml ± SD	12.9±5.3	NA	NA
Total T2 Lesion volume, mean ml ± SD	16.8±15.8	NA	NA

Abbreviations: MS= Multiple Sclerosis; HC= Healthy controls; EDSS= Expanded Disability Status Scale; IQ= Intelligence quotient; IQR= Interquartil range; NA= Not applicable

Standard protocol approvals, registrations, and patient consents

All participants provided written informed consent to participate in the study, which was approved by the local ethics committee.

Clinical assessment

A full medical history and detailed neurologic examination were obtained for all patients. The following clinical and demographic data were collected: age, sex, handedness, years of education, disease duration, and current disease-modifying treatment. Physical disability was evaluated using EDSS [17]. For HC, medical history was obtained by an interview preceding assessment.

Theory of Mind testing

The tests selected to assess ToM were performed by the same investigator blinded to the MRI findings. These tests were administered in a fixed order and included:

1. Revised "Reading the Mind in the Eyes" test

The Revised "Reading the Mind in the Eyes" test (referred to as "Eyes test") consists of 36 black-and-white photographs of the eye region [18]. These are surrounded by four words, one target and three foils, describing complex mental states. Participants were asked to select which words best described the thoughts or feelings of the person in the photograph.

The response options were presented on the screen throughout the test in order to minimise the working memory load. Correct answers were rated 1, incorrect answers were rated 0. The total raw score ranges from 0 to 36.

2. ToM Videos test

This task made use of moving images and consisted of 26 silent color video clips adapted from Sullivan and Ruffman [19], that showed characters interacting. For 3 seconds before the clip appeared on the screen, two words were presented on the screen, one at the bottom left hand side and the other at the bottom right-hand side. These words remained on the screen as the clip played to reduce working memory load. Participants were instructed in advance to choose

the word that best described the thoughts or feelings of the person in the video. Correct answers were rated 1, incorrect answers were rated 0. The total raw score ranges from 0 to 26.

Other Neuropsychological Testing

Premorbid intelligence was estimated using the Irregular Word Reading Test (TeLPI) [20]. The Beck Depression Inventory (BDI) [21, 22] and the Modified Fatigue Impact Scale (MFIS) [23, 24] were used to determine the influence of depression and fatigue, respectively. Results for BDI range from 0 to 63 and the MFIS ranges from 0 to 84, with higher scores indicating more depression and fatigue, respectively.

MRI Acquisition and Analysis

All subjects were examined on a 3Tesla Siemens Magnetom TrioTim scanner (Erlangen, Germany) using a 12-channel birdcage head coil. Scans were prescribed in an axial-oblique orientation, parallel to the sub-callosal line. For each participant the following acquisitions were obtained: (1) sagittal 3D Fluid Attenuated Inversion Recovery (FLAIR): TR 5s, TE 388 ms, TI 1.8s, FoV 250x250 mm^2, yielding 160 slices with 1×1×1 mm^3 voxel size; (2) Diffusion Tensor Imaging (DTI): TR 7800 ms, TE 90 ms, number of excitations (NEX)1; matrix, 96x96x63 contiguous axial slices; isotropic voxel resolution of 2x2x2mm^3; bandwidth of 1628 Hz/pixel and echo spacing of 0.72ms. The DTI was acquired along 63 non-collinear directions (b=1000s/mm^2), with one scan without diffusion weighting (b=0s/mm^2, b0).

The DTI images were processed using Oxford University's FMRIB Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl) version 5.0.9, on a linux-based platform, following the TBSS pipeline [25]. Pre-processing included correction of eddy current distortions and motion

artifacts, followed by removal of extra cerebral tissue using FSL's brain extraction tool. The diffusion tensors were fitted to each voxel and quantitative measures of fractional anisotropy (FA) and mean diffusivity (MD) were derived voxelwise, for each subject. Values for FA range between 0 and 1, with lower values reflecting decreased directionality of diffusion along one axis. MD reflects the the magnitude of the self-diffusion of water molecules, with higher values generally corresponding to increased water diffusion and less cellular density.

Individual FA maps were visually inspected for the presence of significant residual motion or other artefacts. Of the 120 participants included in the study, 2 MRI datasets of MS group were excluded due to significant artefacts that precluded TBSS analysis. The FA images were then nonlinearly transformed to a 1x1x1mm^3 standard space image (FMRIB58_FA). When creating the FA skeleton a threshold of 0.2 was applied to the tract "thinning" process to exclude non-WM voxels. By creating a mean FA skeleton, representing the center of all fiber tracts common to every subject, inter-subject variability was minimized [25].The MD data were processed similarly, using nonlinear warps and skeleton projections previously obtained in the FA processing steps.

A fully automated method, based on a lesion growth algorithm [26], was used to assess the T2-hyperintense WM lesion load from FLAIR images. This algorithm is implemented in the Lesion Segmentation Toolbox (http://www.applied-statistics.de/lst, version 1.2.3) for SPM. Once lesion probability maps have been calculated, binary lesion masks were visually inspected and, if necessary, manually corrected using MRIcron software (www.mricro.com/mricron). Total T1-hypointense WM lesion load was estimated through the conventional semi-automatic pipeline of the FreeSurfer (version 5.3.0, http://surfer.nmr.mgh.harvard.edu/).

Statistical Analysis

Group comparisons were performed using the *t* test for unpaired samples, the nonparametric Mann-Whitney U test, and Chi-Square Test, when appropriate. Correlations between ToM tests, demographic, clinical and MRI variables were examined using Pearson's coefficients or Spearman's rank order coefficient when appropriate. These analyses were conducted using SPSS for Windows version 20.0 (SPSS Inc, Chicago). All tests were performed two-tailed and statistical threshold was set at $p<0.05$ corrected through false discovery rate (FDR) for multiple testing. Effect sizes of group comparisons were calculated according to the Hedges g formula in which the difference between the means is divided by the pooled standard deviation (SD) and weighted for sample size.

Voxelwise statistics for each skeleton voxel were calculated using FSL's randomize tool, which combines permutation testing and general linear modeling – a two sample unpaired t-test for MS patients versus HC's FA and MD, and a correlation analysis between ToM tests and these diffusion metrics for the MS patients group, while treating age as a covariate of no interest. WM voxels with lesions were excluded from the analyses (after transforming the binary lesion masks to the same standard space as the FA images) using the procedure described in the lesion masking section of randomise's user guide (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise/UserGuide). Therefore, only the NAWM was considered for the purpose of these analyses.

A total of 5000 permutations were used with Threshold-Free Cluster Enhancement (TFCE), corrected for multiple comparisons by controlling for family-wise error (FWE) rates[27]. P-values <0.05 were considered statistically significant.

The skeletal regions with significant results were labeled anatomically by mapping the TBSS FWE-corrected statistical maps to the JHU-ICBM-DTI-81 WM atlas [28].

The mean FA skeleton was also mapped onto the aforementioned atlas, and the total number of voxels per tract was calculated, which was used to determine the percentage of significant voxels within each labeled tract. The mean p-values per significant labeled region were determined. The mean and standard deviation of both diffusion metrics were obtained per subject and for each region.

RESULTS

Theory of Mind performance in MS: clinical and conventional MRI correlates

Patients with MS had significantly lower percentage of correct responses on both tasks of ToM compared to HC, i.e. Eyes Test (58.7±13.8% vs. 81.9±10.4%, $p<0.001$, Hedges g:-1.886) and Videos Test (75.3±9.3% vs. 88.1±7.1%, $p<0.001$, Hedges g:-1.537).

Performance on ToM tests in MS patients was not associated with age, sex, years of education, disease duration, EDSS, depression (BDI score) or fatigue (MFIS score) ($p>0.05$ FDR corrected).

On the other hand, Eyes Test and Videos Test performance were both negatively correlated with T2 lesion volume ($r=-0.505$, $p<0.001$; -0.362, $p=0.005$, respectively) and with T1 lesion volume ($r=-0.484$, $p<0.001$; -0.314, $p=0.017$, respectively).

TBSS analysis

1. Voxel-based comparison between MS patients and HC

Compared with HC, MS patients showed widespread abnormalities throughout the WM skeleton in both hemispheres defined by significant FA decrease (in 60.1% of skeleton voxels) and/or MD increase (in 50.7% of skeleton voxels). The damage was most extensive (≥90% of voxels within each tract with significant FA reduction and/or MD increase) in the following tracts: corpus callosum; fornix; posterior thalamic radiations (optical radiations); sagittal stratum;

tapetum; superior fronto-occipital fasciculus, posterior and superior corona radiate; inferior cerebral peduncle (Figure 1; Table e-1: please see at the end of this chapter).

