COGNITIVE FUNCTION IN MULTIPLE SCLEROSIS ASSESSED WITH NEUROPSYCHOLOGICAL AND MULTI-MODAL MRI METHODS

PhD Thesis

Andrea Mike, M.D.

Department of Neurology

Medical School of Pécs

University of Pécs, Hungary

Leader of the project: Prof. Zsolt Illés M.D., Ph.D.

Leader of the program: Prof. Sámuel Komoly M.D., Ph.D.

Leader of Doctoral School: Prof. Sámuel Komoly M.D., Ph.D.

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ABBREVIATIONS

3D: three-dimensional

BVMT-R: Brief Visuospatial Memory Test-Revised

BA: Brodmann area

BDI: Beck Depression Inventory

BOLD: blood oxygen level dependence

CES-D: Center for Epidemiologic Studies Depression Scale

CL: cortical lesion

CLN: cortical lesion number

CLV: cortical lesion volume

CNS: central nervous system

COWAT: Controlled Oral Word Association Test

CSF: cerebrospinal fluid

CVLT-II: California Verbal Learning Test, second edition

DIR: Double Inversion Recovery

D-KEFS: Delis-Kaplan Executive Function System Sorting Test

DTI: diffusion tensor imaging

EDSS: Expanded Disability Status Scale

FFA: fusiform face area

FLAIR: Fluid-attenuated Inversion Recovery

fMRI: functional MRI

FOV: field of view

GLM: general linear model

GM: gray matter

HC: healthy controls

H-WAIS: Hungarian version of the Wechsler Adult Intelligence Scale-Revised

IR-SPGR: inversion recovery spoiled gradient-recalled echo

JLO: Judgment of Line Orientation Test

MACFIMS: Minimal Assessment of Cognitive Function in Multiple Sclerosis

MS: multiple sclerosis

MPRAGE: Magnetization Prepared Rapid Gradient Echo

MRI: magnetic resonance imaging

MS: multiple sclerosis

NAART: North American Adult Reading Test

PASAT: Paced Auditory Serial Addition Test

ROI: region of interest

RR: relapsing-remitting

SDMT: Symbol Digit Modalities Test

SP: secondary progressive

STAI: Spielberger Trait Anxiety Inventory

T1LV: T1 lesion volume

ToM: Theory of Mind

WM: white matter

WMLV: white matter lesion volume

I. INTRODUCTION: Background of the Theses

"If you want the truth to stand clear before you, never be for or against. The struggle between 'for' and 'against' is the mind's worst disease."

Seng-ts'an, c. 700 C.E.

1. Multiple sclerosis

1.1. Epidemiology, clinical features, and disease course

In the western hemisphere of the Earth MS is the most common CNS disease in young adults leading to disability. Prevalence of the disease in Hungary is 62/100.000, i.e. about 6000 Hungarians are affected by the disease (1).

Occurrence of MS is twice as many in females as in males with onset of clinical symptoms between 15-50 years of age. MS is a chronic progressive disease, characterized by a broad range of sensory-motor, cognitive and neuropsychiatric symptoms, and accompanied by fatigue. The majority (~85%) of MS patients have a biphasic disease course, beginning with the primary phase termed relapsing-remitting MS (RRMS). During this disease course patients experience alternating episodes of neurological disability and recovery that can last for many years (2). Within 25 years, ~90% of RRMS patients transform into a secondary-progressive disease course (SPMS), which is characterized by steady neurological decline (2). About 10% of MS patients also exhibit a disease course with steady decline in neurological function without recovery and are classified as primary progressive MS (PPMS). A small minority of MS patients (~5%) suffers from a disease course with progressive neurological decline accompanied by well-demarked acute attacks with or without recovery. This disease course is classified as progressive-relapsing (PRMS).

1.2. Diagnosis and therapy

The International Panel on diagnosis of MS has created the guidelines termed "McDonald Criteria" for the diagnosis of the disease (3). Diagnosis of MS is focused on the demonstration of spatially and temporally disseminated multifocal inflammatory demyelinated plaques in the CNS using clinical data, MRI, cerebrospinal fluid (CSF), and evoked potential examinations.

Clinical data alone may be sufficient for a diagnosis if the patient has suffered at least two separate episodes of different neurologic symptoms characteristic of MS. The Kurtzke Expanded Disability Status Scale (EDSS) (4) is used as a measure of physical disability by assigning a severity score (0-10) to the clinical status of the patients.

MRI is a valuable tool to investigate MS mainly due to its high sensitivity for detecting focal CNS abnormalities of the disease. In addition to conventional MRI, which includes dual-echo, fast fluid-attenuated inversion recovery (FLAIR), and T1-weighted imaging with and without gadolinium administration, several quantitative MR techniques including magnetization transfer MRI, diffusion-weighted and diffusion tensor MRI, and proton MR spectroscopy (^{1}H-MRS) have been used to study the structural changes associated with MS. These techniques have the great advantage of being more specific towards the heterogeneous pathological substrates of the disease than conventional MRI. In addition, they also allow us to quantify and monitor the extent of damage not only in lesions but also in normal-appearing tissues. Functional MRI is a novel approach in MS to study mechanisms of cortical reorganization following the accrual of tissue damage, which have the potential to limit its clinical consequences.

CSF examination can support the diagnosis. Oligoclonal band pattern on the CSF immunoelectrophoresis can reveal intrathecal production of antibodies. Evoked potentials can demonstrate the presence of lesions in the central visual, auditory, and sensory nerve pathways.

The fundamental role of inflammatory immune processes in the pathology of MS provides the rationale for immunodulatory therapies that attempt to shift the immune system from pro-inflammatory to anti-inflammatory pathways and induce regulatory mechanisms. Growing understanding of immune cellular and molecular mechanisms together with modern biotechnology generated promising immunomodulatory treatment

strategies, with novel mechanisms of action and different levels of specificity. These include inhibitory molecules, monoclonal antibodies, cell therapies and agents that are administrated orally or by infrequent infusions. Several of these treatments have demonstrated impressive efficacy in Phase II and III clinical trials by reducing disease activity and accumulation of disability. However, with the advent of potent therapies, rare but severe adverse effects, such as CNS infections and malignancies, have occurred.

1.3. Etiology and pathogenesis

Pathologically, MS affects the CNS with multifocal and diffuse inflammatory and neurodegenerative changes, but their exact etiology and pathogenesis remains uncertain (5). Currently, MS is considered to be a result of immunological, genetic, and environmental factors, including viral infection. Autoimmune origin of the disease is strongly supported by the composition of inflammatory infiltrates in the brain, which parallels with the T-cell mediated animal model of demyelinating diseases called experimental allergic encephalomyelitis (EAE). The presumed immune reaction consists of activation of brain reactive T-cells, migration of inflammatory cells into the CNS, penetration of the blood-brain-barrier, and recognition of myelin proteins, such as myelin basic protein (MBP), proteolipid (PLP), and myelin oligodendrocyte glycoprotein (MOG) as foreign. Typically, MS lesions include breakdown of the blood-brain barrier, multifocal inflammation, demyelination, oligodendrocyte loss, reactive gliosis, and axonal degeneration (6).

Traditionally, inflammatory demyelination has been seen as the main disease process in MS, however, axonal loss is increasingly being documented to occur early in the disease and results in permanent disability (5). Current hypotheses support primary inflammatory demyelination as the underlying cause of axonal loss during earlier stages in MS. The transition from RRMS to SPMS is thought to occur when a threshold of axonal loss is reached and the compensatory capacity of the CNS is surpassed, resulting in steady progression of neurological symptoms.

Genetic susceptibility of MS is associated with the human leukocyte antigen (HLA) DRB1*1501 allele. Infectious agents have been proposed as inciters of MS, in particular, infection with Epstein-Barr virus appears to raise the risk of disease development.

Exposure to a virus may initiate immunpathological processes associated with molecular mimicry between viral and CNS proteins. Recently, vitamin D deficiency has been suspected as environmental risk factor in MS. Studies have shown that MS prevalence is higher where environmental supplies of vitamin D is lower.

1.4. Gray matter pathology

Both MRI and histopathological studies have shown that GM pathology is already present in the earliest disease stages (7-9), but it becomes only prominent with progressive disease (7, 10). GM damage is clinically relevant in terms of conversion from clinically isolated syndrome to MS, and in the short and long term accrual of disability. GM pathology in MS mainly results from primary local cortical demyelinating lesions (11, 12), and from GM atrophy secondary to axonal transections in destructive WM plaques or severe diffuse axonal damage in the normal-appearing WM leading to Wallerian (anterograde or transsynaptic) degeneration, and retrograde degeneration (5, 13, 14). However, the exact relationship between primary and secondary processes leading to GM damage remains to be cleared. Finally, other primary neurodegenerative components developing independently of focal WM or GM lesions has been presumed that may eventually play a role in the early development of GM atrophy in MS (13).

MS is characterized by a relevant number of focal cortical lesions (CLs) (7, 11, 12, 15). Pathology of CLs strikingly differs from that of WM lesions. Lymphocyte infiltration, complement deposition, and blood-brain barrier disruption, all typical hallmarks of WM lesions, are not usually found in CLs. Cortical demyelinisation occurs in part in conjunction with subcortical WM plaques or as small perivascular intracortical lesions. In addition, the most extensive cortical demyelinisation is produced by the widespread and band-like subpial lesions, which span the cortex over long distances, affecting several adjacent gyri and sulci. This type of CL is associated with chronic inflammation of the meninges.

In vivo assessment of CLs is of great significance because it is still not fully elucidated how CLs impact clinical manifestation and progression of MS, and how CLs are related to WM pathology and GM atrophy. To understand these questions would improve our

understanding of MS pathogenesis, and might open new perspectives for novel therapeutic innovations (16, 17).

In vivo visualization of CLs is challenging (18). On conventional imaging techniques CLs are missed in up to 95% of the cases (19). In the past few years, large effort was devoted to the development of MRI techniques capable of visualizing at least a portion of CLs in vivo (18, 20-26). Recently, novel MRI methods have been deployed to address these challenges, including novel pulse sequences, multi-channel and high-resolution imaging, and ultra-high magnetic field strength (20-26). None of these techniques have demonstrated broad clinical applicability or vendor deployment as applied to routine 3 Tesla MR imaging. Recently, the application of the double inversion recovery (DIR) sequence convincingly demonstrated that CLs are frequent findings in MS patients (27, 28). Recent MRI studies to visualize CLs suggest significant impact of CL load on physical disability in MS (27). In addition, preliminary studies link CLs to cognitive dysfunction (26, 29-31).

2. Cognitive impairment in multiple sclerosis

2.1. Clinical characteristics and neuropsychological assessment

Cognitive impairment is a common concomitant of MS, with prevalence rates ranging from 43 to 70% (32, 33) at both the earlier and later stages of the disease (34, 35). Cognitive impairment can be seen irrespective of the duration of the disease and is only mildly associated with physical disability (34, 35). However, cognitive disturbances seem to be more severe in patients with chronic progressive disease compared to those in the RR stage (35). Both fatigue and depression have been identified as important contributors to cognitive impairment in MS patients (36, 37).

MS has a detrimental effect on everyday life activities, employment status, and integration into the society (38), thereby considerably impairs the quality of life (QoL) (38, 39). The extent to which an individual has poor QoL is particularly determined by the neuropsychological deficits (39). Studies have shown that physical disability and demographic factors account for less variance in employment status of MS patients (40), than does the presence of cognitive deficit (38).

MS affects various aspects of cognitive functioning, including efficiency of information processing, verbal and visuo-spatial memory, executive functioning, attention, and visual perceptual processing. Processing speed, and visual learning and memory seem to be most commonly affected (32, 33). Manifest dementia is rare, the most common clinical presentation is one of the specific and subtle cognitive deficits that can vary substantially among patients (38).

Information processing efficiency refers to the ability to maintain and manipulate information in the brain for a short time period (working memory) and to the speed with which one can process that information (processing speed). Reduced speed of information processing is the most common cognitive deficit in MS, and typically occurs concurrently with other cognitive deficits that are common in MS, such as deficits in working memory and long-term memory. Long-term memory is one of the most consistently impaired cognitive functions in MS (41). Long-term memory refers to the ability to learn new information and to recall that information at a later time point. Attention deficit is associated with deficits of processing speed and working memory. Impairment in sustained attention and decrements in divided attention have been reported in MS patients. Executive functioning refers to cognitive abilities needed for complex goal-directed behavior and adaptation to environmental changes or demands. This includes the ability to plan, anticipate outcomes, and direct resources appropriately. Deficits in executive functions (i.e., abstract and conceptual reasoning, fluency, planning, and organization) occur in patients with MS, albeit less frequently (17%) than deficits in memory and information processing efficiency (42). Visual perceptual processing not only includes recognition of a visual stimulus, but also the ability to perceive the characteristics of that stimulus accurately. Difficulties in primary visual processing (e.g., from optic neuritis) in MS can have a detrimental effect on visual perception processes, although perceptual deficits that are independent of primary visual or other cognitive abnormalities can also occur.

As the cognitive deficits in MS frequently fall within specific cognitive domains and can be subtle and vary considerably among patients, a carefully selected neuropsychological test battery is essential. The Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS) is an MS experts developed test battery consisting of seven tests

that assess word fluency, visuospatial ability, verbal memory, visuospatial memory, processing speed, working memory, and executive function (32). The MACFIMS has been shown to be sensitive to the cognitive profiles characteristic of MS patients (32). The paced auditory serial test (PASAT) is a test of working memory with substantial demands on processing speed. The PASAT is the cognitive component of the widely used multiple sclerosis functional composite (MSFC), which is a multidimensional quantitative outcome measure designed specifically for use in MS patients. Fluency tests evaluate the spontaneous production of words under restricted search conditions, such as words that begin with a specific letter and words from a particular category. Fluency assessment is a sensitive measure of neuropsychological impairment, and MS patients has been shown have substantial deficits in both phonemic and semantic fluency. Measures of executive functioning are particularly susceptible to the effects of depression in MS patients, and this should always be considered in the interpretation of poor performance on executive tasks in individuals with MS.