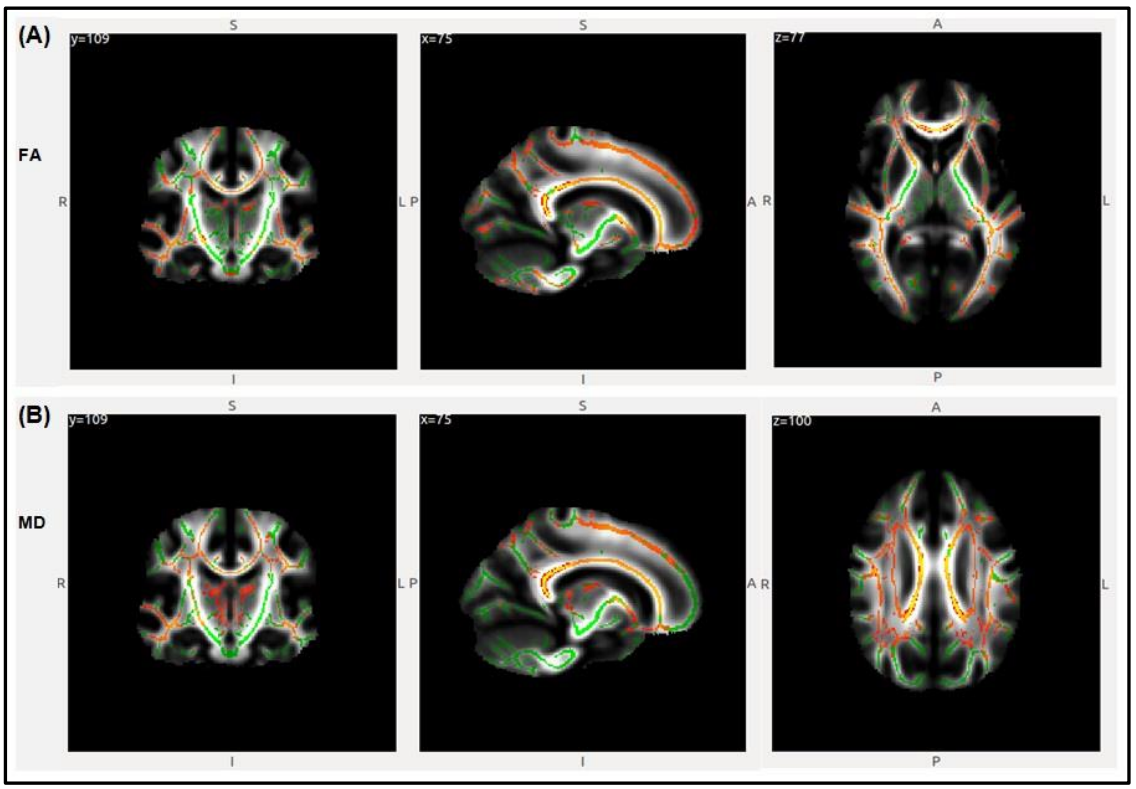

Figure 1. White matter tracts with significantly (A) FA reduction and (B) MD increase in patients with multiple sclerosis relative to healthy controls.
Significant regions are displayed in red/yellow colour according to the lower and higher significance respectively (to aid visualization, significant regions were thickened using the tbss_fill script implemented in FSL). Results are shown overlaid on the mean FA skeleton (A) or on the mean MD skeleton (B), both displayed in green colour. White matter voxels with lesions were excluded and therefore only the NAWM was considered for the analysis. Cluster-based thresholding corrected for multiple comparisons controlling for family-wise error (FWE) rates, with p-values <0.05 considered statistically significant. Abbreviations: FA: fractional anisotropy; MD: Mean diffusivity; NAWM: Normal appearing white matter; A: Anterior; P: Posterior; L: Left; R: Right; S: Superior; I: Inferior.

2. White matter tracts associations of Theory of Mind performance in MS patients

Maps of DTI metrics correlated with performance on ToM tasks in patients with MS are shown in Figure 2. All reported results are thresholded at p<0.05 after FWE correction for multiple testing over the whole brain. Performance on ToM tests was correlated with higher values of FA and lower values of MD across widespread WM tracts of the two hemispheres.

Considering the spatial extent of each significant WM cluster, for Eyes Test the largest effects were found in the body and genu of corpus callosum, stria terminalis of fornix, tapetum, uncinate fasciculus, and left inferior cerebellar peduncle (Table 2, Figure 3.a). On the other hand, for Videos Test the largest effects were found in the genu and splenium of corpus callosum, stria terminalis of fornix, uncinate fasciculus, left tapetum, and right superior fronto-occipital fasciculus (Table 2, Figure 3.b).

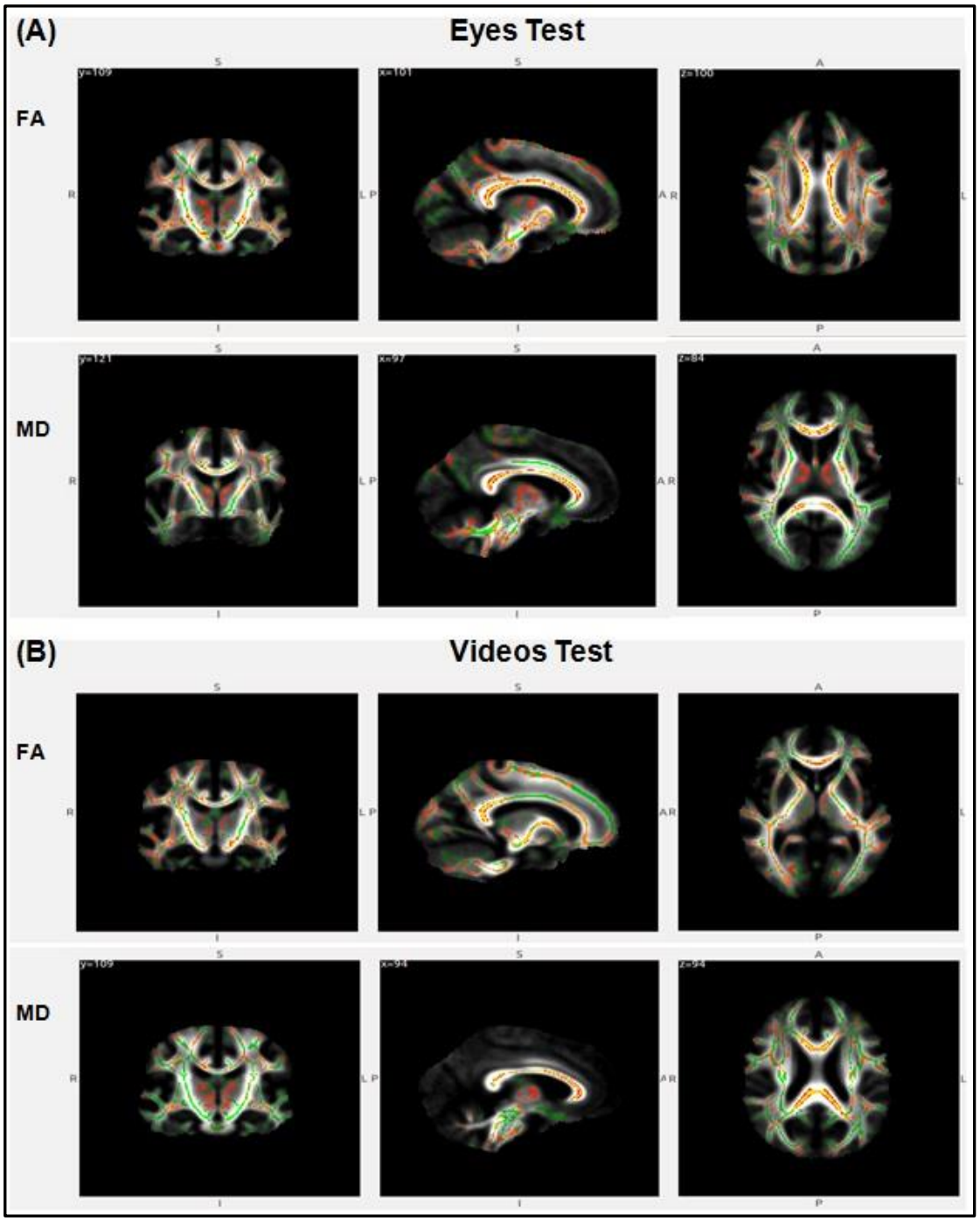

Figure 2. White matter tracts significantly correlated with ToM performance in patients with multiple sclerosis.
Significant regions correlated with (A) Eyes Test scores and with (B) Videos Test scores, treating age as a covariate of no interest, are displayed in red/yellow color. Results are shown overlaid on the mean FA skeleton (top) or on the mean MD skeleton (bottom), both displayed in green color. White matter voxels with lesions were excluded and therefore only the NAWM was considered for the analysis. Cluster-based thresholding corrected for multiple comparisons controlling for family-wise error (FWE) rates, with p-values <0.05 considered statisticaly significant. Abbreviations: ToM: Theory of Mind; FA: Fractional anisotropy; MD: Mean diffusivity; NAWM: Normal appearing white matter; A: Anterior; P: Posterior; L: Left; R: Right; S: Superior; I: Inferior.

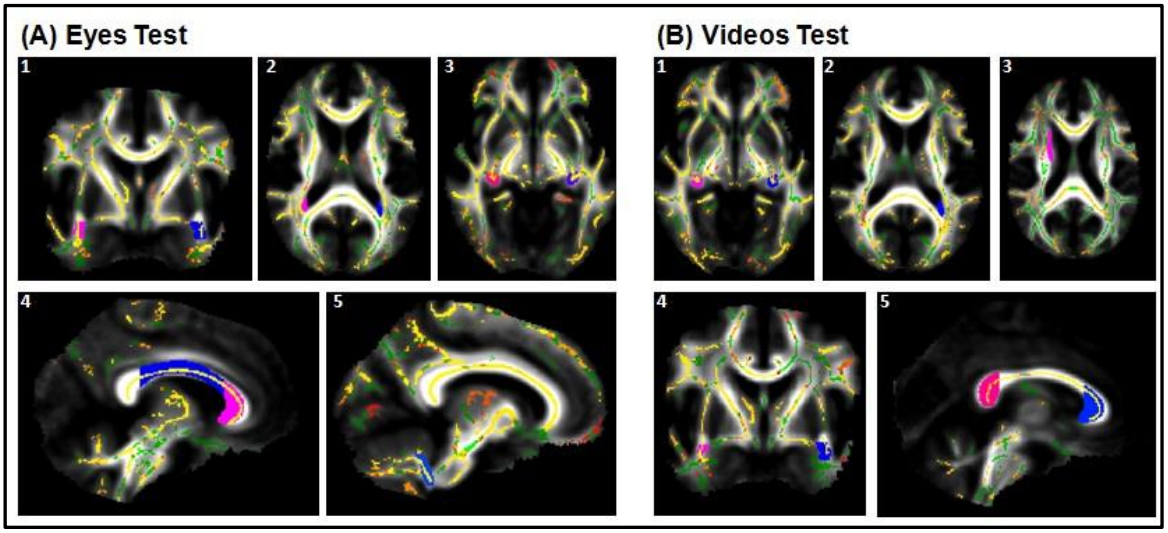

Figure 3. Summary of white matter tracts correlated with ToM performance in patients with multiple sclerosis, with the largest effects in terms of spatial extent
For illustrative purposes, the white matter clusters correlated with ToM tests which have the largest effects in terms of spatial extent (≥90% of significant voxels within each tract) are displayed in pink and blue colors. (A) For Eyes Test the largest effects were found in the uncinate fasciculus (1), tapetum (2), stria terminalis of fornix (3), body and genu of corpus callosum (4), and left inferior cerebellar peduncle (5). (B) For Videos Test the largest effects were found in the stria terminalis of fornix (1), left tapetum (2), right superior fronto-occipital fasciculus (3), uncinate fasciculus (4), genu and splenium of corpus callosum (5).
Abbreviations: ToM: Theory of Mind

Table 2. Summary of WM tracts correlated with ToM performance in MS patients (N=58), with the largest effects in terms of spatial extent[1]

		FA		MD	
	WM tracts	**Sign. Voxels**	**p value***	**Sign. Voxels (%)†**	**p value***
Eyes Test	Body Corpus Callosum	92.68	0.004	76.52	0.006
	Genu Corpus Callosum	98.38	0.002	82.92	0.005
	Tapetum L	100.00	0.006	71.43	0.010
	Tapetum R	100.00	0.003	96.00	0.012
	Fornix Stria L	93.61	0.006	81.74	0.006
	Fornix Stria R	94.36	0.003	85.34	0.005
	Uncinate fasciculus L	100.00	0.002	73.81	0.006
	Uncinate fasciculus R	100.00	0.003	61.54	0.025
	Inferior cerebellar peduncle L	94.30	0.004	75.00	0.006
Videos Test	Genu Corpus Callosum	95.58	0.004	88.86	0.010
	Splenium Corpus Callosum	90.39	0.004	75.69	0.010
	Tapetum L	100.00	0.011	57.14	0.021
	Fornix Stria L	94.52	0.006	83.11	0.011
	Fornix Stria R	90.98	0.007	77.07	0.011
	Uncinate fasciculus L	90.48	0.006	78.57	0.016
	Uncinate fasciculus R	100.00	0.004	65.38	0.019
	Superior fronto-occipital fasciculus R	52.50	0.009	97.50	0.026

[1] ≥90% of significant voxels within each tract

* P-values<0.05 were considered statistically significant (corrected for multiple comparisons by controlling for family-wise error (FWE) rates)

† Percentage of significant voxels within each labeled tract

Abbreviations: ToM: Theory of Mind; MS: Multiple Sclerosis; DTI: Diffusion Tensor Imaging; WM: White matter; FA: Fractional anisotropy; MD: Mean diffusivity

DISCUSSION

The present study aimed to assess the contribution of microstructural damage of WM for social cognition impairment in MS patients using DTI with TBSS analysis. Considering that social cognition is a multidimensional function which relies on a large scale neural network [13, 29], it is reasonable to hypothesize that MS, as a prototypal disease of WM, may be associated with reduced connectivity within the social brain network and, as a result, with social cognition impairment.

In line with previous studies [2-9], we confirmed that patients with MS exhibited deficits in ToM, which is considered one of the essential domains of social cognition. Indeed, patients presented worse performance both on Eyes Test and Videos Test, indicating that they have difficulties on decoding other persons' mental states either from static or dynamic visual stimuli. Accordingly, in a recent meta-analysis [9] deficits in ToM and facial emotion recognition were identified among patients with MS relative to HC and the largest deficits were observed for visual ToM tasks. However, the effect sizes found in our study are higher than those reported in the meta-analysis.

It is also noteworthy that ToM impairment was independent of disease duration or EDSS suggesting it may occur even in earlier stages of the disease or in patients without significant physical disability. On the other hand, ToM performance was negatively correlated with T1 and T2 lesion volumes, supporting the hypothesis that WM damage is a potential mechanism underlying ToM impairment. This finding is in agreement with a recent study in which ToM performance in MS correlated with both total and regional T1-lesion load of association fiber tracts interconnecting cortical regions related to visual and emotion processing [30].

In order to assess the contribution of microstructural NAWM damage for ToM impairment in MS we used TBSS for whole-brain voxelwise analysis. As expected, patients with MS showed widespread abnormalities throughout the majority of NAWM skeleton characterized by reduced FA and/or MD increase. More importantly, we found that NAWM damage contributed for ToM

impairment in MS and that the pattern of tract involvement was widely dispersed across both hemispheres, reflecting the complex and distributed nature of the brain networks that support social cognition processes [13]. Nonetheless, the most robust associations were identified within tracts of limbic pathways (uncinate fasciculus, fornix) and callosal interhemispheric fibers (corpus callosum, tapetum).

The uncinate fasciculus is a long-range WM tract that connects anterior temporal lobes and the amygdala to the orbitofrontal cortex through a direct and bidirectional monosynaptic pathway [31]. At the centre of this loop is the amygdala, where the social relevant information converges and is integrated, constituting the main hub of the social brain network [13]. Hence, due to its unique position, the uncinate fasciculus is a plausible WM tract underpinning connectivity of the social brain network. In line with this, previous studies suggest that the uncinate fasciculus plays an important role in social-emotional processing by allowing temporal lobe-based mnemonic associations (e.g. a person's name, face, voice or feelings about a person) to modify behaviour through interactions with the orbitofrontal cortex [32]. Furthermore, abnormalities in the uncinate fasciculus have been associated with several disorders characterized by altered social behaviour such as autism, antisocial personality disorder, and frontotemporal dementia [32].

Regarding the fornix, even though its role is best established within the domain of episodic memory [33], there has been some evidence linking fornix damage with altered processing of emotion and abnormal emotional behaviours [34, 35].

Recent studies indicate a critical role of callosal interhemispheric fibres for social-communicative abilities. Accordingly, interhemispheric connectivity disturbances caused by structural abnormalities in the corpus callosum have been suggested to play a major role in the pathophysiology of schizophrenia and autism [36, 37]. In addition, emotion processing impairments have been documented in patients with agenesis of the corpus callosum [38]. Likewise, the damage of tapetum, a WM tract composed of fibers from the body and splenium of the corpus callosum connecting the bilateral temporal lobes, including the superior temporal

gyrus which is a crucial region for social cognition [36], has been associated with non-verbal communication deficits in autism [36, 39].

The results of our study indicate that a diffuse pattern of NAWM damage in MS patients contributes for social cognition deficits by a putative mechanism of disconnection within the social brain network. In line with this, many of the injured WM tracts herein identified which predicted ToM performance are consistent with the known functional anatomy of the social brain network, including uncinate fasciculus, fornix, corpus callosum and tapetum. From another perspective, we cannot exclude the additional role of disconnection of compensatory cortical activity previously shown to occur in MS patients during cognitive tasks in functional MRI [40].

Ultimately, the contribution of GM damage for the pathophysiology of social cognition impairment in MS must be taken into account. As a matter of fact, GM pathology is a well-recognized mechanism underlying cognitive dysfunction in MS and the strength of this association exceeds that related to WM lesions [15]. Accordingly, in a recent study of our group [10] we found that ToM impairment in MS was associated with a multifocal pattern of GM atrophy involving cortical and subcortical regions that are considered key neural nodes of the social brain. More importantly, of all the GM regions involved, amygdala atrophy was the main predictor.

The present study has some limitations that suggest directions for future work. First, this is a cross-sectional study and therefore definite temporal relations cannot be established. Second, ToM was assessed with tests that rely exclusively on visual stimuli and in real life other sources are important cues, particularly the voice intonation. Finally, we did not assess other MRI variables that may have accounted for variance in ToM performance such as grey matter lesions and compensatory cortical adaptive responses by functional MRI.