2.2. Neuroimaging correlates of cognitive dysfunction in multiple sclerosis

Several MS pathologies seem to contribute to the presence and worsening of cognitive deficits in MS. The advent of MRI to assess possible clinico-pathological relationships has highly contributed to better understanding of cognitive dysfunction in MS. The relationship between cognitive changes and MRI findings involve WM lesion volume, whole brain atrophy, cortical and deep GM atrophy, and abnormalities in brain tissue appearing normal on conventional MRI.

Moderate to strong correlations have been reported between cognitive performance and T2-lesion load (43-46). This suggested that widespread damage to the WM might lead to functional disconnection of different cortical areas and deep GM structures, such as the thalamus, which in turn, might cause development of cognitive deficits. Better correlations have been obtained with assessment of T1 hypointense lesions (black holes), which reflect more directly the severity of axonal loss in the lesions (47, 48). The analysis of regional lesion load, particularly in the frontal lobe indicated better association with the specific pattern of cognitive decline in MS patients (49, 50).

Several MRI studies have demonstrated that quantification of atrophy in the whole brain or in selected brain regions, such as the corpus callosum and frontal lobe, provides more robust correlates of MS-associated cognitive dysfunction (43, 51-54). Brain atrophic changes better explain cognitive impairment in RRMS patients than the total T2 WM lesion volume (55, 56). In addition, the rate of brain atrophy progression was a good predictor of cognitive impairment approximately five years later (48). Neocortical volume loss was more closely associated with cognitive decline than whole brain atrophy and WM lesion volume (54, 56-58). Increase in neocortical volume loss was higher in deteriorating than in stable MS patients after 2.5 years of follow-up (59). Recent studies demonstrated that regional distribution of cortical atrophy differs between cognitively impaired and preserved patients, and is related to the overall cognitive performance and deficits of specific cognitive domains (44, 56). Thalamic atrophy was also found as a reliable predictor of cognitive impairment in MS (60, 61). CLs have been identified among the possible causes of cognitive impairment in MS (26, 29-31). Changes in the normal appearing brain tissue detected by magnetization transfer imaging (52, 62), DTI (63, 64), or ^{1}H-MRS (65, 66) have all been found to correlate with the severity of the cognitive impairment. Functional MRI studies have shown that beside degenerative events in parallel massive cortical reorganization processes are taking place in the MS brain enabling a functional adaptation and compensation of the structural damages (67).

3. Social cognition, Theory of Mind

Social cognition is a high-level mental capacity evolutionary developed for successful adaptation to the social environment, and enables coping with socially relevant information both in complex social groups and in close relationships. Although many species including primates can accurately predict the goals of their conspecific's behavior, it appears that only humans can separate a mental perspective of their own actions from that of others' actions (68). We can recognize that mental state of others does not necessarily correspond to our own interpretation or to reality (69). In social psychology the capacity to interpret and predict mental states of other peoples in terms of thoughts, intentions, desires and beliefs is known as theory of mind (ToM), also referred as mentalizing and mindreading. ToM ability involves social-perceptual processes that

enable mental state decoding from nonverbal cues (70), such as from facial expression, eye gaze, body postures; and social cognitive processes that enable more abstract reasoning about mental state of others, such as prosody and social content of speech (71). Social cognition might be independent, or dissociable from general intelligence (72).

Previous studies demonstrated that living with MS carries high risk of poor quality of life (38) resulting from several features of the disease. Most significant factors include physical status, duration and progression rate of the disease, presence of mood disorders, and cognitive decline (38). Deficits of general intellectual abilities significantly interfere with everyday life activities and decision-making. In addition, loss of employment status, restricted social activities, and difficulties in inter-personal relationships frequently occur during the disease. Deficits of the social cognition may additionally account for all of these functional limitations. Only a few studies investigated social cognition in MS; all demonstrated deficits in facial emotion recognition (73-77), in addition, decline in complex cognitive inferences relating to the content of the mental state was also indicated (73, 77).

Neural basis of ToM abilities has been widely investigated using advanced neuroimaging methods in healthy subjects and in clinical conditions showing social cognitive impairment, particularly in high-functioning autism or Asperger syndrome (78), and in schizophrenia (79-81). These studies support the hypothesis that integrated fronto-temporal and temporo-parietal circuits are dedicated to mentalizing. Main hubs of these networks were found distributed in the posterior superior temporal sulcus, temporo-parietal junction, temporal pole, medial prefrontal cortex, anterior cingulate cortex, orbitofrontal cortex, and inferior parietal cortex, as well as in subcortical areas, particularly in the amygdala (71, 82, 83). Another important neural mechanism participating in the social cognitive processes is the activation of mirror neurons during observation of movements of others (84, 85). Mirror neuron firing described first in macaque monkeys (86, 87) replicates internally the neural activation required for the execution of the observed action (simulation), thereby enables the understanding of its intended goal. Electrophysiological studies of monkeys and human fMRI studies found mirror neurons in the premotor cortex (frontal mirror system), as well as in the inferior parietal lobule and posterior temporal cortex (parietal mirror system) (84, 85).

Previously, only one MRI study investigated neural correlates of social cognitive dysfunction in MS in vivo. Krause et al. (76) using an emotional face expression recognition task found decreased insular and ventrolateral prefrontal cortex BOLD activation in MS patients with impaired recognition performance compared to the unimpaired group. In addition, poor performance correlated with left temporal lesion load suggesting interruption of information processing to the prefrontal cortex during emotional face recognition.

II. AIMS

Aims of the studies were to investigate cognitive aspects of MS using neuropsychological evaluation and quantitative MRI methods.

Our hypotheses were:

1. In MS, cognitive dysfunction develops both in fields of general intelligence and social cognition. These deficits may manifest independently or dissociated from each other.

2. In MS, pathological processes of both the GM and WM independently contribute to the manifestation of the cognitive decline.

3. In MS, evolution of cortical GM pathology relates both to processes primary taking place in the cortex and to WM pathology resulting in secondary neurodegeneration.

We tested these hypotheses by:

Identification and clinical impact of cortical lesions in multiple sclerosis

1. We evaluated the ability of a routinely available MRI protocol to capture CLs and their different subtypes.

2. We studied the relationship between CL load and WM lesion load.

3. We investigated the association between CLs and physical disability, as well as CLs and cognitive performance of MS patients.

Study of social cognition in multiple sclerosis

4. We evaluated the social cognitive performance of MS patients compared to healthy control subjects using comprehensive social cognitive tests.

5. We studied the impact of physical disability, disease duration, and disease progression rate on the social-cognitive performance.

Neuroanatomical substrates and connectivity of mentalization: a multi-modal MRI study in multiple sclerosis

6. We tested whether brain pathological changes of MS patients impact the mentalization ability. Without a priori hypothesis about possible anatomical substrates relating to social cognitive performance using multi-modal MRI methods we assessed cortical thickness of the whole neocortex and regional WM lesion loads in each interconnecting fiber bundles to find correlations between WM as well as cortical GM changes and mentalization.

7. To extend the general neuroanatomical knowledge about mentalization we identified

neural substrates in MS brain, which if damaged, associate with impaired social cognitive performance in individuals, whose primary brain development was normal.

8. We investigated the relationship between regional WM lesion load and focal cortical atrophy related to the same cognitive function to better understand neurodegenerative processes in MS.

III. IDENTIFICATION AND CLINICAL IMPACT OF MULTIPLE SCLEROSIS CORTICAL LESIONS AS ASSESSED BY A ROUTINE 3 TESLA MRI

Material and Methods

Patient population

Twenty-six patients with MS according to McDonald criteria (3) were recruited from the Partners MS Center at Brigham and Women's Hospital, Boston, MA. Each patient had a clinical exam, neuropsychological test and brain MRI. The interval between clinical exam and neuropsychological testing ranged between 0-126 (mean±SD: 28.2±28.8) days, and between MRI and neuropsychological testing ranged between 0-21 (1.8±5.0) days. Patients with three different clinical subtypes were studied: 1. EarlyRR (n=7): RR disease duration ≤5 years; 2. LateRR (n=13): RR with disease duration ≥10 years; 3. SP (n=6): SP disease course with at least 3 years duration in this stage. Clinical and demographic characteristics are presented in Table 1. Patients with history of alcohol or drug dependence, major psychiatric illness, neurological disease (other than MS) and gross visual impairment were excluded. Physical disability was measured with the EDSS (4). None of the patients were cognitively tested or scanned until at least 1 month had elapsed following an exacerbation. At the time of the study, 22 patients (84.6%) had received immunomodulatory or immunosupressive therapy (glatiramer acetate, interferon beta-1b, interferon beta-1a, mitoxantrone, methotrexate, mycophenolate mofetil, daclizumab, IVIG). The ethical review board approved the study and all patients provided written informed consent.

Neuropsychological testing

Twenty-five MS patients completed the MACFIMS, a test battery, which has been shown to be sensitive to cognitive functions commonly impaired in MS (32). MACFIMS is composed of the following tests:

1. PASAT measures working memory and speed of information processing. Subjects hear a series of digits every 2 seconds and are required to add sequential pairs so that each

digit is added to the digit immediately preceding it. The score is the total number of correct responses.

2. SDMT is a measure of working memory and speed of information processing that requires subjects to substitute numbers for symbols according to a look-up table. The score is the total number of correct items.

3. CVLT-II measures verbal learning and memory using a 16-item word list. There are 5 learning trials. Two scores are used: CVLT-II T1-T5, which is total recall across the 5 learning trials and CVLT-II Long-Delay Free Recall, which is recall after a 25-minute delay.

4. BVMT-R tests spatial learning and memory by requiring subjects to learn a matrix of six simple abstract designs. Two scores are calculated: Total Recall, which is the total score across the 3 learning trials and Delayed Recall, which is recall after a 25-minute delay.

5. D-KEFS assesses executive function by requiring subjects to sort cards using perceptual stimuli and printed words. The score is the number of Confirmed Correct Sorts.

6. JLO estimates visual perception and spatial processing. Subjects are required to match the angle defined by two stimulus lines. The score is the total number of correct responses.

7. COWAT tests verbal fluency by asking subjects to generate as many words as they can that begin with three stimulus letters. The score is the total number of words generated.

The NAART was used to estimate pre-morbid intelligence (88). Depressive symptoms were assessed by the CES-D (89). Cognitive tests were conducted in a single session by a trained research assistant under supervision of an experienced clinical neuropsychologist. Both were blinded to MRI findings.

MRI

MRI acquisition

High-resolution brain MR images were acquired using two pulse sequences on a 3 Tesla GE Signa scanner (General Electric Medical Systems, Milwaukee, WI, USA) with 8-channel head coil: T1-weighted 3D IR-SPGR (138 contiguous 1mm thick sagittal slices,

TR/TE=7.48/2.984ms, TI=450ms, matrix size=256x256, in-plane pixel spacing=1mmx1mm, field of view=256x256mm, Flip Angle=15 degree, scanning time ~9 minutes); single-slab 3D fast spin-echo FLAIR (136 contiguous 1.2mm thick sagittal slices, TR/TE=6200/200ms, TI=1901ms, matrix size=256x256, in-plane pixel spacing=1.2mmx1.2mm, field of view=307x307mm, Flip Angle=90 degree, scanning time ~6 minutes).

MRI analysis

Cortical lesion detection and typing:

Image interpretation was performed by a neurologist with clinical and imaging experience in MS [AM] in collaboration with a neuroradiologist. Both raters were blinded to clinical and neuropsychiatric findings. The 3D IR-SPGR and FLAIR images were spatially co-registered for concordant image analysis using FLIRT (Functional Magnetic Resonance Imaging of the Brain's Linear Image Registration Tool; http://www.fmrib.ox.ac.uk/flirt) (90). This step required approximately 5 minutes of computer time. OsiriX software (version 3.3, http://www.osirix-viewer.com) was used to view the co-registered FLAIR and IR-SPGR images concurrently in multiple reformatted planes. Initially, hyperintense signal abnormalities in the proximity of the cortical mantle were detected on the FLAIR sequence. To be counted, a lesion had to have a minimum diameter of 3mm in any of three orthogonal views. Each lesion detected on FLAIR was then also required to demonstrate hypointensity on the IR-SPGR sequence. In addition, the lesion was required to involve the cerebral cortex with or without involvement of the underlying WM as defined on IR-SPGR images. Lesions involving only the subcortical WM at the GM-WM junction without extension into the cortical mantle (juxtacortical lesions) were excluded. Precise visualization of the spatial extension of CLs was enabled by the 3D IR-SPGR isotropic images (Figures 1, 2).

CL classification was based on a histopathological scheme (11): Type I lesion involved both the cortex and adjoining subcortical WM (mixed GM-WM lesion); Type II lesion was restricted purely to the cortex (intracortical lesion); Type III lesion was bandlike, extending from the pial surface into the cortex (subpial lesion).

During quality control we found 7 cases (27%) with some degree of degradation related to motion or flow artifacts. However, the quality of all images was sufficient for our analysis. We registered each patient's total CLN, the number of lesions assigned to each subtype, and also distinguished CLs in the left versus right cerebral hemispheres. CL detection and characterization required 4-6 hours/exam of expert analyst time depending on the lesion load.

The two raters reviewed all lesions they identified and came to a consensus with regards to number and lesion types. Five cases were randomly chosen from the clinical subgroups (1 early RR, 2 late RR, 2 SP) to test intra- and inter-rater variability of CLN assessment. For both evaluations the coefficient of variation was defined as the standard deviation divided by the mean. The mean inter-rater variability was 4.47% for total CLN and 4.85% for Type I CLN. The mean intra-rater variability was 4.53% for total CLN and 4.21% for Type I CLN. Reproducibility of Type II lesion evaluation was not analyzed separately due to its small proportion (see results).