Nevertheless, the results herein reported further the knowledge about the complex constellation of neurobehavioral symptoms in MS and extend our insight into the neural basis of

social cognition. The damage of WM, particularly NAWM, appears to be one of the underlying mechanisms contributing for social cognition impairment in MS.

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COMPETING INTERESTS

S. Batista has received honoraria for serving on scientific advisory boards of Biogen and Novartis Pharma, and for speaking in scientific meetings of Teva, Merck Serono, Genzyme, Biogen, and Novartis Pharma.

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O. C. d'Almeida, A. Afonso, Sandra Freitas, M. Castelo-Branco, I. Santana and L. Cunha have nothing to disclose.

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Table e-1. WM tracts with significant changes in DTI indices (reduced FA and/or increased MD) in MS patients (N=58) compared to HC (N=60)

	FA		MD	
WM tracts	**Sign. Voxels (%)†**	**p value***	**Sign. Voxels (%)†**	**p value**
Anterior corona radiata L	80.33	0.003	84.78	0.002
Anterior corona radiata R	87.64	0.002	88.36	0.002
Anterior limb internal capsule L	76.52	0.017	68.81	0.004
Anterior limb internal capsule R	82.05	0.002	72.65	0.004
Body Corpus Callosum	97.01	0.002	94.36	0.001
Cerebral peduncle L	48.69	0.003	7.85	0.007
Cerebral peduncle R	35.86	0.002	10.86	0.008
Cingulum gyrus L	78.59	0.002	48.56	0.006
Cingulum gyrus R	87.20	0.003	47.47	0.008
Cingulum hippocampus L	53.71	0.006	29.26	0.010
Cingulum hippocampus R	51.05	0.007	13.39	0.024
Corticospinal tract L	36.43	0.031	0.00	1.000
Corticospinal tract R	22.19	0.038	0.00	1.000
External capsule L	74.17	0.010	68.18	0.004
External capsule R	72.84	0.003	74.02	0.005
Fornix body	100.00	0.002	100.00	0.002
Fornix Stria L	98.88	0.003	94.03	0.001

Fornix Stria R	98.42	0.001	89.56	0.002
Genu Corpus Callosum	95.71	0.001	89.89	0.002
Inferior cerebelar peduncle L	94.85	0.018	0.00	1.000
Inferior cerebelar peduncle R	96.11	0.021	0.00	1.000
Medial lemniscus L	81.82	0.017	0.00	1.000
Medial lemniscus R	81.65	0.021	0.00	1,000
Middle Cerebellar Peduncle	58.54	0.023	0.00	1.000
Pontine crossing tract	86.85	0.025	0.00	1.000
Posterior corona radiata L	91.51	0.001	99.46	0.004
Posterior corona radiata R	90.57	0.001	95.82	0.001
Posterior limb internal capsule L	13.63	0.006	23.25	0.007
Posterior limb internal capsule R	15.28	0.005	34.89	0.008
Posterior thalamic radiations (OR) L	99.73	0.002	89.01	0.001
Posterior thalamic radiations (OR) R	99.00	0.003	88.40	0.002
Retrolenticular internal capsule L	73.01	0.002	76.14	0.003
Retrolenticular internal capsule R	69.87	0.001	81.67	0.002
Sagittal stratum L	100.00	0.003	98.60	<0.001
Sagittal stratum R	97.81	0.004	97.64	0.001
Splenium Corpus Callosum	98.22	0.001	98.68	0.004
Superior cerebelar peduncle L	32.76	0.022	0.00	1.000
Superior cerebelar peduncle R	40.91	0.021	0.00	1.000
Superior corona radiata L	54.86	0.005	90.86	0.001

Superior corona radiata R	61.53	0.003	93.67	0.002
Superior frontooccipital fasciculus L	92.86	0.017	98.81	0.002
Superior frontooccipital fasciculus R	92.86	0.001	95.71	0.001
Superior longitudinal fasciculus L	66.98	0.003	80.64	0.002
Superior longitudinal fasciculus R	60.55	0.005	86.31	0.002
Tapetum L	100.00	0.002	100.00	0.002
Tapetum R	100.00	0.002	100.00	0.002
Uncinate fasciculus L	67.35	0.010	0.00	1.000
Uncinate fasciculus R	52.63	0.005	22.81	0.004

* p values <0.05 were considered statistically significant (corrected for multiple comparisons by controlling for family-wise error (FWE) rates)

† Percentage of significant voxels within each labeled tract

Abbreviations: WM: White matter; DTI: diffusion Tensor Imaging; MS: Multiple Sclerosis; HC: Healthy controls; FA: Fractional anisotropy; MD: Mean diffusivity

CHAPTER 3 Theory of Mind and Executive functions are dissociated in multiple sclerosis

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ABSTRACT

Objectives: To investigate the relationship of executive functions (EF) and theory of mind (ToM) in multiple sclerosis (MS), clarifying whether ToM impairment in MS is related to EF dysfunction or whether they represent dissociable processes which can be independently affected in MS.

Methods: We enrolled 60 consecutive MS patients and 60 healthy controls (HC) matched on age, gender, and education level. All participants underwent ToM testing using the Eyes Test and the Videos Test and neuropsychological testing tapping different EF subdomains. A hierarchical cluster analysis was used to determine the similarity between explained variance between EF measures and ToM tests.

Results: MS patients had lower scores on both tasks of ToM compared to HC, i.e. Eyes Test (21.1± 5.0 vs. 29.5± 3.8, $p<0.001$; Cohen's d: 1.9) and Videos Test (19.6± 2.4 vs. 22.9± 1.8, $p<0.001$; Cohen's d: 1.6). They also performed significantly worse on different measures of EF. ToM performance was not significantly correlated with EF tests. The hierarchical cluster analysis showed that ToM measures were clustered separately from the EF measures, revealing three executive clusters (Attention/working memory cluster; Inhibitory control/shifting ability cluster; Verbal Initiative/Abstract reasoning cluster) and one ToM cluster.

Conclusion: This study suggests a dissociation of EF and ToM in MS, meaning that the MS-related neurobehavioral symptoms may be associated with a significant impairment in ToM independent of the level of EF performance. Ultimately, this discrimination of ToM deficits in MS may help to identify the appropriate cognitive and behavioural interventions.

KEYWORDS

Multiple Sclerosis, Social Cognition, Theory of Mind, Executive Functions, Cognitive impairment, Neurobehavioral symptoms

INTRODUCTION

Changes in social conduct and personality have been recognized for more than a century as frequent neurobehavioral symptoms of multiple sclerosis (MS) [1]. However, this cluster of symptoms originally called "euphoria sclerotic" has received little attention afterwards despite their negative impact on social life, including friendship, family roles and doctor-patient communication [2]. Hence, the origin of social behaviour disturbance in MS remains poorly understood.

Executive functions (EF) and theory of mind (ToM) are essential cognitive processes for adaptive social behaviour but the nature of their relationship is still debated . Previous studies in patients with neurological pathology have yielded variable results, either supporting a complete dissociation or a dependency between EF and ToM [3]. Traditionally, neurobehavioral symptoms in neurological diseases were considered to be a part of a cognitive dysexecutive syndrome. However, numerous clinical reports have accumulated over the past years which indicate that patients with florid behavioural symptoms may perform within the normal range on classic tests of executive functions [4]. On the other hand, there is growing evidence that ToM impairment is associated with social behaviour maladjustment in patients with neurological pathology, including frontotemporal dementia [5, 6], Huntington's disease [7], and Parkinson's disease [8].

In MS, cognitive impairment typically consists of domain-specific deficits rather than global cognitive decline**,** with EF being recognized as one of the core cognitive domains involved [9]. Likewise, recent studies revealed that ToM is impaired in patients with MS since the early stages of the disease [10-16]. Partially different results have been reported by Ouellet et al. (2010) who found that only MS patients with mild to moderate cognitive deficits had deficits on cognitive ToM, whereas MS patients without cognitive impairment did not differ from healthy controls. More recently, a thorough investigation of cognitive and affective ToM abilities in MS patients

was performed by Raimo et al. [17] who reported that both components were affected in MS patients, in verbal and nonverbal modalities.

However, the relationship between EF and ToM in MS remains to be understood as previous studies reported contradictory results. While some authors observed an association between ToM and EF impairment [5, 10, 16, 17], others did not [12, 14, 18].

In the study of Henry et al. [10], ToM was assessed using a non-verbal ToM test (Reading the Mind in the Eyes test) and EF with a measure of verbal abstraction from the Screening Examination for Cognitive Impairment. They reported that MS patients were significantly impaired on ToM compared to a demographic-matched healthy group, and that this impairment was associated with deficits in executive control. The main limitations of this study were the reduced sample sized and the heterogeneity of the patient cohort. Ouellet and colleagues [18] examined ToM and cognitive abilities, including EF, of patients with definite diagnosis of MS but also with probable diagnosis of MS, increasing the heterogeneity of the sample. They subdivided the MS group into a cognitively impaired group and cognitively intact group. MS patients with cognitive impairments were found to have more difficulties attributing mental states to others, while there were no differences between cognitively intact MS patients and normal controls on the two ToM measures which were the short stories and video clip. There was no association of ToM with EF which was assessed with a large battery including the Trails-Making Test, the Oral Word Association Test, the Zoo Map Test, the Mazes subtest of the WISC-III and the Stroop Test. Other study [14] analysed the cognitive and affective aspects of ToM individually in patients with mild relapsing-remitting MS and found that they showed deficits in cognitive ToM, but their affective ToM seemed to be spared. Although some executive tests correlated with the patients' ability to detect a social faux pas, none correlated specifically with their ability to infer other people's intentions. More recently, Raimo et al. [17] found different results and reported that both affective and cognitive aspects of ToM were impaired in MS patients and significantly related to EF. However, EF were only assessed by the Word List Generation test and Stroop colour-word interference test.