Lesion load measurement:

Field inhomogeneity correction was performed by applying an automated algorithm based upon entropy minimization (91), requiring approximately 5 minutes of computer time. To estimate WMLV and CLV from FLAIR images, we outlined lesions using a semi-automated thresholding technique with the software 3D-Slicer (Version 3.4, http://www.slicer.org). The method relies on user-guided specification of the intensity threshold range, which is manually adjustable during lesion segmentation. The algorithm labels all pixels with signal intensity within the set threshold range in the lesion area identified manually by the user. When outlining lesions in the cortex, care was taken not to include FLAIR hyperintense signal abnormalities, which did not have corresponding IR-SPGR hypointensities. Lesion volume determination by an expert required 20-40 minutes/case.

Statistical Analysis

All statistical analyses were performed using SPSS software version 13.0 (SPSS, Chicago, IL, USA). Prevalence of lesions in each hemisphere was compared with the Wilcoxon signed rank test. Non-parametric tests were used because distributions of the MRI variables were not normal. Kruskal-Wallis test was used for comparisons between the three patient subgroups. If a significant difference was observed, the Mann-Whitney U-test was performed for pairwise comparisons and a Bonferroni correction was used to account for multiple comparisons. The non-parametric Spearman rank correlation test was applied to investigate univariate correlations between the three MRI measures (CLN, CLV, WMLV), neuropsychological test performance, and physical disability. In addition, multiple linear regression was used to assess the cognitive-MRI relationship adjusting for age, pre-morbid IQ (NAART), and depressive symptoms (CES-D). Due to small sample sizes the univariate modeling was not performed within individual clinical subgroups. A two-tailed level of $p<0.05$ was the significance threshold.

Results

Cortical lesion frequency and typing

Descriptive statistics of the MRI data are listed in Table 1. Overall, 249 CLs were detected in 24/26 patients (92.3%). Lesions per patient ranged between 0-30, mean±SD: 9.6±8.8. Two hundred thirty-five lesions (94.4%) were classified as Type I, 14 (5.6%) as Type II, and none as Type III. All 24 patients with CLs had Type I lesions, while the 14 Type II CLs were confined to 7 patients (2 EarlyRR; 3 LateRR; 2 SP). No statistically significant hemispheric differences in CLN were found.

Table 1.

Demographic, clinical and MRI data of multiple sclerosis patients

Variables	All patients (n=26)			EarlyRR (n=7)			LateRR (n=13)			SP (n=6)			Comparison between the three patient subgroups (Kruskal-Wallis test) (p-value)
Gender Female/Male (%)	19/7 (73/27)			4/3 (57/43)			10/3 (77/23)			5/1 (83/17)			
	Min.	Max.	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.	Mean±SD	
Age (y)	33	54	43.27±6.8	33	48	39.43±6.2	34	54	43.69±7.3	42	54	46.83±4.4	
Disease duration (y)	2	28	13.27±7.8	2	5	3.57±1.1	10	28	16.38±5.8	8	24	17.83±6.2	
EDSS	0.0	6.0	2.6±2.0	0.0	3.5	1.4±1.2	0.0	4.5	1.6±1.3	3.5	6.0	5.2±1.3	
Whole brain CLN	0	30	9.58±8.8	0	23	6.00±7.9	1	24	9.31±8.2	0	30	14.33±10.3	0.353
Type I number	0	26	9.04±8.3	0	20	5.43±7.0	0	24	9.00±8.2	0	26	13.33±9.1	N/A
Type II number	0	4	0.54±1.1	0	3	0.57±1.1	0	2	0.31±0.6	0	4	1.00±1.7	N/A
Right sided CLN	0	18	5.15±5.0	0	12	3.00±4.2	0	15	5.00±4.6	0	18	8.00±6.2	N/A
Left sided CLN	0	15	4.42±4.7	0	11	3.00±4.0	0	15	4.31±4.4	0	15	6.33±5.9	N/A
CLV (ml)	0.000	0.692	0.161±0.21	0.000	0.322	0.092±0.14	0.004	0.652	0.157±0.20	0.000	0.692	0.253±0.27	0.382
WMLV (ml)	0.54	51.47	15.22±13.8	1.12	32.49	7.69±11.2	0.54	51.47	16.89±14.00	3.49	41.98	20.38±14.6	0.120
TLV (ml)	0.55	51.93	15.38±13.94	1.14	32.81	7.78±11.31	0.55	51.93	17.04±14.10	3.49	42.67	20.64±14.86	0.132

EarlyRR: Early relapsing remitting subgroup

LateRR: Late relapsing remitting subgroup

SP: Secondary progressive subgroup

EDSS: Expanded Disability Status Scale

CLN: Cortical lesion number, divided into each hemisphere

CLV: Cortical lesion volume

WMLV: White matter lesion volume

TLV: Total lesion volume=CLV+WMLV

Comparison of the clinical subgroups

Mean and median values of CLN, CLV and WMLV were higher in LateRR compared to EarlyRR and higher in SP compared to the LateRR subgroup, however these differences were not statistically significant (Table 1).

A statistically significant difference in the BVMT-R Total Recall was observed among the three subgroups (Kruskal-Wallis p-value: 0.04). For pairwise comparisons, SP patients performed worse than LateRR patients on BVMT-R Total Recall (Bonferroni corrected p-value=0.029).

Associations among cortical lesions, white matter lesions, physical disability and cognitive performance

Results of univariate correlation analyses between CLs and WMLV as well as between the lesion metrics versus physical disability and neuropsychological test performance are summarized in Table 2.

Table 2.

Univariate correlations between physical disability, neuropsychological tests and MRI measures.

| | | EDSS | PASAT | SDMT | CVLT-II | | BVMT-R | | D-KEFS | JLO | COWAT | CES-D | CLV | WMLV |
					T1-T5	Long-Delayed Free Recall	Total Recall	Delayed Recall						
CLN	r_S (p-value)	0.472 (0.015)	ns	-0.404 (0.045)	-0.436 (0.029)	-0.409 (0.042)	ns	ns	ns	ns	ns	ns	0.887 (<0.001)	0.652 (<0.001)
CLV		0.404 (0.041)	ns	-0.425 (0.034)	ns	ns	-0.446 (0.025)	-0.400 (0.048)	ns	ns	ns	ns	N/A	0.705 (<0.001)
WMLV		ns	ns	-0.492 (0.013)	ns	ns	-0.507 (0.010)	-0.483 (0.014)	ns	ns	ns	ns	0.705 (<0.001)	N/A

r_S: Spearman's correlation coefficient

CLV and CLN showed significant correlations with WMLV. EDSS score significantly correlated with CLN and CLV, but not with WMLV. In univariate analyses all three MRI variables (CLN, CLV, WMLV) correlated with SDMT. CLN correlated with both immediate and delayed CVLT-II scores. CLV and WMLV correlated with both

immediate and delayed BVMT-R scores. None of the MRI variables correlated with PASAT, D-KEFS, JLO, or COWAT.

After correction for age, depression and pre-morbid IQ, in multiple linear regression analyses SDMT remained associated with CLN (overall R^2=0.513, partial regression coefficient for standardized data (St ß)= -0.566, p=0.002), CLV (overall R^2=0.449, St ß= -0.492, p=0.008), and WMLV (overall R^2=0.418, St ß= -0.461, p=0.014). CLN remained associated with CVLT-II T1-T5 (overall R^2=0.542, St ß= -0.421, p=0.013), and CVLT-II Long-Delayed Free Recall (overall R^2=0.461, St ß= -0.363, p=0.043). However, in the final adjusted models MRI variables no longer correlated with BVMT-R.

Discussion

We employed a high-resolution 3 Tesla brain MRI protocol that combined multiplanar display of 3D FLAIR and T1-weighted 3D IR-SPGR sequences. This approach took advantage of the high contrast sensitivity of FLAIR for imaging CLs combined with IR-SPGR to delineate the boundary between cortex and WM. These sequences are widely available on clinical scanners from multiple vendors and can be integrated in clinical routine with reasonable scan time.

Using this clinically applicable MRI method we assessed CLs in MS patients at different disease stages. The detected CL load is comparable with the bulk of CLs assessed by specialized MRI methods developed for sensitive CL delineation (22-25, 28). While it has been reported that CLs are more conspicuous on 3D DIR compared to 3D FLAIR images (18, 25), the former is not yet widely available on clinical scanners.

FLAIR hyperintensities might represent lesions, perivenular spaces, or CSF-related flow artifacts. Our lesion detection required that any FLAIR hyperintense lesions also show concurrent hypointensity on IR-SPGR images. This rule minimized the inclusion of non-lesional hyperintensities (e.g. artifacts, Figure 1). The possibility of misclassifying small micro-ischemic lesions as MS lesions exists with our applied method; however, the prevalence of ischemic lesions was likely low given the relatively young age of our MS cohort. Our dual image-based CL identification method leads to a trade off with higher specificity and lower sensitivity than conventional methods.

We detected CLs in 92.3% of patients, supporting the notion that focal CLs are highly prevalent in MS (12, 19, 20, 23-25, 27). CLs occurred in all studied clinical subgroups, including 85.7% of patients with EarlyRR MS. These results are consistent with recent MRI studies, which detected CLs from the earliest clinical stages of MS, even in patients with clinically isolated syndrome (20, 25, 27, 30, 92). We found a trend towards higher number and volume of CLs with disease duration and progression when comparing EarlyRR vs. LateRR vs. SP subgroups (Table 1). However, these differences did not reach statistical significance, possibly because of small sample sizes. Technical factors might also influence lesion detection: images from more disabled patients may contain more motion artifacts and in more advanced disease diffuse cortical damage may blur the contrast between GM and WM (93), thereby obscuring juxtacortical and cortical-subcortical lesions. Both of these factors could result in underestimation of CLs in SP MS. Several histopathological and MRI studies have found an increasing number of CLs with advancing disease duration and clinical stage (7, 27, 30, 92). One study was unable to confirm these findings (20) and a 7T MRI study showed differences between RR and SP patients only for subpial lesions (22).

We classified the majority of CLs as cortico-subcortical type (Type I), while intracortical lesions (Type II) made up 5.63%, and no subpial lesions (Type III) were detected. In histopathological studies, subpial lesions were the most prevalent type (prevalence: 44-70%), followed by cortico-subcortical lesions (10-34%), and small intracortical lesions (13-26%) (7, 11, 12, 94, 95). We believe, we had much higher sensitivity in detecting Type I lesions vs. both Type II and Type III lesions. This resulted in our disproportionate estimation of the distribution of lesion subtypes, using histological reports as the standard of reference. There are probably two major reasons for this: Type II (intracortical) lesions are relatively small and difficult to detect even at the ~1 mm isotropic image resolution employed in our protocol. Type III lesions escaped our detection most likely because severe subpial cortical tissue damage results in MR signal closely approximating that of the adjacent subarachnoid CSF limiting their conspicuity on IR-SPGR. Histopathologically, the intensity of inflammation in cortico-subcortical lesions is higher than in lesions confined purely to the cortex resulting in a more conspicuous MRI appearance (11). A further complicating factor relates to the different cytoarchitecture of

the cortex compared to WM, in that subpial lesions are located in the myelin-sparse upper layer of the cortex (11, 12, 15, 96). While ultra-high field MRI allowed depiction of subpial lesions (21, 22) previous MRI studies at field strengths ≤4.5T were similarly to our study unable to visualize them (18, 20, 23, 24, 97). The multiplanar display of our isotropic resolution IR-SPGR images allowed precise anatomic lesion classification in relation to the GM-WM junction and cortical folding patterns. We believe that this is a critical step in assigning lesions to their proper subtype. Thus, our method might have more accurately classified lesions as Type I, while previous methods would have classified these lesions as Type II or juxtacortical (Figure 1, 2).

Figure 1.

Type I cortical lesion shown on multiplanar images.

A-F: Unmagnified images. G-L: Magnified images. A-L: The same Type I lesion (arrows) is shown on FLAIR (A-C, G-I) and IR-SPGR (D-F, J-L) images, reconstructed in sagittal (A, D, G, J), axial (B, E, H, K), and coronal (C, F, I, L) planes. Note that the lesion is conspicuous on FLAIR, while its anatomic localization is well defined on the IR-SPGR images. On the IR-SPGR images, GM involvement of the lesion is clear on the sagittal and coronal views, while WM involvement is visible on the sagittal and axial views. Arrowheads indicate FLAIR hyperintense areas without corresponding hypointensity on the IR-SPGR images, suggesting the possibility of artifacts; these were not counted as cortical lesions.

	Sagittal	Axial	Coronal

FLAIR

SPGR

FLAIR

SPGR
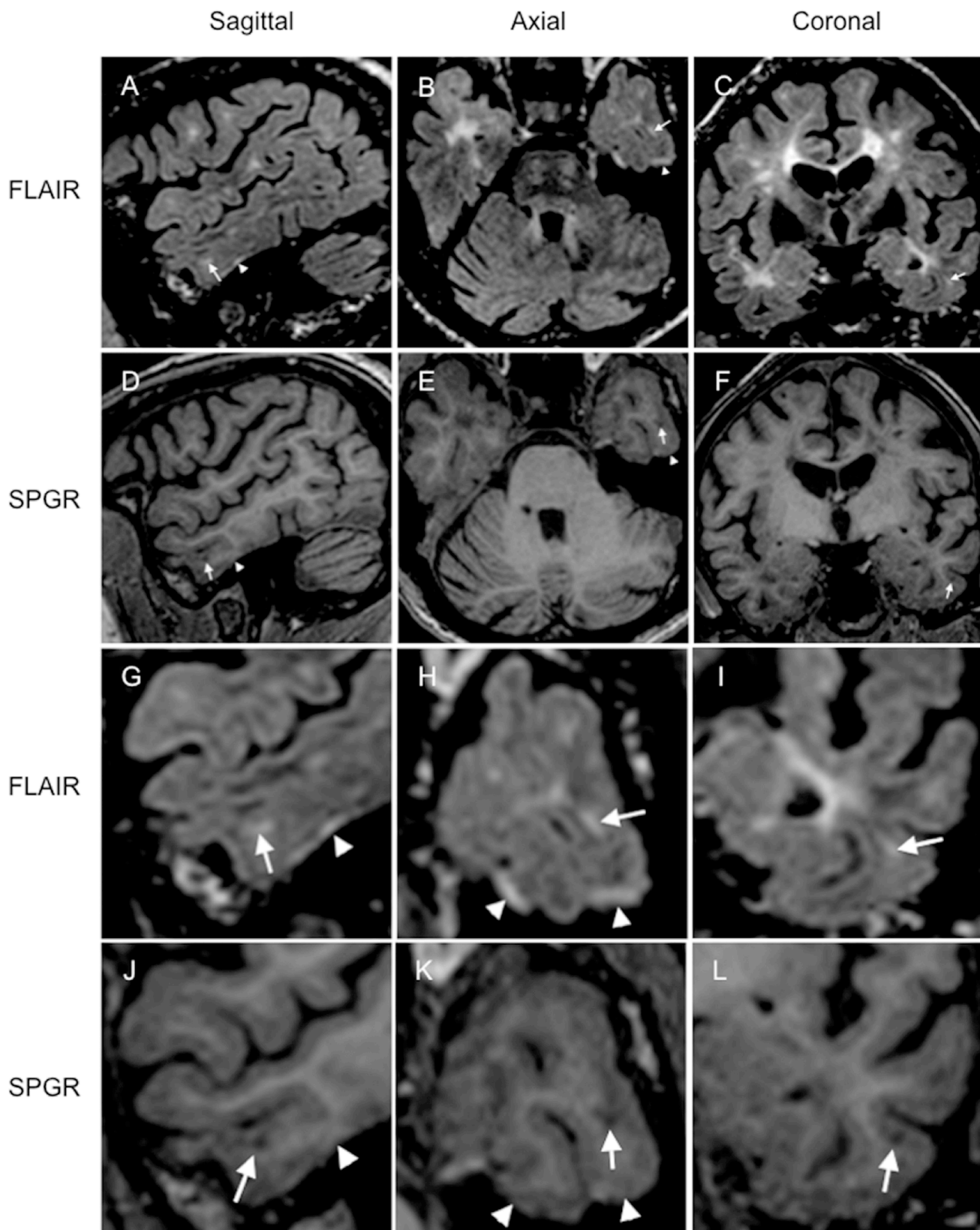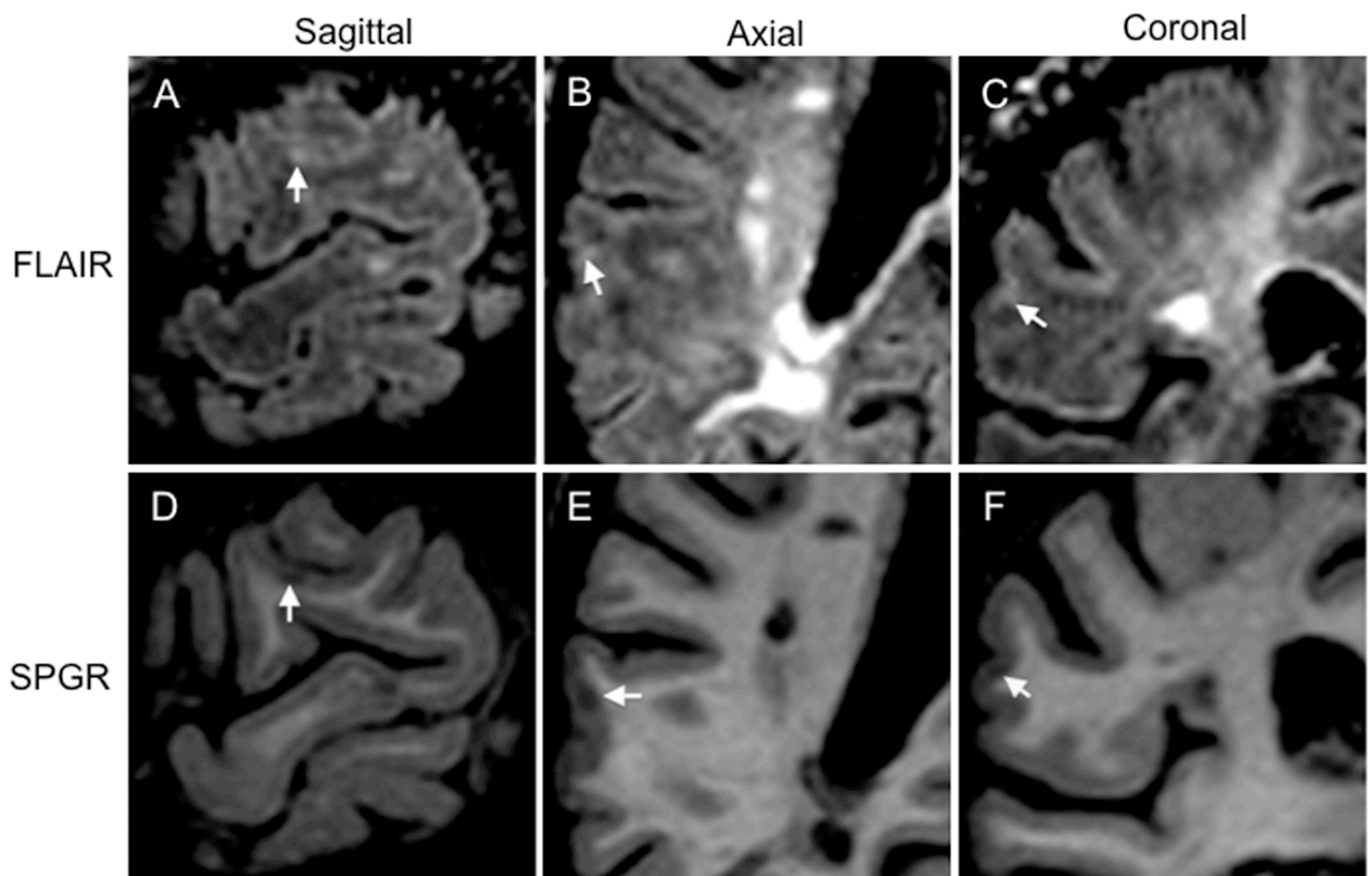
Figure 2.

Type II cortical lesion shown on multiplanar images.

A Type II lesion (arrows) is shown on FLAIR (upper row) and IR-SPGR (bottom row). A and D: sagittal view; B and E: axial view; C and F: coronal view. Note that the lesion is confined to the cerebral cortical GM and does not involve the subcortical WM, which is clearly seen in the 3 orthogonal planes of the IR-SPGR images.

Of fundamental importance is whether similar pathological processes drive focal lesion formation in the WM and in the GM (7, 11, 12, 16, 17, 95). In our study, CL measures reflected nearly entirely cortical-subcortical types and showed moderate correlations with WMLV, suggesting that the underlying pathogenic processes might be related. The relationship between inflammatory demyelinating lesions in WM and GM has previously demonstrated conflicting results, some studies found correlation (27, 30, 31, 98), while others did not (22, 92, 99). Unfortunately, the approach we used was unable to shed light on the relationship with subpial lesions.

In univariate analysis, CL metrics showed significant correlations with EDSS score while WMLV did not, although a trend was apparent. A large cross-sectional study using 1.5 Tesla 2D DIR sequence showed correlation between CLN and EDSS score in patients with RRMS, SPMS and clinically isolated syndrome (27). Applying the same MRI method, predictive value of baseline CL load was reported in a 2-year longitudinal study on the progression of physical disability in primary progressive MS patients (98), and during a 3-year follow-up in patients with RR and SP MS (99). However, using other imaging methods no correlation was found between physical disability and CL load (20, 23). Using 7T MRI, subpial lesions correlated with physical disability, but total CLs did not (22). Larger studies enabling multivariate regression analysis of CL subtypes and WM lesions will be necessary to understand the specific contribution of CL subtypes towards the clinical outcomes.

We explored the relationship between cognitive performance and lesion load in WM and cortical GM compartments of MS patients. Very few studies have examined this previously (30, 31). Therefore we wanted to assess a broad range of neuropsychological variables, which could potentially include cortical contributions (e.g. information processing speed, new learning, verbal and non-verbal memory, executive function). After controlling for age, depression and pre-morbid intelligence, CLN, CLV and WMLV independently predicted the performance of information processing speed and working memory (SDMT). In addition, CLN also predicted verbal learning and memory (CVLT-II). One limitation of our study is the lack of cognitive data on normal controls. Thus, we cannot ascertain the prevalence and severity of cognitive impairment in our cohort.

Previous studies demonstrated that information processing speed, working memory, and verbal memory are commonly affected in MS (32, 38, 100). Performance on SDMT involves diverse mental functions including complex scanning and visual tracking, requiring the integrity of widely dispersed cortical regions (100). PASAT also measures information processing speed and working memory, however compared to SDMT lower accuracy and sensitivity have been found in predicting cognitive deficit in MS (100), and weaker correlations with MRI metrics have been previously reported (54, 66, 101).

Our findings suggesting the importance of CLs with regard to CVLT-II are in line with previous studies showing associations between GM atrophy and verbal learning (57, 102).

The absence of significant correlation between CLs and other cognitive tests (BVMT-R, COWAT, JLO, and D-KEFS) may be related to their lower sensitivity to show associations with distributed multiple lesions (54, 55, 66, 100), however regional CLs were not assessed in our study. Other factors may have contributed to our failure to find significant correlations across the spectrum of cognitive domains commonly affected in MS: the small sample size and the high proportion of RR compared to SP patients may have limited the severity of the deficits observed.

Our findings suggest that both WM and GM lesions influence cognitive performance in MS. However, CLs may have a particularly important contribution to this relationship, as beside information processing speed performance, CLN also significantly correlated with verbal learning abilities. Two recent studies using DIR sequence yielded similar results (30, 31). Future studies using this clinically applicable MRI protocol in a larger sample size and in longitudinal fashion may further extend our knowledge about the pathogenic and clinical relevance of CLs in MS, even though there remains a prominent need for clinically applicable MR methodology capable of clearly depicting subpial lesions in MS.

Conclusion

The combination of 3 Tesla high-resolution T2-FLAIR and T1-IR-SPGR MRI detected many CLs (Type I/II) and allowed precise anatomic lesion classification. Our data suggest that focal cortical demyelination is a significant contributor to both the physical disability and cognitive ability of MS patients.

IV. SOCIAL COGNITION AND THEORY OF MIND IN PATIENTS WITH RELAPSING-REMITTING MULTIPLE SCLEROSIS

Materials and Methods

Participants

Multiple sclerosis patients

Forty patients with MS according to McDonald Criteria (3) were recruited from the Department of Neurology, University of Pécs. All patients had relapsing-remitting (n=37) or secondary progressive (n=3) disease course. Patients were eligible for the study, if they were clinically stable for at least 30 days. Severity of physical disability was estimated using the Expanded Disability Status Scale (EDSS) (4) rated by a neurologist specialized in MS care and blinded to cognitive data. Patients with mild and moderate disability were included in the study (EDSS≤4.5). Patients with history of alcohol or drug dependence, major psychiatric illness, neurological disease (other than MS) and gross visual impairment were excluded. Neuropsychological testing and clinical exam were performed on the same day.

Healthy controls (HC)

Thirty-five gender- and age-matched healthy volunteers served as controls for the neuropsychological testing with no previous history of neurologic dysfunction and a normal neurological examination.

Demographic and clinical characteristics are presented in Table 3.

Written informed consent was obtained from each participant. The local ethical standards committee approved the study and written informed consent was obtained from all participants.

Social cognitive testing and psychometric assessment

All tests have been validated and were executed in Hungarian. For assessment of the mentalization and empathic ability socio-perceptual tasks (non-verbal) and complex perspective taking tasks (verbal), as well as an empathy questionnaire were applied.

Socio-perceptual ability testing

Computerized version of the Faces Task (103), and the Eyes test (104, 105) were used. The stimuli were presented on separate slides after each other. The protocol was completed in a single session without time limit.

Faces test

The Faces test (103) consists of 20 photographs of the face of an actress portraying different facial expressions. Subjects were instructed to choose from two emotions (one correct, one incorrect) presented on the left and right side of the photograph the one most appropriately describes thoughts and feelings of the actor. Each correct choice scored one point.

Eyes Test

The Eyes test (105) consists of 36 photographs depicting just the eye region of different Caucasian individuals expressing various mental states. A rectangular area of approximately 15x6cm delineates the eye region encompassing the entire width of the face from midway up the nose to right above the brow. Four mental state terms were presented simultaneously (one target word and three foils) at each corner of the photograph. Participants were asked to choose the word best describes the thought or feeling displayed. Scores are calculated as the total number of correct discriminations for all 36 items.

In a control trial, subjects had to judge the gender of each person on the photograph to control for possible impairments in face perception, which could interfere with facial expressions recognition.

Perspective taking tasks

Perspective taking and reasoning about mental and emotional states of others was evaluated with the Faux Pas test. In a social faux pas situation somebody unwittingly makes an awkward comment not realizing that it might hurt another person. To detect a faux pas, the participant has to understand simultaneously the knowledge or beliefs of both characters in the situation and appreciating the emotional state associated to it.

We applied 5 Faux pas task (78), and 5 control tasks presented in random order, and in the same design. A short verbal story was displayed on computer screen containing a faux pas situation. After reading a story, participants received two statements one after

another, and were asked to indicate whether the offered interpretation was correct. Correct comprehension scored one point. Control task was used to assess basic story comprehension.

Empathy testing

Empathic abilities were measured with the self-report Empathy Questionnaire (106). The questionnaire contains 40 empathy items and 20 filler items. On each item, a person can score 2, 1, or 0, thereby the maximum score is 80. The questionnaire evaluates the ability to detect and appropriately react in response to emotion of other peoples.