Overall, the main limitation of the aforementioned studies was the use of a statistical approach based on binomial correlation analyses or analysis of variance (ANOVA), which do not test directly the dependency of variables with each other. Cluster analysis is a technique that allows to investigate the relationship between cognitive measures more accurately, by determining the similarity of variance [6, 19].

In the present study, using a hierarchical cluster analysis, we aimed to investigate whether ToM impairment in MS is related to EF dysfunction or whether they can occur independently.

METHODS

Participants

We enrolled 60 consecutive patients with MS regularly followed in our department and 60 healthy volunteers recruited from the community who served as healthy controls (HC), matched on age, gender and educational level (Table 1). All participants provided written informed consent to participate in the study, which was approved by the ethics committee of Faculty of Medicine of Coimbra´s University (CE-027/2011).

Patient´s inclusion criteria for the present study were: age between 18 to 55 years old, definite diagnosis of MS according to the 2010 McDonald criteria [20], and relapsing-remitting or secondary-progressive subtypes. Exclusion criteria for all participants were history of neurological (other than MS in patient group) or systemic disease; history of psychiatric illness, with the exception of stable mild to moderate depressive symptoms; history of head injury resulting in loss of consciousness; a severe visual, auditory or language impairment that would negatively affect their ability to satisfactorily complete tests or understand test instructions(the exclusion of a deficit of oral comprehension was done by the neuropsychologist and by the neurologist based on the clinical interview, the neurological exam and the overall comprehension

of the neuropsychological test instructions); current or prior use of antipsychotic medication; alcohol, drug or substance abuse; starting or stopping antidepressants in the previous 2 months; and, for MS patients, a relapse or steroid treatment within 8 weeks preceding evaluation.

Clinical assessment

A full medical history and detailed neurologic examination were obtained for all patients. The following clinical and demographic data were collected: age, sex, handedness, years of education, disease duration, number of relapses in the last year and current disease-modifying treatment. Physical disability was evaluated using the detailed Kurtzke Expanded Disability Status Scale (EDSS) [21]. The Global MS severity score (MSSS) was used as a measure of disease severity. Individual MSSS were calculated using the MSSS test program of Roxburgh et al. [22]. This algorithm adjusts disability as measured by the EDSS for disease duration. Disease duration was measured as the number of years between the year of onset of first symptom and year of examination. For HC, medical history was obtained by an interview preceding assessment.

Theory of Mind testing

The same investigator (SB) was responsible for testing the subjects on the ToM battery. She was blind as to the performance in neuropsychological testing. These tests were administered in a fixed order and included:

1. Revised "Reading the Mind in the Eyes" test

The Revised "Reading the Mind in the Eyes" test [133] (referred to as "Eyes test") consists of 36 black-and-white photographs of the eye region. These are surrounded by four words, one target and three foils, describing complex mental states such as friendly, irritated, worried and sarcastic. A handout with definitions for all the target and foil words in the

photographs was prepared for participants' use before and during the task. Participants were asked to select which words best described the thoughts or feelings of the person in the photograph. The response options were presented on the screen throughout the test in order to minimize the working memory load.

Correct answers were rated 1, incorrect answers were rated 0. The total raw score ranges from 0 to 36 with higher scores indicating better performance.

2. ToM Videos test

This task is designed to mirror the real demands of live social situations. The test consisted of 26 silent colour video clips adapted from Sullivan and Ruffman [23] showing characters interacting. Participants were instructed in advance to choose the word that best described the thoughts or feelings of the person in the video. The clips were 2 to 7 seconds long. Two choice words were presented on the bottom right and left side of the screen, for 3 seconds before the clip appeared on the screen. These words remained on the screen as the clip played to reduce working memory load. For example, in one video clip there was a young woman speaking with a man. Taking into account her body posture and facial expression the participant's task was to decide whether she was "flirtatious" or "*sad*".

Correct answers were rated 1, incorrect answers were rated 0. The total raw score ranges from 0 to 26 with higher scores indicating better performance.

Neuropsychological Testing – Executive Functions Measures

Neuropsychological (NP) testing was performed by a neuropsychologist (AA) blinded to the performance in ToM tests. The same battery was administered to all subjects in a fixed order. For the purpose of the current study, it was comprised of tests tapping different EF processes: Controlled Oral Word Association Test (COWAT)[24] is a verbal fluency test used to measure

mental flexibility; the Wisconsin Card Sorting Test (WCST)[25], was used to evaluate mental set shifting ability; the Stroop Test [26] was used to assess inhibitory control; Trail Making Test (TMT) A and B [27] to evaluate attention and reactive mental flexibility; Interpretation of Proverbs [28] and Raven's Advanced Progressive Matrices (RAPM) [29] to assess verbal and non-verbal abstract reasoning, respectively.

1. The Controlled Oral Word Association Test

COWAT is a verbal fluency test that measures spontaneous production of words belonging to the same category or beginning with some designated letter. In this study we used three trials of letter/phonemic fluency and one trial of category/semantic fluency. The letter fluency stimuli was "F-A-S" and the category cue was "animals". Participants were instructed to say as many words as they can in one minute for each trial. The total number of correct words produced in the three trials of letter fluency and in the category fluency trial were retained both as variables for analysis.

2. The Wisconsin Card Sorting Test

The WCST was administered in accordance with the test manual [25]. Each of the 128 cards displays a varying number of symbols in colors of red, yellow, blue, or green. The participant was asked to match each new card to one of four target cards. After each response, the examiner signaled whether the participant was correct or incorrect. After 10 consecutive correct sorts, the concept for sorting (i.e., color, form, number) changed. Number of categories achieved and perseverative responses were retained for analyses. A category was achieved when 10 consecutive sorts were made according to the correct sorting principle. Perseverative responses were defined as responses that matched a previous category.

3. The Stroop Test

The Stroop test version used in this study consisted of a page with written color names different from the color ink they are printed in [26]. In the first trial (Stroop-Color), the participant was instructed to read the written words. In the second trial (Color-Word), the participant named the ink colors instead. In both trials, participants were instructed to go down each sheet reading words or naming the ink colors as quickly as possible. The score used was the time that it took to complete each of the trials.

4. The Trail Making Test

The TMT is composed of two parts: TMT-A and TMT-B [27]. In TMT-A, the participant was asked to connect numbers placed randomly on a page in a consecutive order. In TMT-B, the participant was asked to connect consecutive numbers and letters in an alternating sequence. Participants were instructed to work as quickly and accurately as possible without lifting the pencil; if an error is made, it is pointed out by the examiner for correction. The total time required to complete each part is measured and used for analysis.

5. Interpretation of proverbs

This test consists on the interpretation of three Portuguese proverbs, containing, each one, two abstract concepts [28]. The maximum score for each proverb (3) is considered when there is a clear abstract interpretation of the two key concepts; the score 2 is attributed when only one of the concepts is explained; the score 1 when there is a pragmatic interpretation of all the proverb (without any abstract reasoning) and the score 0 is considered when the patient simply repeats the proverb or does not answer. The total score, ranging from 3 (poor performance) to 9 (completely correct interpretation), was used for analysis.

6. The Raven's Advanced Progressive Matrices

We administered only the set I from the RAPM [29], as a relatively brief evaluation of nonverbal abstract reasoning, to reduce the length of the testing session and therefore to minimize fatigue in MS patients. The test includes 12 matrices of different geometrical patterns, with the bottom right cell missing. Participants were asked to select the correct pattern that completes the matrix from a set of six alternatives displayed below the matrix. The score used corresponds to the number of correct responses. The minimum score is zero and the maximum score is 12.

Depression and Fatigue measures

Depression and fatigue were evaluated as potential covariables of cognitive performance. The Beck Depression Inventory (BDI)[30, 31] and the Modified Fatigue Impact Scale (MFIS)[32, 33], were used to measure depression and fatigue respectively. Results for BDI range from 0 to 63 and the MFIS ranges from 0 to 84, with higher scores indicating more depression and fatigue, respectively.

Statistical Analysis

Group comparisons were performed using the t test for unpaired samples, the nonparametric Mann-Whitney test, and a Chi-Square Test, when appropriate. A One-way MANCOVA was conducted to evaluate the differences between MS patients and HC on the performance of ToM tasks and EF tests controlling for depression and fatigue.

Correlations between ToM tests and EF tests were examined using Pearson's coefficients or Spearman's rank order coefficient when appropriate.