Psychometric assessment

The Hungarian version of the Wechsler Adult Intelligence Scale-Revised (H-WAIS) (107) was applied to estimate verbal (VQ) and performance skills (PQ). Depression severity was rated with the Beck Depression Inventory (BDI) (108), a validated screening tool for depressive symptoms. The BDI contains self-reported response to a multi-choice questionnaire on 21 items. Anxiety was measured with the 20-item Spielberger Trait Anxiety Inventory (20-item STAI) (109). The STAI consists of anxiety trait and anxiety state evaluation on a 4-point rating scale. The STAI is well validated in a variety of populations. Cut-off values were 16 and 54 for BDI and STAI, respectively.

Statistical analysis

Statistical analyses were performed using SPSS version 15.0 software. Mann-Whitney U-test was used to detect differences in gender, and *t* test to evaluate age, IQ, depression, anxiety, and ToM performance differences between groups of MS patients and HC.

MS cohort was dichotomized based on the degree of physical disability measured by the EDSS representing mild (EDSS 1-2, mean±SD 1.13±0.35) versus moderate (EDSS 2.5-4.5, mean±SD 2.98±1.01) disability corresponding to previous reports (110). In addition, MS subgroups were created using the median value of length of the disease duration (median: 7 years, mean±SD 7.57±6.29) resulting in short-term (1-7 years, mean±SD 3.8±2.88) and long-term (8-18 years, mean±SD 13.86±5.3) disease duration subgroups. Physical disability did not differ significantly between MS subgroups with different disease duration length (EDSS 2.26±1.18 vs. 2.33±1.33). To analyze the effect of MS, physical disability represented by EDSS, and disease duration on mentalization MS

subgroups and HC were compared using one-way ANOVA, and p-values of Scheffe post-hoc tests were calculated. To control for the confounding effect of age, gender, depression, anxiety, IQ, PQ and VQ, multiple logistic regression analyses were performed. We tested the predictive value of MS, physical disability, disease duration, and disease progression rate on different domains of mentalization. Age-, gender-, depression-, and anxiety-adjusted odds ratios (OR) were calculated after dichotomizing the outcome parameters of each mentalization domains by the median. OR<1 indicates poorer, while OR>1 indicates better mentalization performance compared to the average.

Results

I. Comparisons of demographic variables, neuropsychiatric performance, mentalization and empathic ability between patients with multiple sclerosis and healthy controls

Age and gender distribution between MS and HC groups were statistically comparable. The two groups did not differ when IQ was compared (Table 3). Except age, these parameters showed no difference when MS subgroups were compared either to HC or to each other (data not shown). Patients with MS showed significantly higher scores in BDI and STAI compared to HC (Table 3).

MS patients performed significantly poorer in the Faces test and in the Eyes test compared to HC (Table 3).

Table 3.

Comparisons of demographic data, neuropsychological performance, mentalization and empathic ability between patients with multiple sclerosis (MS) and healthy controls (HC)

Variables	HC (n=35)	MS (n=40)	P-value
Gender	Female=18	Female=29	ns
Age	33.4±7.8	36.2±9.4	ns
IQ	112.9±6.4	107.7±9.4	ns
Depression	4.14±3.4	9.5±7.4	<0.001
Anxiety	40.0±6.3	48.2±6.9	<0.001
Faux Pas	7.3±1.9	7.8±1.4	ns
Eyes test	25.2±4.0	22.1±4.2	<0.001
Faces test	18.8±1.2	17.1±1.4	0.01
Empathy	34.1±8.6	36.7±10.8	ns

Mean ± SD are indicated.

IQ= intelligence quotient.

After controlling for confounding effect of age, gender, IQ, PQ, VQ, and co-morbidities (anxiety and depression), MS patients performed significantly poorer in the Eyes test: proportion of subjects who achieved higher scores than the median in Eyes test was reduced by 79 % in the MS group compared to HC (OR=0.21, p=0.02). All other mentalization domains were unaffected by MS (Table 4).

Table 4.

Multiple logistic regression analysis of the effect of multiple sclerosis (MS) on mentalization performance and empathic ability compared to healthy controls (HC).

	Odds ratio (OR)[a]	P-value	r^2
Eyes test	0.21	0.002	0.297
Faces test	1.23	ns	0.247
Faux Pas	0.41	ns	0.076
Empathy	3.34	ns	0.202

[a]Age, gender, depression, anxiety, VQ, PQ, and IQ adjusted OR; MS patients: n=40, HC: n=35.

II. Effect of physical disability on mentalization and empathic ability

Groups of moderately and mild disabled MS patients were compared to HC and to each other. Age, gender, and IQ were comparable between the two MS subgroups, and HC (data not shown). Compared to HC, patients in both MS subgroups were more depressed and anxious. Both MS subgroups performed significantly poorer in the Eyes test compared to HC. In addition, patients with mild physical disability performed poorer in the Faux pas task. Performance of the two MS subgroups did not differed significantly in any of the mentalization tests, and in the empathy questionnaire. (Table 5)

Table 5.

Neuropsychological performance, mentalization and empathic ability of multiple sclerosis (MS) subgroups differing in the degree of physical disability were compared to each other, and to healthy controls (HC).

	HC n=35	MS with low EDSS[a] n=15	MS with high EDSS[b] n=25	P-value
Depression	5.2±5.2	6.3±5.9	13.8±9.6	<0.001[c], 0.01[d], 0.01[e]
Anxiety	41±7.7	46.5±4.5	50.0±7.7	<0.001[c], <0.001[d], ns[e]
Faux Pas	7.8±1.4	7.7±1.2	6.6±2.3	<0.05[c], ns[d], ns[e]
Eyes test	25.6±3.3	23.9±1.7	20.4±5.5	0.02[c], 0.007[d], ns[e]
Faces test	18.6±1.2	18.2±1.4	16.3±3.3	ns[c,d,e]
Empathy	54.4±10.8	56.7±6.3	57.1±9.6	ns[c,d,e]

Mean ± SD, and results of one-way ANOVA and Scheffe pos-hoc analysis are indicated.

ns= not significant.

[a]EDSS: ≤2

[b]EDSS: >2

[c]MS with low EDSS vs. HC

[d]MS with high EDSS vs. HC

[e]MS with low EDSS vs. MS with high EDSS

After correction for age, gender, IQ, PQ, VQ, depression, and anxiety, more disabled patients (moderate physical disability) performed poorer in the Eyes test, and Faux pas test compared to HC (OR=0.17, p=0.02, r^2=0.301 and OR=0.17, 0.01, r^2=0.181, respectively). In addition, more disabled patients showed a strong trend toward higher empathic ability compared to HC (OR=5.52, p=0.05, r^2=0.221). MS subgroups did not perform significantly different in any of the mentalization tests, and in the empathy questionnaire.

III. Effect of disease duration on mentalization and empathic ability

MS subgroups created by dichotomization of the MS cohort by the median of the disease duration were compared to HC and to each other. Age, gender, and IQ were comparable between the two MS subgroups and HC (data not shown). Compared to HC, patients in both MS subgroups were more depressed and anxious. Both MS subgroup performed poorer in the Eyes test, compared to HC. In addition, patients with shorter disease duration showed a strong trend to make more errors in the Faces test, and empathize better. No statistical differences were found between the MS subgroups in any of the variables (Table 6).

Table 6.

Neuropsychological performance, mentalization and empathic ability of multiple sclerosis (MS) subgroups differing in disease duration were compared to each other, and to healthy controls (HC).

	HC n=35	MS with short disease duration[a] n=25	MS with long disease duration[b] n=15	P-value
Depression	5.2±5.2	10.8±8.8	12.2±9.5	<0.001[c], 0.01[d], ns[e]
Anxiety	41±7.7	49.7±6.7	48.1±7.4	<0.01[c], <0.05[d], ns[e]
Faux Pas	7.8±1.4	6.9±1.5	7.1±2.5	ns[c, d, e]
Eyes test	25.6±3.3	20.6±4.6	22.4±5.1	0.007[c], 0.002[d], ns[e]
Faces test	18.6±1.2	16.6±2.7	16.8±3.2	0.05[c], ns[d,e]
Empathy	54.4±10.8	57.7±10.3	56.3±6.6	0.05[c], ns[d,e]

Mean ± SD, and results of one-way ANOVA and Scheffe pos-hoc analysis are indicated.

ns= not significant.

[a]short-term disease duration: ≤7 years, [b]long-term disease duration: >7 years

[c]MS with short-term disease duration vs. HC

[d]MS with long-term disease duration vs. HC

[e]MS with short-term disease duration vs. MS with long-term disease duration

Adjusting for age, gender, depression, anxiety, PQ, and VQ, patients with shorter disease duration showed significantly higher empathic ability compared to HC (OR=7.75, p=0.03, r^2=0.249). In addition, a strong trend was noticeable for poorer Eyes test performance among patients with longer disease duration compared to HC (OR=0.13, p=0.05, r^2=0.303).

IV. Effect of disease progression rate on mentalization and empathic ability

Multiple logistic regression analyses showed that in MS both degree of physical disability and length of disease duration significantly impact the mentalization and empathic ability. To investigate the combined effect of these two factors defining the progression rate of the disease MS patients were divided into four subgroups: low EDSS and short-term disease duration, low EDSS and long-term disease duration, high EDSS and short-term disease duration, high EDSS and long-term disease duration. After adjusting for the confounding factors patients with rapid disease progression (high EDSS and short-term disease duration subgroup) performed poorer in the Eyes test and Faux Pas test compared to HC (OR=0.13, p<0.03; OR=0.14, p<0.02, respectively). In contrast, patients in this subgroup attributed 14-times better empathizing ability to themselves compared to HC (OR=13.73, p<0.05). Other domains were not significantly different between MS subgroups and HC.

<u>Discussion</u>

Living with MS frequently associates with disturbed social life including insufficiency in partnerships and family roles, as well employment status (110). Intact social cognition is fundamental for successful social functioning (80). We aimed to investigate whether mentalization and empathy two major mechanisms of social cognition are affected in MS, and thereby may contribute to social difficulties perceivable in everyday life of MS patients. In addition, we studied the effect of physical disability, disease duration, and disease progression rate on social cognitive abilities of MS patients.

Depression is a common symptom in MS and interferes considerably with cognitive functioning (36). In addition, social cognitive performance of bipolar depressive patients was reported impaired (111, 112). In our MS cohort depressive and anxious symptoms

occurred significantly more frequent compared to HC potentially affecting the ToM performance. To remove confounding effect of the affective state multivariate statistical models were used.

Compared to HC, MS patients showed social-perceptual dysfunction. Performance of MS patients in the Eyes test was significantly poorer even after removing confounding effects of demographic factors, general intelligence, and affective state. The Eyes test is an advanced and sensitive tool to detect subtle dysfunction in the mindreading ability of adults with normal intelligence. The complex musculature patterns of the human face, prominently around the eyes are rich source of cues for accurate emotion decoding (113, 114). However, interpretation is difficult when viewing only the eye region separated from the entire facial expression especially when complex emotions and mental state instead of basic emotions are required to decode (82, 103, 104). Supporting our results facial emotion recognition was reported deficient in recent studies of MS patients (74-77).

The degree of physical disability profoundly impacted mentalization: patients with higher EDSS showed poorer performance both in social-perception (Eyes test) and cognitive perspective taking (Faux pas test) tasks independently from the confounding factors. Previous studies concluded that cognitive abilities of MS patients decline independently from physical disability (33, 115-117), however another studies found correlation between cognitive impairment and EDSS (118, 119).

After correction for confounding factors, longer disease duration associated with poorer emotion recognition ability in the Eyes test, however this relationship did not reach statistical significance, only a trend was noticeable (p=0.05). Previously, cognitive impairment was reported irrespective of the duration of the disease (35).

Similarly to the effect of physical disability, disease progression rate was related to both aspects of mentalization: compared to HC, more rapidly progressing patients performed significantly poorer in visual (Eyes test) and verbal (Faux pas test) mind reading tasks independently from the confounding factors. Association between cognitive decline and disease activity, as well as physical disability progression was also demonstrated in patients with MS (35, 115, 120). These results are consistent with the hypothesis that

cognitive impairment is generally caused by the same type of brain damage responsible for other MS deficits.

MS patients with shorter disease duration, and with faster disease progression rate reported higher level of empathic ability. The style of the empathy questionnaire is self-rating and reflects the level of empathic ability attributed by the subjects to themselves. ToM dysfunction itself might result in a false self-judgment of own empathic skill leading to higher scoring on the empathy questionnaire. Recently, elevated empathic ability has been found in bipolar depression disorder (112). Although in our MS cohort depression was more frequent than in HC, the increased empathic ability was independent from depression in the multivariate analysis. It is also possible, that increased empathic ability is related to the emotional stress associated with MS resulting in a more focused emotional processing. The higher rate of depressive and anxiety symptoms among MS patients may support this hypothesis, as certain affective states may influence meta-representational skills (121, 122).

Fatigue is a frequent manifestation of MS and may be present before significant disability develops (123). MS patients with social cognitive dysfunction may need to exert more cognitive effort to perform successfully in social situations. This higher cognitive demand in everyday life situations may contribute to cognitive fatigue. In addition, constant cognitive compensation of social cognitive disabilities may interfere with other cognitive domains, such as information processing speed, verbal and visuospatial memory, and executive function known most frequently impaired in MS. Future studies investigating the relationship between social cognition and other cognitive domains, as well as fatigue may broaden our knowledge about evolvement of fatigue and different aspects of MS cognitive disability.

In summary, our study demonstrated impaired mind reading ability from facial expressions in MS. Deficient judgments of the thoughts and feelings of other people from facial cues associates with insufficient interpersonal communication skill and may belong to the factors contributing to poor quality of life of MS patients. More advanced physical disability and faster progression rate of the disease predicted more extended mentalization deficit affecting both the social-perceptual and the complex perspective

taking abilities. This result suggests that development of cognitive dysfunction in MS parallels with the pathological processes defining general progression of the disease.