These tests were performed two-tailed and statistical threshold was set at $p< 0.05$ corrected through false discovery rate (FDR) for multiple testing.To determine the relation between EF

and ToM measures, a hierarchical cluster analysis (Ward's method) was performed. During the analysis, each variable was defined as an individual cluster and was standardized (z-scores). Clusters were then sequentially merged according to their similarity, or distance (squared Euclidean distance) in a geometric space where the number of variables set the number of dimensions. A dendrogram was computed to represent the relationships of similarity among the group of variables.

Analyses were conducted using SPSS for Windows version 20.0 (SPSS Inc, Chicago, Ill).

RESULTS

Sample characteristics

As a result of the matching procedure, MS patients and HC did not differ significantly in age, sex or education level (Table 1). Similarly, handedness was not significantly different across the two groups and all participants were Caucasian. Regarding the MS population (Table 1), the disease course was relapsing–remitting (RR) in 50 patients (83.3%) and secondary progressive (SP) in 10 patients (16.7%). The mean disease duration was 10.6 ± 6.6 years, the median EDSS score was 2.0 (IQR 1.5) and the median MSSS score was 2.8 (IQR 3.1). All patients were under treatment with disease-modifying drugs.

Table 1. Clinical-demographic data of multiple sclerosis patients and healthy controls

	MS (n =60)	HC (n=60)	p value
Age, mean years ±SD	37.2± 7.5	36.1 ± 9.4	0.475
Education, mean years ±SD	13.2± 4.0	14.0 ± 3.9	0.258
Female, n (%)	40 (66.7)	40 (66.7)	1.0
Right handedness, n (%)	56 (93.3)	58 (96.7)	0.539

Table 1. Clinical-demographic data of multiple sclerosis patients and healthy controls

	MS (n =60)	HC (n=60)	p value
Disease course, n (%)			
Relapsing-remitting	50 (83.3)		
Secondary progressive	10 (16.7)		
Disease duration, mean years±SD	10.6 ± 6.6		
EDSS, median (IQR)	2.0 (1.5)		
MSS, median (IQR)	2.8 (3.1)		

Abbreviations: MS= patients with MS; HC= healthy controls; EDSS= Expanded Disability Status Scale; IQR= Interquartil range; MS= Multiple Sclerosis Severity Scale

Theory of Mind testing and neuropsychological results in patients with MS and HC

Patients with MS had significantly lower scores on both tasks of ToM compared to HC, i.e. Eyes Test (21.1± 5.0 vs. 29.5± 3.8, p<0.001 FDR-corrected; Cohen's d: 1.9) and Videos Test (19.6± 2.4 vs. 22.9± 1.8, p<0.001 FDR-corrected; Cohen's d: 1.6). They also performed significantly worse on EF tests, except for proverbs interpretation test and number of categories on WCST, and presented higher scores on BDI and MFIS (Table 2).

After controlling for the effect of depression and fatigue using a one-way MANCOVA analysis, the difference between MS patients and HC on ToM and EF performance was still significant (Pillai's Trace = 0.552, F (12,103)= 10.6, p< 0.001). The univariate F tests showed there was a significant difference between MS patients and HC for the Eyes test, F(1,116)=88.6, p<0.001 FDR-corrected; and the Videos test, F(1,116)=50.9, p<0.001 FDR-corrected. Regarding the EF tests, only the COWAT-FAS F(1,116)=12.7, p=0.004 FDR-corrected, the TMT-B (1,116)= 8.8,

p=0.012 FDR-corrected, and the Stroop Color F(1,116)= 8.1, p=0.012 FDR-corrected, remained significant.

Table 2. Neuropsychological results in MS patients and HC

		MS (n =60)	HC (n=60)	Cohen's d	p value*
ToM measures	**Eyes Test**	21.1± 5.0	29.5±3.8	1.9	<0.001
	Videos Test	19.6±2.4	22.9±1.8	1.6	<0.001
Executive Functions measures	**COWAT –FAS (total)**	27.2±8.2	34.3±9.2	0.8	<0.001
	COWAT- Animals (total)	18.7±4.7	20.4±4.3	0.4	0.041
	WCST-Categories	5.2±1.4	5.7±1.0	0.4	0.055
	WCST- Perseverative responses	28.3±22.9	19.7±18.0	0.4	0.034
	Stroop Color (time)	72.4±30.9	56.8±8.3	0.7	<0.001
	Stroop Color-Word (time)	143.0±40.1	120.5±20.9	0.7	<0.001
	TMT-A (time)	46.6±20.6	38.0±13.5	0.5	0.012
	TMT-B (time)	125.8±66.2	81.5±32.3	0.9	<0.001
	Proverbs (total)	7.6±1.7	7.6±1.9	0	0.881
	RAPM	10.8±2.0	11.4±0.9	0.4	0.041
Depression	**BDI**	9.5±7.0	3.8±3.9	1.0	<0.001
Fatigue	**MFIS**	33.8±19.6	16.5±15.1	1.0	<0.001

Data are given as mean raw scores ±SD

*p-values are corrected for multiple comparisons through False Rate Discovery method

Abbreviations: MS= patients with multiple sclerosis; HC= Healthy controls; ToM = Theory of Mind; COWAT= Controlled Oral Word Association Test; WCST= Wisconsin Card Sorting Test; TMT= Trail Making Test; RAPM= Raven's Advanced Progressive Matrices; BDI= Beck Depression Inventory; MFIS= Modified Impact Fatigue Scale.

Possible ranges of scores: Eyes Test= [0-36]; Videos Test= [0-26]; COWAT- FAS = [0;∞[; COWAT- Animals = [0;∞[; WCST categories= [0, 6]; WCST perseverative responses= [0, 128]; Stroop-Color (time) = [0;∞[; Stroop-Color-word (time) = [0;∞[; TMT-A (time) = [0;∞[; TMT-B (time) = [0;∞[; Proverbs=[3, 9]; RAPM = [0;12]; BDI= [0-63]; MFIS= [0-84]

Correlation of Executive Functions and Theory of Mind with clinical variables in MS patients

Performance of MS patients on ToM tests was not correlated with age, years of education, disease duration, EDSS or MSSS ($p>0.05$ FDR corrected) (Table 3).

In order to analyze the effect of depression on ToM performance, we divided the MS sample on two groups: those patients scoring above the cut-off for depression on BDI ("depressed patients"= 18, 30%) and those scoring below the cut-off for depression on BDI ("non-depressed patients"= 42, 70%). We found that ToM performance was not significantly different when comparing "depressed patients" with "non-depressed patients": Eyes Test 21.6 ±5.9 vs 20.9 ±4.5, $p= 0.617$; Videos Test 19.6 ±3.4 vs 19.6 ±1.9, $p=0.963$. Moreover, scores on ToM tests were not significantly correlated with BDI score (Eyes Test $r=-0.042$, $p>0.05$; Videos Test $r=-0.095$, $p>0.05$).

Regarding fatigue, there were 26 patients (43.3%) scoring positively in the Modified Fatigue Impact Scale (MFIS). However, ToM performance was not significantly different between the patients with fatigue and those without fatigue (Eyes Test 21.0 ±5.5 vs 21.2 ±4.6, $p= 0.916$; Videos Test 19.1 ±3.1 vs 19.9 ±1.7, $p=0.269$). Moreover, scores on ToM tests were not significantly correlated with MFIS score (Eyes Test $r=-0.079$, $p>0.05$; Videos Test $r=-0.226$, $p>0.05$).

On the other hand, some EF tests were significantly correlated with age, years of education, EDSS, MSS and MFIS (Table 3).

Table 3. Correlation of ToM and Executive Functions measures with clinical-demographic variables in MS patients

		Age	Education	Disease Duration	EDSS	MSSS	BDI	MFIS
ToM measures	**Eyes Test**	-0.143	0.229	0.293	-0.197	-0.056	-0.042	-0.079
	Videos Test	-0.034	0.120	0.042	-0.082	-0.171	-0.095	-0.226
Executive functions	**COWAT FAS**	-0.005	0.245	-0.059	0.002	-0.015	-0.071	-0.107
	COWAT animals	-0.066	0.426**	-0.058	-0.241	-0.279	-0.198	-0.370*
	WCST PR	0.095	-0.354*	0.051	0.126	0.138	0.005	0.150
	Stroop C	0.143	-0.202	0.097	0.388*	0.308*	0.403**	0.347*
	Stroop CW	0.321*	-0.224	-0.022	0.376*	0.352*	0.222	0.403*
	TMT A	0.308*	-0.187	0.210	0.423**	0.189	0.278	0.207
	TMT B	0.293	-0.384*	0.120	0.265	0.224	0.105	0.205
	RAPM	-0.143	0.137	-0.136	-0.288	-0.225	-0.185	-0.351*

All correlations are Pearson r coefficients except for EDSS and MSSS (Spearman's rank correlation coefficient)

*p<0.05; **p<0.01; ***p<0.001 (corrected for multiple testing through False Discovery Rate method)
Abbreviations: EDSS= Expanded Disability Status Scale; MSSS= Multiple Sclerosis Severity Scale; BDI= Beck Depression Inventory; MFIS= Modified Fatigue Impact Scale; ToM = Theory of Mind; COWAT= Controlled Oral Word Association Test; WCST PR= Wisconsin Card Sorting Test perseverative responses; Stroop C= Stroop color; Stroop CW= Stroop color-word; TMT= Trail Making Test; RAPM= Raven's Advanced Progressive Matrices

Relationship of Executive Functions and Theory of Mind in MS patients

Correlations between ToM and EF measures in MS group, after controlling for the effect of age, years of education and fatigue, are presented in Table 4. The EF measures are significantly intercorrelated while the correlations of ToM tests with EF tests are not significant.

Table 4. Correlation matrix including ToM and Executive Functions measures, controlled for age, education and fatigue in MS patients

	Videos Test	COWAT FAS	COWAT animals	WCST PR	Stroop C	Stroop CW	TMT A	TMT B	RAPM
Eyes Test	0.371*	-0.051	0.127	-0.021	-0.041	-0.063	-0.259	-0.207	0.184
Videos Test		0.114	-0.057	-0.329	-0.048	-0.121	-0.204	-0.311	0.156
COWAT FAS			0.354	-0.251	-0.330	-0.341	-0.148	-0.293	0.327
COWAT animals				-0.247	-0.302	-0.478***	-0.201	-0.198	0.244
WCST PR					0.130	0.472***	0.169	0.412**	-0.501***
Stroop C						0.569***	0.697***	0.510***	-0.213
Stroop CW							0.400**	0.563***	-0.399**
TMT A								0.434**	-0.254
TMT B									-0.534***

All correlations are partial correlations coefficients

P values are corrected for multiple testing through False Rate Discovery method

*p<0.05; **p<0.01; ***p<0.001

Abbreviations: COWAT= Controlled Oral Word Association Test; WCST PR= Wisconsin Card Sorting Test perseverative responses; Stroop C= Stroop color; Stroop CW= Stroop color-word; TMT= Trail Making Test; RAPM= Raven's Advanced Progressive Matrices

In order to investigate more accurately the relationship between executive functions and ToM, measuring specifically the similarity of variance, we performed a hierarchical cluster analysis.

The hierarchy resulting from clustering the data is shown in Figure 1. Four clusters were recognized by the analysis, three of these were executive clusters and one was a pure ToM cluster: (1) Attention/working memory cluster, composed by TMT-A and Stroop C; (2) Inhibitory control/shifting ability cluster, including TMT-B, Stroop CW and WCST-perseverative responses; (3) ToM cluster, including Eyes Test and Videos Test; (4) Verbal Initiative/Abstract reasoning cluster, composed by COWAT-FAS, COWAT- animals and RAPM.

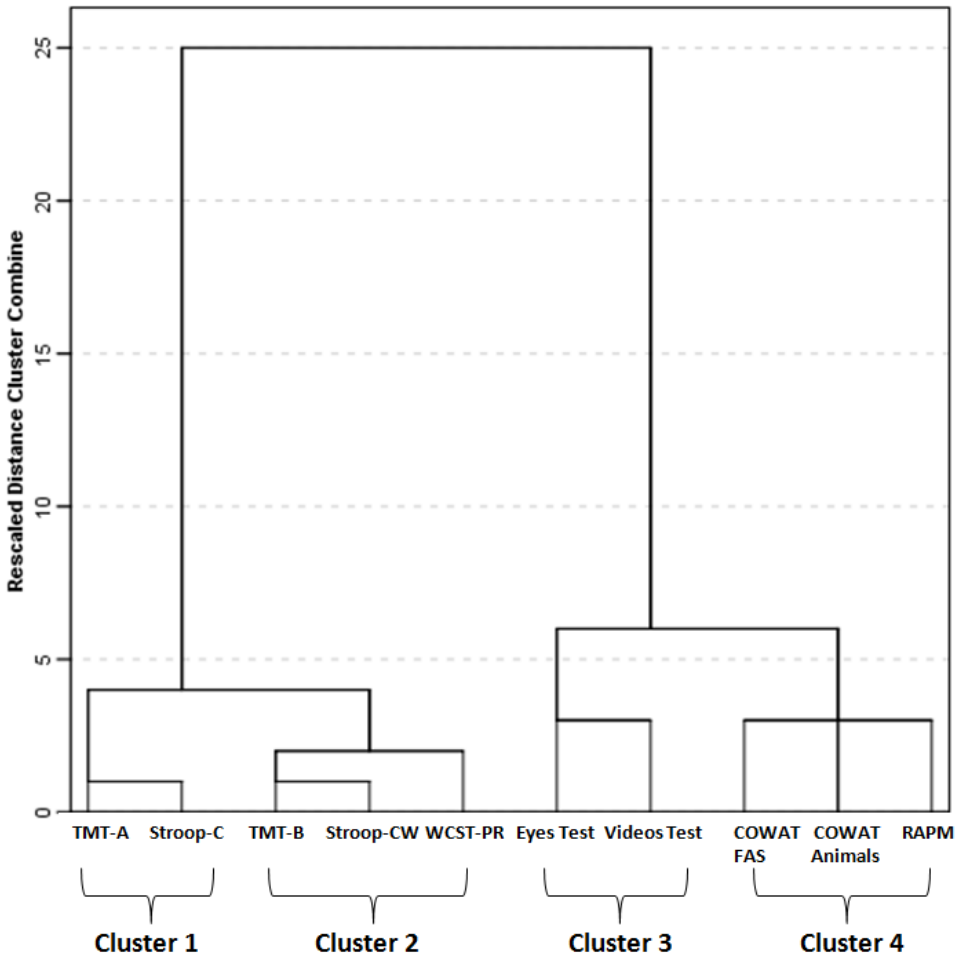

Figure 1. Dendrogram showing the cluster architecture of executive functions and Theory of Mind tests in MS patients

(Cluster 1) Attention/working memory cluster, composed by TMT-A and Stroop C; (Cluster 2) Inhibitory control/Shifting ability cluster, including TMT-B, Stroop CW and WCST-perseverative responses; (Cluster 3) ToM cluster, including Eyes Test and Videos Test; (Cluster 4) Verbal Initiative/Abstract reasoning cluster, composed by COWAT-FAS, COWAT-animals and RAPM.

Abbreviations: COWAT= Controlled Oral Word Association Test-FAS; WCST PR= Wisconsin Card Sorting Test perseverative responses; Stroop C= Stroop color; Stroop CW= Stroop color-word; TMT= Trail Making Test; RAPM= Raven's Advanced Progressive Matrices

DISCUSSION

The relationship between ToM and EF has been increasingly studied in different populations, such as healthy children and adults, as well as patients with different psychiatric and neurological pathologies. In MS, this subject is particularly relevant because it is associated with neurobehavioral symptoms whose origins are still not clearly understood[2]. As EF and ToM are both essential for a healthy social behaviour [6], it would be important to understand if social behaviour problems associated with ToM impairment in MS patients may occur independently of the level of EF performance. Ultimately, this conceptual definition will allow to distinguish MS patients with social behavioural problems related to ToM impairment from those related to EF and consequently help planning individualized treatment with appropriate cognitive and behavioural interventions.

The results of this study showed that ToM measures were clustered separately from EF measures, arguing in favour of the independence between ToM and EF. Previous studies investigated this relationship in MS and the results were contradictory. While some studies [12, 14, 18] reported that ToM performance was not significantly correlated with EF, others [10, 11, 16, 17] found that deficits in ToM tasks were mostly correlated with EF deficits.

In order to overcome this methodological limitation we used cluster analysis, a technique that allows investigating more directly the relationship between cognitive measures. Moreover, we used a large battery of EF tests covering its different subdomains.

The independence between ToM and EF was originally suggested by Baron-Cohen et al in one of the earliest studies of ToM in humans [34]. They observed that children with Down's syndrome presented a good performance on ToM tasks despite the impairment on global intellectual functions, while in children with autism the performance on ToM tests was disproportionately impaired compared with global intellectual and EF. Later, the dissociation of EF and ToM was reported in single case studies with neurological pathology. Particularly, Fine et

al [35] described a patient with intact EF and impaired ToM who had a congenital lesion in the amygdala, and Lough et al [4] reported the case of a patient with a diagnosis of frontotemporal dementia and severe behavior disturbance presenting a relatively intact EF assessment but a poor performance on ToM tasks. More recently, Bertoux et al replicated the dissociation between the two functions in a large sample of patients with behavioural variant frontotemporal dementia using a cluster analysis [6]. In contrast, other studies results supported the hypothesis that ToM and EF are significantly related [3, 36, 37], which led some authors to consider ToM impairment as part of a cognitive dysexecutive syndrome.

From a neurodevelopmental perspective, even though ToM and EF jointly develop during infancy, there is evidence of modularity in ToM. It is culturally invariant in its development with little individual variation and narrow fame times, independently of other cognitive domains [38].

At a neural level, even though some of the neurocircuitry are probably shared by EF and ToM, like those associated with domain-general attention, the dissociation of these functions may be explained by additional recruitment of different functional networks [3]. The neural nodes of ToM network includes orbitobasal and ventromedial prefrontal cortices, temporo-parietal junction, fusiform face area, cingulate cortex, and amygdala as the main hub [39], while EF rely on a diffuse cortical and subcortical network anchored on the dorsolateral prefrontal cortex [40].