V. NEUROANATOMICAL SUBSTRATES AND CONNECTIVITY OF MENTALIZATION: A MULTI-MODAL MRI STUDY IN MULTIPLE SCLEROSIS

Material and Methods

Participants

Forty-nine Caucasian patients with MS according to McDonald Criteria (3) were recruited from the Department of Neurology, University of Pécs. Patients were eligible for the study, if they were clinically stable for at least 30 days. Patients with history of alcohol or drug dependence, major psychiatric illness, neurological disease (other than MS) and gross visual impairment were excluded. Each patient had clinical exam, neuropsychiatric test and brain MRI on the same day. Physical disability was measured with the EDSS (4). At the time of the study 40 patients (82%) received immunomodulatory or immunosupressive therapy (glatiramer acetate, interferon beta-1b, interferon beta-1a, azathioprine). Twenty-four gender- and age-matched healthy volunteers served as controls for the neuropsychological testing with no previous history of neurologic dysfunction and a normal neurologic examination. Demographic and clinical characteristics are presented in Table 7.

The local ethical standards committee approved the study and written informed consent was obtained from all participants.

Table 7.

Demographic and clinical data of the participants

Variables	MS patients (n=49)	Healthy subjects for neuropsychological testing (n=24)
Gender Female/Male (%)	31/18 (63/37)	13/11 (54/46)
Age (y)	20-61 (39.82±9.3)	24-51 (36.71±7.3)
Disease course RR/PP/SP (%)	44/1/4 (90/2/8)	
Disease duration (y)	0-21 (9.48±6.2)	
EDSS	0.0-6.0 (2.43±1.7)	
tT1LV	112-20000 (median: 1524)	
Faces test	8-20 (median: 17)	16-20 (median: 19)
Eyes test	16-30 (22.47±3.37)	19-31 (25.67±3.05)
Faux pas test	3-10 (median: 8)	5-10 (median: 8)
Irony test	3-10 (median: 10)	4-10 (median: 8)
Depression	0-28 (9.00±6.46)	0-11 (3.33±3.19)
Anxiety	26-71 (48.12±12.11)	29-55 (37.87±6.38)

range (mean±SD)

RR: Relapsing remitting

PP: Primary progressive

SP: Secondary progressive

EDSS: Expanded Disability Status Scale

tT1LV: total T1 lesion volume (mm^3)

Social cognitive testing and psychometric assessment

All tests applied have been validated in Hungarian.

Socio-perceptual ability testing

Social-perceptual ability was evaluated with the Faces and Eyes tests performed as described in the previous study (see page 35).

Perspective taking testing

Perspective taking and reasoning about mental and emotional states of others was evaluated with social faux pas, and irony comprehension tests.

Faux pas detection was evaluated as described in the previous study (see page 35).

Irony is a statement with an intended meaning opposite to its literal meaning. Usually, ironic utterances express implicit criticism and disapproving attitude of the speaker. To comprehend irony, the listener has to interpret beliefs and intentions of the speaker and use that knowledge to generate the non-literal interpretation. Irony comprehension was tested with 5 Irony task (79) in an identical way as the assessment of the Faux pas and control tasks.

Psychometric assessment

Psychometric assessment of depressive and anxious symptoms equals with the fashion described in the previous study (see page 36).

Descriptive data of social cognitive testing and psychometric assessment of the participants are presented in Table 7.

MRI

MRI acquisition

MRI was performed on a 3.0 T clinical MRI scanner (Magnetom TIM Trio, Siemens Medical Solutions, Erlangen, Germany) with a field gradient strength of 40mT/m and a 12-channel phased array head coil. The following sequences were measured: T1-weighted three-dimensional (3D) MPRAGE (TR/TI/TE=2300/900/1.9ms, Flip Angle=9º, 160 sagittal slices, slice thickness=1.2mm, no interslice gap, FOV=240x256mm^2, matrix size=240x256, bandwidth=240Hz/pixel, scanning time ~5 minutes); diffusion tensor data were measured with single-shot diffusion-weighted echo-planar imaging (TR/TE=9600/93ms, 63 axial slices, slice thickness=2mm, no interslice gap,

FOV=208x256 mm^2, matrix size=104x128, diffusion was measured in 20 directions with a b-value of 700 s/mm^2, bandwidth=1395 Hz/pixel, number of averages 3, scanning time ~12 minutes).

MRI analysis

Total lesion load measurement

A neurologist [AM] with clinical and imaging experience in MS blinded to clinical and neuropsychiatric findings performed the lesion volume assessments. Total T1 lesion volume (tT1LV) was estimated from MPRAGE images. Lesions were outlined and T1 binary lesion maps were generated for MPRAGE images using a semi-automated thresholding technique with the software 3D-Slicer (Version 3.4, http://www.slicer.org). The method relies on user-guided specification of the intensity threshold range, which is manually adjustable during lesion segmentation. The algorithm labels all pixels with signal intensity within the set threshold range in the lesion area identified manually by the user (Figure 3).

Analysis of regional white matter lesions in the white matter fiber bundles

MRI images of all patients were processed with FSL software of the FMRIB Software Library tools (http://www.fmrib.ox.ac.uk/fsl). For parcellation of the WM lesions a WM fiber tract atlas was applied (ICBM DTI-81 Atlas) (124) originally developed from a DTI database of 81 normal subjects and normalized to the MNI-International Consortium for Brain Mapping (ICBM) template. The atlas parcellates the WM of the two hemispheres into 50 fiber bundles (Figure 3). During MRI processing brain extraction was performed using Brain Extraction Tool (BET) (125) on the MPRAGE image. MPRAGE images were then spatially registered into standard space reference brain (MNI152_T1_1mm_brain, 1x1x1mm^3/voxel) using a two-step process. First, brain-extracted images were registered to the MNI152 standard brain image (12 degrees-of-freedom linear fit, correlation ratio cost function) using FLIRT (90). Second, the registration was further refined using FNIRT nonlinear registration. Subsequently, an inverse transformation was applied to warp the ICBM DTI-81 Atlas labels into the native space of MPRAGE images. Quality of all registrations was visually evaluated. The T1

binary lesion maps were labeled in their own native space using the transformed ICBM DTI-81 Atlas. The labeled lesion maps were used to measure regional T1 WM lesion volume (rT1LV) in the individual major fiber bundles (Figure 3). We assessed the volume of each individual fiber bundle to calculate their relative lesion content. All volume measures were performed in the native space to avoid possible volume changes due to any spatial transformation.

Figure 3.

Example illustrating the procedure of total and regional T1 lesion volume assessment. Panel A: T1 hypointense lesions are shown on a grayscale MPRAGE image (white arrows). Panel B: Binary lesion map generated from outlined T1 hypointense lesions visualized in green. Panel C: WM fiber tract atlas registered into native space of the MPRGE image. Panel D: Labeled lesion map derived from the binary lesion map after parcellation of the lesions with the use of the WM fiber tract atlas. The labeled lesion map was used to measure regional T1 white matter lesion volumes in each individual fiber bundles.
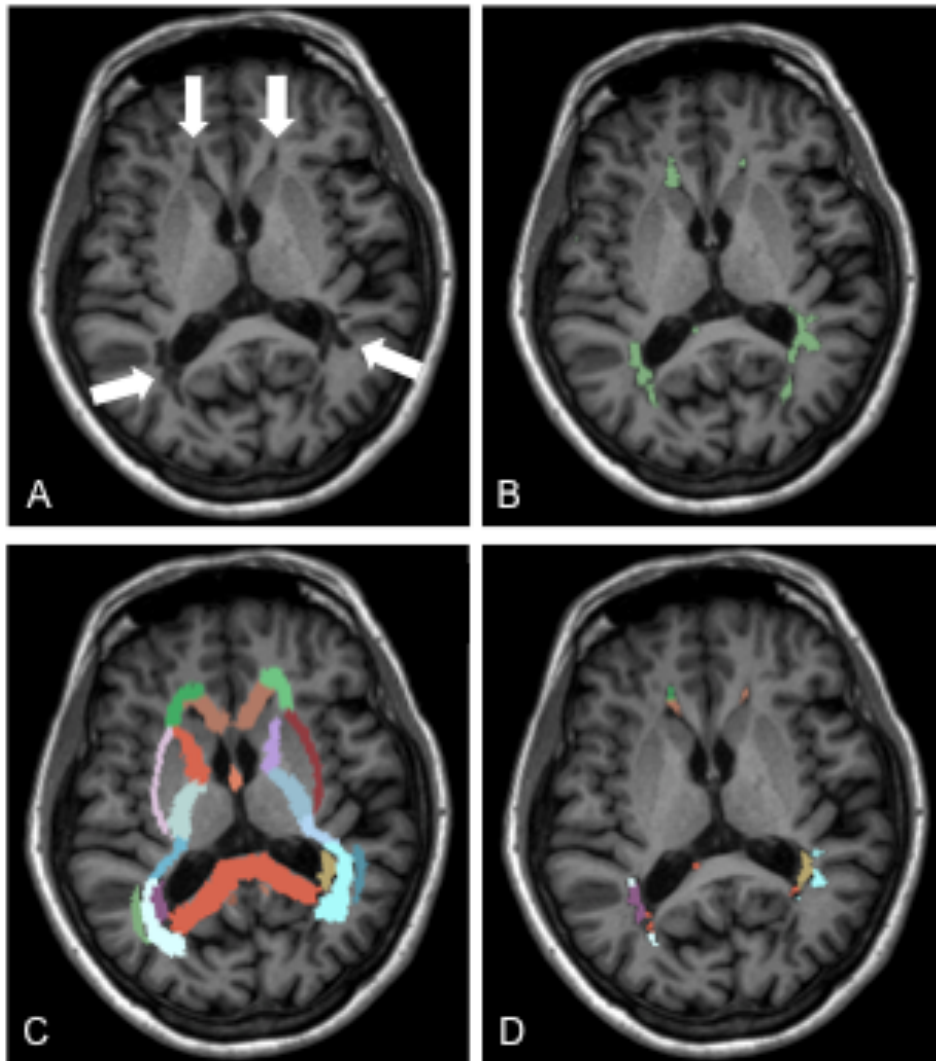
DTI-based fiber tractography

Using FSL software, brain-extracted MPRAGE images were registered to b0 DTI images (6 degrees-of-freedom linear fit, mutual information cost function). The derived transformation matrix was applied to transform the T1 binary lesion maps to the space of the b0 DTI images. The ICBM DTI-81 Atlas labels were warped to the native space of b0 images using the nonlinear transformation from standard space to native MPRAGE image space concatenated with the transformation from native MPRAGE space to native b0 DTI space. The transformed T1 binary lesion maps were labeled using the transformed ICBM DTI-81 Atlas in each subject. Fiber tractography was obtained using 3D-Slicer, Version 3.6 (http://www.slicer.org). The co-registered labeled lesion maps served as seed areas to reconstruct the fiber bundles one at a time. For reconstruction default tracking parameters were used. In each patient tractography tracking was performed for every fiber bundles, which lesion content showed in previous statistical analysis significant correlation with the social cognitive tests. Co-registered MPRAGE images were used to identify cortical endings of the tracts. We registered tract projections to the prefrontal cortex, premotor and motor cortex, anterior cingulate cortex, posterior cingulate cortex, insula, parietal lobe, temporal lobe and occipital lobe. For anatomical identification of the cortical areas, a cortical parcellation map was used individually calculated for each subject's brain, as part of the FreeSurfer reconstruction algorithm (see below) (126). The cortical parcellation map was viewed side by side with the tractography results both separately overlaid on the MPRAGE image.

Cortical thickness measures

We measured the thickness of the neocortex throughout the whole brain on 3D MPRAGE images using the automated reconstruction algorithm of the software FreeSurfer version 4.5 (http://surfer.nmr.mgh.harvard.edu). Technical details of the procedure have been described previously (127, 128). Briefly, the implemented processing stream includes removal of non-brain tissue, transformation to Talairach-like space, and segmentation of GM/WM tissue. The GM/WM boundary was tessellated with a mesh of triangles creating up to 160000 vertex points for each hemisphere. Topographical defects were automatically corrected. After intensity normalization, the GM/WM and pial boundaries

were detected by finding the greatest shift in intensity as the surface is deformed. The entire cortex of each subject was then visually inspected and errors due to misclassifications of WM lesions located close to the cortex were manually edited and automatically corrected. After creation of the cortical representations, the cerebral cortex was parcellated into anatomical structures. Cortical thickness was computed by finding the shortest distance between vertex points of the estimated pial surface and the GM/WM boundary and vica versa. These two values were then averaged (129). To measure cortical thickness at each vertex, each participant's brain surface was mapped onto a common spherical coordinate system using a spherical transformation to align cortical folding patterns. This procedure provides accurate matching of morphologically homologous cortical locations among participants. Maps were smoothed with a full-width-half-maximum Gaussian kernel of 10 mm. The maps produced are not restricted to the voxel resolution of the original data, thus, are capable of detecting sub-millimeter differences. FreeSurfer methodology has been previously validated against histological (130), and manual measurements (in schizophrenia: (131)); sensitivity has been demonstrated in MS patient groups (132), and reproducibility has been reported for detection regional cortical thickness correlates of cognitive performance in normal older adults (133).

Effect of each social cognitive test on cortical thickness was computed at each vertex point using a GLM implemented in the FreeSurfer software package. Age was entered as covariate to control for its effect on the cortical thickness. The resulted significance maps were displayed on the FreeSurfer averaged brain surface. GLMs were generated separately for each social cognitive test in both hemispheres separately. We identified continuous focal cortical areas (ROIs) with a surface area of more than $25mm^2$ showing significant correlation at $p < 0.001$ (uncorrected for multiple comparisons). The ROIs were manually outlined, and automatically mapped back on the brain surface of each participant using the spherical morphing to find homologous regions across subjects and the average thickness of each ROI was calculated for every participant.

Statistical analysis

Statistical analyses were performed using SPSS software version 18.0 (SPSS, Chicago, IL, USA). Normality testing using the Shapiro-Wilk test showed normal distribution for the Eyes test; other cognitive tests (Faces, Irony, Faux pas), and tT1LV were not-normally distributed.