Even though our findings support the theory that ToM is a distinct module, independent of EF, this should be put in perspective since other cognitive functions such as semantic (eg, social rules or conventions) or episodic memory (eg, previous similar experience) could also have an effect on ToM processing [6]. Moreover, given the multifaceted nature of ToM and EF, the dissociation of these domains needs to be considered cautiously, since not all the executive domains or ToM subdimensions were assessed. In this regard, it would be particularly relevant to discriminate between cognitive ToM and affective ToM, since tasks tapping these different components may load on different cognitive abilities. This is a reasonable hypothesis taking into account that

cognitive and affective aspects of ToM seem to be subserved by dissociable brain networks [41]. The cognitive ToM network primarily engages the dorsomedial prefrontal cortex, the dorsal anterior cingulate cortex and the dorsal striatum, while the affective ToM network primarily engages the ventromedial and orbitofrontal cortices, the ventral anterior cingulate cortex, the amygdala and the ventral striatum[41]. Therefore, it would be important to further explore the relationship between EF and ToM using additional tests which allow to assess separately the cognitive and affective dimensions of ToM because they may have a different relationship with EF.

Regarding EF, we identified three separable clusters in our analysis that are in line with the current perspective of its non-unitary nature [42, 43]: Attention/working memory cluster that corresponds to the ability of monitoring and coding information; Inhibitory control/Shifting ability cluster that requires holding back preponderant or automatic responses when necessary and the aptitude to engage and disengage attention from different tasks sets and rules; Verbal Initiative/Abstract reasoning that globally measures whether and how well subjects organize their thinking, with verbal initiative depending on the ability to organize output in terms of clusters of meaningfully related words [44], and abstract reasoning involving the ability to think in useful generalizations, identifying and forming concepts (i.e. generating cognitive schemas to organize information)[45].

Consistent with earlier studies, we found that our patients had executive deficits particularly on measures of attention and cognitive flexibility (TMT-B); verbal fluency (COWAT-FAS) and inhibitory control (Stroop Test), with relatively preservation of planning and abstract reasoning [9, 46].

There are some limitations of this study to be considered. First, this cohort of patients with MS was clinically heterogeneous because it includes both RR and SP forms. Even though it increases the ability to generalize the results to the target population, it also enhances the variation of extraneous variables that might influence the results. Therefore these results should be

replicated using a homogeneous cohort of MS patients. Secondly, we used only two non-verbal tests to assess ToM although it involves multiple sub-processes. Therefore, it would be important to further explore the relationship between EF and ToM using additional verbal and non-verbal tasks of ToM. Likewise, it is not possible to clearly distinguish which component of ToM was evaluated by Eyes Test or by Videos Test because both tasks included questions addressing feelings and emotions (affective ToM) and other questions addressing thoughts and mental states (cognitive ToM). Therefore, future studies should use tests which allow to assess separately the cognitive and affective dimensions of ToM because they may have a different relationship with EF.

In conclusion, this study suggests a dissociation of EF and ToM in MS, meaning that ToM impairment may occur independently of the level of EF performance, and thus contribute to relevant everyday problems in interpersonal relationships of MS patients. Clinicians need to be aware that patients in this situation may be able to compensate in their everyday executive functions but may suffer the social consequences of impaired social cognition. Ultimately, this thorough discrimination of ToM deficits in MS may help the guidance of appropriate cognitive and behavioural interventions.

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PART V

CONCLUSIONS

The following conclusions can be drawn from this thesis:

- **Social cognition, particularly theory of mind (ToM), is impaired in patients with multiple sclerosis (MS) and therefore should be considered in the spectrum of clinical manifestations associated with MS.**

  At present, this finding offers patients, families and health professionals involved in the management of MS, an understanding of why the patient's behaviour may have changed, potentially contributing for an improvement in the interpersonal relationships. In the near future, it is hoped that neurologists may have validated tools to diagnose social cognition deficits in MS patients and, ultimately, that effective symptomatic therapy may be offered to these patients.

- **The social cognitive deficits are independent of the classic MS-related cognitive impairment**.

  This conclusion may be explained by taking into account the multifocal nature of MS pathology, which may disrupt the neural networks involved in social cognition while preserving those responsible for the classic cognitive domains. Therefore, clinicians need to be aware that patients with MS may have preserved cognitive abilities to carry out the activities of daily living and skills related to jobs but may suffer from the social consequences of impaired social cognition which is not captured by classical neuropsychological testing.

- **Performance on ToM and executive functions (EF) is dissociated in patients with MS.**

  Although ToM and EF are both essential cognitive processes for a normal social behaviour and depend on closely related brain circuits, this finding argues in favour that ToM and EF are independent cognitive domains. This means that a patient with MS may have a significant ToM impairment causing abnormalities in social behaviour and still may perform within the normal range on EF testing, contradicting the classical concept that neurobehavioral symptoms in MS are part of a cognitive dysexecutive syndrome.

- **ToM impairment in patients with MS is not associated with disease duration, level of neurological disability (EDSS), depression or fatigue.**

  This finding has important clinical implications since it suggests that social cognitive deficits may occur, and should be suspected, even in the early stages of MS or in mildly disabled patients. Additionally, depression and fatigue do not seem to influence performance on ToM.

- **The impairment of social cognition in MS is related both to grey matter (GM) and white matter (WM) pathology involving the social brain network.**

  Regarding the GM pathology, MS patients have a multifocal pattern of atrophy affecting the main nodes of the social brain network, including cortical regions (fusiform gyrus, entorhinal cortex, superior temporal gyrus, superior parietal gyrus, supramarginal gyrus, medial orbitofrontal cortex, anterior and posterior cingulate gyrus) and subcortical structures (amygdala and putamen). Of all the GM regions involved, amygdala seems to be the main predictor, probably due to its central position within the social brain network, where the social relevant information converges and is integrated.

  The WM damage, caused by focal lesions and by diffuse microstructural pathology throughout normal-appearing WM, also contributes for social cognition impairment in MS probably due to a mechanism of disconnection within the social brain network. The pattern of tract involvement was widely disperse across both hemispheres, reflecting the complex and distributed nature of the brain networks that support social cognition processes. Nonetheless, the most robust associations were identified within tracts of limbic pathways (uncinate fasciculus, fornix) and callosal interhemispheric fibers (corpus callosum, tapetum).

  In summary, the social brain network in MS is affected by two different mechanisms: direct damage of the main cortical and subcortical GM nodes, particularly amygdala, and by

disconnection between the mentioned nodes caused by damage of the interconnecting WM tracts.

Taken together, the results reported in this thesis further expand the knowledge about the complex constellation of cognitive-behavioural symptoms in MS, providing additional evidences regarding social cognition impairment in patients with MS, which ultimately may contribute to improve the global care of these patients. Moreover, from a neuroscience perspective, the findings herein reported increases the understanding of the neural basis of social cognition.

PART VI

FUTURE PERSPECTIVES

Although this dissertation shed light on the factors associated with social cognition impairment in multiple sclerosis (MS), several questions remain unexplored and should be addressed in future research.

To clarify the relative contributions of white (WM) and grey matter (GM) pathology for the social cognitive deficits in MS and better characterize the whole-brain MRI results herein reported, a region-of-interest (ROI) analysis using combined volumetric and tractography methods targeting the main nodes of the social brain network, particularly amygdala, and the major input/ouput patways may prove revealing. Moreover, using ultra high-field MRI for the segmentation of the amygdala in its multiple anatomically nuclei, which have different functions and distinct connectivity patterns, may provide new insights into the functionality of amygdala within the social brain network and eventually to identify particular nuclei more susceptible to the MS-related pathologic mechanisms. Additionally, the use of resting-state functional connectivity analysis (fcMRI) may contribute to better delineate the social brain network and elucidate how it is affected in MS. This is a relatively new method for delineating large-scale networks in the living human brain that are composed of regions that tend to function together and share anatomical connections.

It is also as yet unclear whether there are factors that promote resilience to social cognitive decline. In studies of cognition in MS, cognitive reserve measured by premorbid intelligence, education level and cognitive leisure activities, moderates the negative effect of disease burden on cognition, protecting MS patients against the cognitive decline. Therefore, it is an interesting matter of research to explore if these same factors attenuate the effects of MS on social cognition. Also, the study of compensatory cortical adaptive responses by functional MRI (fMRI) using social cognitive tasks will help to enlighten the role of neural plasticity in the modulation of social cognition decline in MS.

Studies focusing the neurochemistry and receptor pharmacology of molecules important for social behaviour, such as oxytocin and vasopressin, and genetic variants that confer individual

differences in social cognitive skills may offer valuable insights into the basic mechanisms underlying social cognition and how they are affected in MS.

Finally, the ultimate goal of future research about social cognition impairment in MS is to provide validated diagnostic tools and symptomatic treatments. Therefore, concerted effort is now needed to gather normative data and validate social cognitive tests suitable for implementation in clinical practice. Lastly, from a personal perspective, the most important question is whether the dysfunctional social brain is plastic and can be repaired, leading to an improvement in the social cognitive skills. Several clinical trials are being carried out in other pathologies with social cognition impairment, such as autism and schizophrenia, aimed at evaluating new pharmacotherapy approaches, particularly intranasal oxytocin. Non-pharmacological interventions with social cognitive training have also been applied to target social cognitive impairment. Hopefully, this studies will pave the way for the development of similar clinical trials in MS patients diagnosed with social cognitive deficits.