Impact of gender, EDSS, anxiety, and depression on the social cognitive tests were analyzed with non-parametric Spearman rank correlation test, and Mann-Whitney U-test, as well as with parametric t-test. Depression and anxiety were divided by the cut-off values. A two-tailed level of $p<0.05$ was the significance threshold.

Social cognitive performance of MS patients and healthy controls were compared using GLMs controlling for the significant confounding factors. Tests showing not-normally distribution were dichotomized by the median.

Predictive values of tT1LV and rT1LVs on social cognitive test performance were analyzed in GLMs adjusted for the significant confounding factors followed by Bonferroni corrections. Total T1LV were dichotomized by the median.

Correlation analysis between cortical thickness and social cognitive performance was performed in two steps. In the first step, on a vertex-by-vertex basis the effect of social cognitive performance was analyzed on the cortical thickness corrected for age highly influencing cortical thickness (134) using a GLM implemented in the FreeSurfer package. Corrections for multiple comparisons using False Discovery Rate and clusterwise probability Monte Carlo simulation were performed. In the second step the identified ROIs were further analyzed to test for relationships between social cognitive performance and cortical thickness of the ROI accounted for the significant confounding factors. These second step analyses were performed in SPSS using GLMs followed by a Bonferroni correction.

Forward stepwise linear regression analysis was performed with p-value for entry of 0.05, and a p-value for removal of 0.1 to assess the contributions of rT1LVs and cortical thickness of focal cortex areas to the variance of social cognitive performance corrected for the confounding variables. Finally, in linear regression analyses predictive value of the rT1LV were assessed on focal cortical thinning corrected for age.

Results

I. Significance of the confounding variables

In the univariate correlations gender and EDSS did not show significant correlation with any of the social cognitive tests, therefore these parameters were not considered as confounding factors in further statistical models. Anxious subjects performed poorer in the Faces (p=0.027) test, and depressed subjects performed poorer in the Eyes test (p=0.013), and Faces tests (p=0.01).

II. Comparison of social cognitive performance between multiple sclerosis patients and healthy controls

After correction for anxiety and depression compared to healthy controls MS patients performed significantly poorer in the Irony test (B=-0.481, p=0.008, R^2=0.285) and in the Eyes test (B=2.876, p=0.035, R^2=0.225). Further MRI analysis was performed with social cognitive tests significantly differing between MS patients and healthy controls.

III. Association of T1 white matter lesion volume with cognitive performance of multiple sclerosis patients

1. Effect of total T1 white matter lesion volume

After correction for the confounding factors tT1LV did not correlate with any of the social cognitive tests.

2. Effect if regional T1 white matter lesion volumes localized in regions of major inter-connecting tracts

Tracts containing rT1LV>0.001 mm^3, in ≥25% of the patients (n=12) were considered for statistical analysis: 21 from 50 fiber bundles of the ICBM DTI-81 Atlas fulfilled these criteria. After correction for confounding variables, and using Bonferroni adjustment (p<0.0024), the Eyes test significantly correlated with rT1LV of the splenium of corpus callosum (SCC) (B=-0.886, p<0.001, R^2=0.299).

IV. Fiber tractography

Splenium of corpus callosum:

Since we observed correlation between rT1LV of the SCC and Eyes test performance, fiber tractography was used to explore cortical projections from T1 lesions of the SCC (Figure 4). Seventy-one percent (35/49) of MS patients had T1 lesions in the SCC. In one patient no tracts, and in one patient no cortical projections were traceable during the

tractography. In the remaining 73% (24/33) of patients tracts projected to the occipital lobe (right vs left hemisphere: 75% vs 92%), and similarly, in 73% to the parietal lobe (right vs left hemisphere: 92% vs 96%). In 61% (20/33) of the patients tracts were traceable to the temporal lobe (right vs left hemisphere: 65% vs 95%), in 39% (12/33) to the prefrontal cortex (right vs left hemisphere: 46% vs 77%), and in 21% (7/33) to the premotor-motor area (right vs left hemisphere: 57% vs 57%).

Figure 4.

Examples of DTI fiber tractography to reconstruct the tracts of the splenium of corpus callosum affected by macroscopically visible lesions and to trace their cortical projections. Lesions in the splenium of corpus callosum served as seed areas. Note that the bulk of the traced tracts indicates the amount of the damaged tracts. Panel A and B shows the traced tracts of the same patient posteriorly (A) and laterally (B). Regional T1 lesion volume of the splenium of corpus callosum in this patient was 3.546 mm^3. Panel C and D shows patients with fewer regional T1 lesion volume, 0.027 mm^3 and 0.028 mm^3, respectively. Panel E: Patient with regional T1 lesion volume of 3.588 mm^3 in the splenium of corpus callosum. Red circle indictes the fusiform face area, which cortical thickness corelated with social-perceptual performnce of the MS patient cohort.
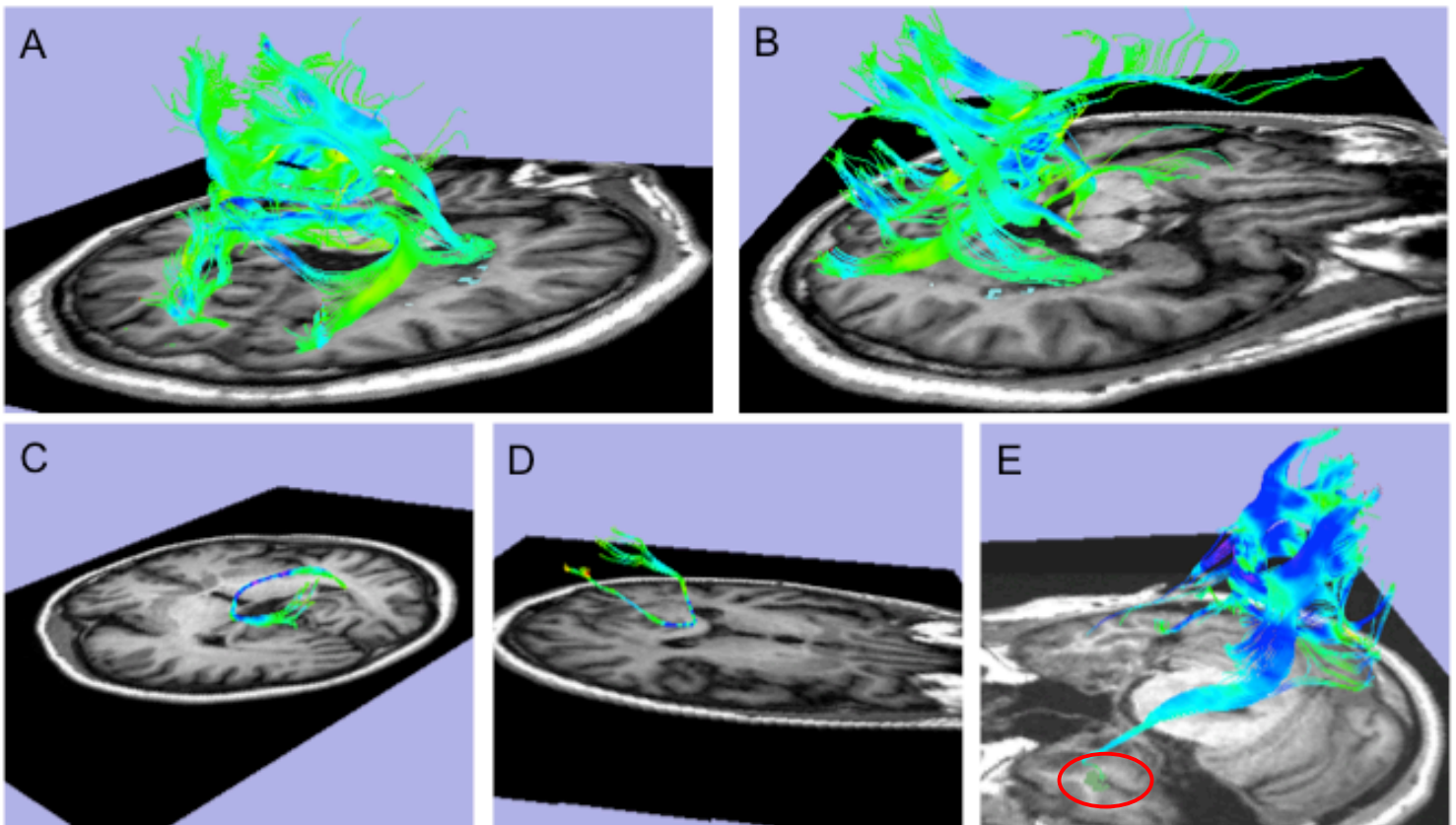
V. Cortical thickness analysis

Regardless of the cortical tract projections from T1 lesions, first we explored the entire cortex to examine cortical thinning related to mentalization performance. The two-step regression analysis between cortical thickness and social cognitive performance controlling for age ($p<0.001$), and confounding factors followed by a Bonferroni correction ($p<0.01$) showed statistically significant relationship between Eyes test performance and cortical thickness of the anterior pole of the left anterior inferior temporal gyrus (BA20), left fusiform gyrus in the fusiform face area (FFA) (BA19), and right caudal middle frontal gyrus (BA8) (Table 8), (Figure 5). The Irony test did not correlate with cortical thickness of any brain area. Correction for multiple comparisons with false discovery rate and Monte Carlo simulation tests did not result significant cortical areas.

Figure 5.

Results from the general linear model analysis (p<0.001, uncorrected) of cortical thickness data from 49 multiple sclerosis patients displayed at each vertex of the inflated standardized brain. Significant regions of interest, which cortical thickness show correlation with the Eyes test performance when effect of age is controlled for are located within the left fusiform gyrus, left anterior inferior temporal gyrus, right caudal middle frontal gyrus, and right precentral gyrus. P-values are presented in the color bar (logarithmic value). The relationship between average cortical thickness of the right precentral region of interest did not remain significant after effect of depression and anxiety was removed in the subsequent statistical analysis.

FFA=fusiform face area, TP=temporal pole, PCG=precentral gyrus, cMFG=caudal middle frontal gyrus
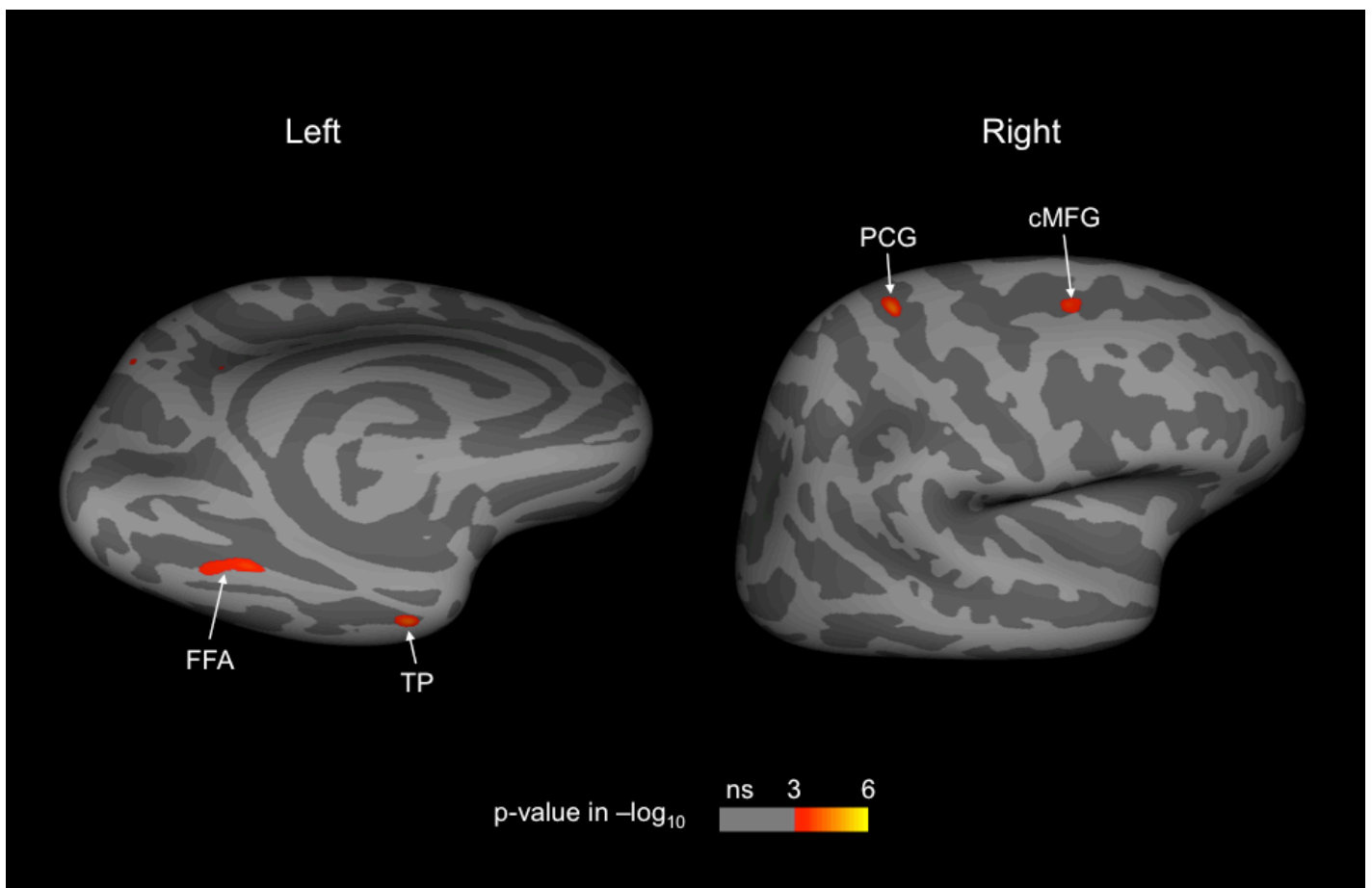
Table 8.

Focal cortical areas, which mean cortical thickness showed significant correlation with the Eyes test performance. General linear models were used to correct for confounding variables (age, anxiety, depression) followed by a Bonferroni correction (Bonferroni corrected p<0.01).

Focal cortical area	Talairach coordinates			Area (mm^2)	p-value	B	R^2
	x	y	z				
Left TP (BA20)	-41	-9	-36	272	<0.001	3.731	0.327
Left FFA (BA19)	-30	-53	-13	465	0.004	3.681	0.234
Right caudal middle frontal gyrus (BA8)	28	11	45	197	0.008	3.517	0.209

TP: temporal pole

FFA: fusiform face area

BA: Brodmann area

VI. Contributions of focal cortical thinnings and regional white matter lesions to social-perceptual performance

Next, we considered whether the observed pathologies in the WM and cortical GM compartments independently contributed to performance in the Eyes test. In a stepwise regression analysis, cortical thicknesses of the left anterior inferior temporal area (St beta=0.391, p=0.001), left FFA (St beta=0.335, p=0.003), and rT1LV of the SCC (St beta=-0.305, p=0.009) independently significantly predicted the Eyes test performance (r^2=0.517, p<0.001).

VII. Relationship between regional lesion load of white matter tracts and cortical thinning of focal areas

Finally, we addressed whether focal cortical thinnings correlating with Eyes test performance were related to regional lesion load of the SCC. Linear regression analysis showed that after correction for age, rT1LV of the SCC significantly predicted cortical

thickness of the left temporal pole area (St beta=-0.316, p=0.003, R^2=0.138), however no correlation was found between lesion load of the SCC and cortical thickness of the left FFA.

Discussion

The present MRI study aimed to investigate the impact of WM lesion burden and cortical atrophy on mentalization ability in MS. In addition, brain pathologies were explored with different MRI methods to identify neural substrates, which if damaged, associates with impaired social cognitive performance in individuals, whose primary brain development was normal. We also aimed to explore the relationship between focal cortical atrophy and regional WM lesion load taking part in the same cognitive function to better understand neurodegenerative processes in MS.

After correction for confounding effect of anxiety and depression, our MS patient cohort performed significantly poorer in verbal and visual mentalization tasks (Irony and Eyes test) compared to healthy subjects. These results are in line with previous reports demonstrating impaired social cognitive abilities in MS (73-77).

After correction for anxiety and depression, no associations were found between total T1 WM lesion load and social cognitive performance. This finding converges with previous studies reporting no or modest correlations between cognitive performance and total WM lesion volume (57, 135). However, assessment of the total lesion load may mask identification of strategically important WM pathways, which if damaged, impairs mentalization abilities. Therefore, to investigate associations between damage of individual inter-connecting WM bundles and mentalization performance, we measured WM lesion volumes in each major fiber bundles anatomically identified by a WM fiber tract atlas (124) (Figure 3). After correction for depression and anxiety, and adjusting for multiple comparisons, rT1LV of the SCC did correlate with the Eyes test performance. Importance of the corpus callosum in cognitive function of MS patients was recently reported in quantitative MRI studies (136-138). Next, using DTI fiber tractography we traced the tracts from the SCC affected by macroscopically visible lesions to explore their cortical projections (Figure 4). DTI tractography is a novel MRI method that provides 3D reconstructions of WM tracts by measuring anisotropic diffusion of water molecules

along the fiber tracts. The major eigenvector of the diffusion tensor ellipsoid denotes the predominant orientation of the fibers in a given voxel. Connection of the vectors results in the fiber streams (139, 140). Our tractography analysis showed projections from the lesions of the SCC to the occipital (in 73% of the patients), parietal (73%) and temporal lobes (61%) in both hemispheres corresponding to the anatomical fiber tract endings in healthy subjects (141). Less projections to the temporal lobe presumably reflect errors associated with fiber tracking. In the temporal lobe callosal fibers cross other various directional fibers (e.g. internal capsule, inferior longitudinal fascicules), and such fiber crossing may reduce the reliability of fiber tracking (141). Callosal fibers connect homologous cortical areas between the hemispheres. The SCC is primarily involved in integration of visual information. Our results suggest that impaired inter-hemispheric integration of visual processing due to damage of the SCC associates with poorer facial emotion recognition performance of MS patients. This finding points out the importance of WM integrity on cognitive processes, and suggests that cognitive function may be deteriorated by disconnection of communicating cortical areas involved in neural networks. Our results are supported by previous studies demonstarting that in patients with complete callosotomy higher order visual functions became impaired (142). Individuals with agenesia of corpus callosum scored significantly lower than controls in emotion recognition face test (143).

Previous MRI studies have convergently demonstarted neocortical volume loss in MS showing strong correlation with cognitive decline (57, 135). To investigate the relationship between mentalization and cortical pathology we measured cortical thickness throughout the whole brain to quantify cortical atrophy as thinning of the cortex. For the assessment FreeSurfer software was applied, which is an automated method for reconstruction and measure of cortical thickness within submillimeter preciosity. After correcting for confounding effects of age on cortical thickness, and depression, anxiety on mentalization significant correlations were found between the Eyes test performance and cortical thickness of focal areas in the right premotor cortex (caudal middle frontal gyrus, BA8), left anterior inferior temporal gyrus, close to the temporal pole (BA20), and in the fusiform face area (FFA) of the fusifrom gyrus (BA19) (Table 8), (Figure 5). The identified cortical areas were also reported in previous studies documenting a widely

distributed brain network engaged in neural processes of facial emotion recognition (82, 83, 144). Simulation theory describes a presumed physiologic mechanism participating in the understanding of observed motor actions, motor learning, and mental state decoding as well as empathizing in social-perceptual processes (85, 145). Firing of mirror neurons in the brain of the observer simulates neuronal activation pattern required to execute the observed movement enabling the understanding of the underlying goal (85, 145). Mirror neurons were identified in prefrontal and premotor areas, and in the parietal and temporal lobe (85, 145). In addition, recent studies found evidence on simulation of observed sensations and emotions through mirror neuron activation in the primary somatosensory cortex, anterior insula, and amygdala contributing to empathic understanding (85, 145). Previous fMRI and lesion studies demonstrated that viewing emotional facial expressions associates with premotor cortex activation presuming a mirror neuron mechanism (145). Inner simulation of the observed facial muscle group contractions is linked to a matching affective state based on personal experiences. Main function of the premotor BA8 area is the regulation of voluntary and involuntary eye movements (146). Majority of the photographs in the Eyes test depict averted eye gaze. We propose that correlation between Eyes test performance and cortical thinning of the premotor eye region reflects simulation of averted eye gazes through activation of somatotopic-organized mirror neurons. This result is in line with the report of Buccino et al. (147) demonstrating somatotopic-organized activation of premotor and parietal areas when viewing mouth, hand, and foot motor actions.

Eyes test performance significantly correlated with cortical thickness of a left inferior temporal area, close to the temporal pole. The temporal pole is involved in high-level integration of social and emotional signals, and in retrieving autobiographical memory and social scripts (83). During mentalization temporal pole integrates the general knowledge about the world with actual new situations to understand mental states of others.

Not surprising is our finding of correlation between FFA and Eyes test performance. Fusiform gyrus is crucial in visual processing of faces, and participates in emotional perception (144).

Stepwise linear regression analysis showed that Eyes test performance was independently predicted from rT1LV of the SCC, and cortical thickness of the left temporal pole and left FFA. This result demonstrates that social-perceptual function is compromised by both a disconnection mechanism caused by WM tract disruptions and cortical damage measured as cortical thinning. Similar mechanisms are presumed in mentalization deficits of patients with autism, where both decreased cortical thickness of areas constituting neural networks dedicated to mentalization, and functional as well as structural underconnectivity of elements of the network have been demonstrated (85).

The exact pathological mechanisms that underlie cortical atrophy measurable in early disease stage, and accelerating over time are not yet clearly understood (135). Secondary Wallerian (anterograd/trans-synaptic) and retrograde neurodegeneration resulted from axonal injury in destructive WM lesions, cortical lesion formation, and a primary neurodegenerative process independent from focal demyelination are presumed to play a part in cortical atrophy (13, 57). To explore the contribution of secondary neurodegeneration to cortical thinning of areas related to mentalization performance, we analyzed the relationship between regional lesion load of the individual WM tracts and cortical thickness of focal areas lying in their projection field, both correlating with the same mental function. This approach quantifying WM lesion load along interconnecting WM pathways provides a specific method to make implications about cortical atrophy development related to anatomically linked WM lesions. Our results suggest that the contribution of WM lesions to cortical atrophy is modest, and processes independent from axonal damage in the WM prevail in the pathogenesis of GM degeneration related to mentalization. Only a very few study investigated relationship between regional lesion load and regional cortical atrophy; a single study addressed cognitive domains. In a recent study, spatial proximity or functional linkage was found between WM lesion and cortical atrophy locations assuming secondary development of GM atrophy (148). However, a longitudinal study found no spatial relationship between areas of significant GM volume reduction and anatomically closely adjacent WM lesion volume increase (149). Similarly, a recent cross-sectional study argues against spatial association between regional GM volume loss and corresponding regional WM lesion occurrence (150). Another MRI study provided evidence for GM atrophy secondary to retrograde axonal

neurodegeneration, however, the importance of other factors contributing to GM volume decrease were emphasized (13).

Our study is not without limitations. After applying conservative statistical approaches to correct for multiple comparisons (false discovery rate, cluster based Monte Carlo simulation) at the initial exploratory whole-cortex correlation analyses no cortical area remained significant. However, the statistical threshold set at a conservative level (p<0.001) as well as the anatomical location and physiological function of cortical areas found to impact Eyes test performance and reasonably compatible with recent knowledge about mentalization, validate our results. Although performance in Irony test was also impaired in MS compared to healthy subjects similar to Eyes test, no significant correlations were found with MRI parameters. It may relate to task difficulty and psychometric properties of Eyes test sensitively detecting a wider range of mentalization performance in MS compared to Irony test thereby showing better correlation with structural brain changes assessed by our MRI methods. Finally, mentalization performance of MS patients might also be affected by the damage of deep GM structures, as well as microscopic injuries in the normal-appearing WM, which were not assessed in our study. Future MRI studies focusing on these pathologies may further extend the knowledge about mentaliziation processes in MS.

In summary, in our study cortical thickness and regional WM lesion loads were assessed with an exploratory approach analyzing the whole cortex on a vertex-to-vertex basis and lesion content of each major fiber bundles without a priori hypotheses about possible anatomic substrates relating to social cognitive performance. Regional WM lesion loads were assessed in each interconnecting fiber bundles, which assured a highly specific strategy to explore associations between regionally damaged WM tracts and social cognitive performance. Our results suggest that MS brain pathological changes significantly impact the mentalization ability. Cognitive performance may equally be compromised by a disconnection mechanism related to WM lesions and direct damage of cortical hubs constituting neural networks processing high-level mental information. In particular, we propose that mind reading ability from facial expressions is dependent on inter-hemispheric visual integration mediated by the SCC, social, emotional, and visual signal processing of temporal cortical areas, and somatotopic-organized mirror neurons

located in the premotor eye region. Finally, our approach specifically investigated the relationship between axonal injury in the WM lesions and atrophy development in the corresponding cortical projection field. Our results suggest that although axonal degeneration in WM lesions associated with declined mentalization mildly relate to focal cortical atrophy correlating with poor mentalization, the dominant pathologic processes of cortical neurodegeneration are independent from WM demyelination.

VII. SUMMARY OF THE THESES

Identification and clinical impact of multiple sclerosis cortical lesions as assessed by a routine 3 Tesla MRI

1. Using a clinically applicable 3 Tesla MRI protocol combining 3D FLAIR and IR-SPGR sequences, the detected CL load was comparable with the bulk of CLs assessed by specialized MRI methods developed for sensitive CL delineation.

2. The multiplanar display of our isotropic resolution IR-SPGR images allowed precise anatomic lesion classification in relation to the GM-WM junction and cortical folding patterns.

3. CLs were detectable in different progression stage of MS including early RRMS.

4. Our CL measures reflecting nearly entirely the cortical-subcortical lesion type (Type I) showed moderate correlations with WMLV, suggesting that the underlying pathogenic processes might be related.

5. CL load showed significant correlations with MS physical disability. We did not found this correlation with the WM lesion load, however a trend was apparent.

6. After controlling for age, depression, and pre-morbid intelligence, CLN, CLV, and WMLV independently predicted the performance of information processing speed and working memory performance. In addition, CLN also predicted verbal learning and memory performances.

Social cognition and theory of mind in patients with relapsing-remitting multiple sclerosis

7. Compared to healthy control subjects, mind reading ability from facial expressions is impaired in MS unrelated to the confounding effects of age, gender, IQ, PQ, VQ, and affective co-morbidities (depression, anxiety).

8. More advanced physical disability and faster progression rate of the disease predicted more extended mentalization deficit affecting both the social-perceptual and the complex perspective taking abilities.

9. MS patients with shorter disease duration, and with faster disease progression rate reported higher level of empathic ability suggesting complex mechanisms contributing to empathic understanding of patients with chronic disease.

Neuroanatomical substrates and connectivity of mentalization: a multi-modal MRI study in multiple sclerosis

10. MS brain pathological changes significantly impact the mentalization ability. Cognitive performance may equally be compromised by a disconnection mechanism related to WM lesions, and direct damage of cortical hubs constituting neural networks processing high-level mental information.

11. Mind reading ability from face expressions involves inter-hemispheric visual integration mediated by the splenium of corpus callosum, social, emotional, and visual signal processing, as well as memory retrieval in focal cortical areas of the temporal lobe, in addition, simulation by somatotopic-organized mirror neurons located in the premotor eye region.

12. We specifically investigated the relationship between axonal injury in lesions of WM tracts and atrophy development in the corresponding cortical projection field. Our results suggest that although axonal degeneration in WM lesions associated with declined mentalization mildly relate to focal cortical atrophy correlating with poor mentalization, the dominant pathologic processes of cortical neurodegeneration are independent from WM demyelination.

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VIII. ACKNOWLEDGEMENTS

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