

An investigation of motor disabilities in people with multiple sclerosis using advanced magnetic resonance imaging

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An investigation of motor disabilities in people with multiple sclerosis using advanced magnetic resonance imaging

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door

Myrte Strik
geboren te Rijssen

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dr. J.O. Cleary

Abstract

Multiple sclerosis (MS) is an autoimmune disorder of the brain and spinal cord, and the most common cause of neurological disability in young adults. The presentation of MS is highly heterogeneous with an unknown aetiology and no known cure, presenting as inflammation, demyelination and axonal injury/loss. MS pathology is disseminated throughout the central nervous system leading to a broad range of symptoms including cognitive dysfunctions, bowel and bladder problems, fatigue, sensory disturbances and difficulties with walking and balance.

Up to 90% of people with MS experience motor impairments that significantly worsen with increasing disease severity, and which can affect both the upper and lower limbs. Motor impairments are often highly debilitating, ranging from muscle weakness, coordination loss, tremors to spasticity. However, while the pathophysiological mechanisms underpinning motor impairments in MS have been widely studied, they are not currently well understood. This is particularly true for early disease, a time when personalised treatment strategies can be formulated and will have maximal effect, preventing future deterioration and accumulation of disability. Consequently, there is an urgent need to understand the pathophysiology underlying motor impairments in MS and elucidate the microstructural and functional changes that occur at their earliest manifestation.

To this end, we investigated the pathophysiology of motor impairments associated with dexterity and mobility in people with MS using advanced magnetic resonance imaging (MRI). Our investigations included functional resting-state (Chapters 2.1 and 2.2) and task (Chapter 3.1) MRI, diffusion weighted imaging (Chapter 3.2) and ultra-high field MRI (Chapters 3.1 and 3.2). Our findings consistently demonstrated a clear link between the development of motor impairments and alterations in the structure/function of the sensorimotor system, a system responsible for the integration of sensory information with motor processing in order to facilitate and maintain movement. Specifically, studying the sensorimotor system in its entirety using network analyses in a large cohort of people with MS, we identified functional disturbances within the sensorimotor

system of patients with serious disabilities (Chapter 2.1), with disturbances particularly predictive of future progression of upper and lower limb impairments (Chapter 2.2). Further, using high resolution ultra-high field MRI and measures of motor behaviour in a cohort of patients with minimal motor impairments (Chapters 3.1 and 3.2), we similarly found a link between changes in the function and microstructure of the sensorimotor system and the presence of subtle impairments in hand function and walking.

These findings provide evidence for the role of the sensorimotor network in the development of motor impairments. Potentially, the sensorimotor network might be central to the development of motor impairments in MS and represent a useful target for the development of imaging biomarkers for use in treatment development as well as understanding and monitoring the evolution of motor impairments. From this and subsequent work, it is hoped that this knowledge will lead to more effective treatments and management of patients, alleviating the burden of these impairments.

Authorship Declaration

I, **Myrte Strik**, certify that:

- i. This thesis is my own work based on original research.
- ii. Due acknowledgements have been made in the text to all other material used.
- iii. This thesis is fewer than 100,000 words in length, exclusive of tables, figures, Bibliographies *and appendices*

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Myrte Strik

Preface

The core theme of this thesis is the investigation of motor disabilities in people with multiple sclerosis (MS); see Table 1 for a summary of the experimental chapters comprised within this thesis. The ideas, methodologies and write up of all experimental chapters were the principal responsibility of myself, the candidate, working within the Departments of Medicine and Radiology, Melbourne, Australia and Anatomy and Neurosciences, Amsterdam, the Netherlands under the supervision of Dr Scott Kolbe (Senior Research Fellow), Dr Menno Schoonheim (Assistant Professor), Prof Jeroen Geurts, Dr Jon Cleary and Dr Leigh Johnston (Associate Professor). The following discussion provides a breakdown of each experimental chapters, specifically pertaining to the arrangements surrounding collection/analysis of data, and contribution by specific collaborators, institutions and funding bodies.

Table 1. Publication status and candidate's contribution to experimental chapters.

Thesis chapter	Publication title	Publication status	Nature and extent of candidate's contribution
2.1	Increased functional sensorimotor network efficiency relates to disability in multiple sclerosis	Published by <i>Multiple Sclerosis Journal</i> on 26-10-2020	Project design, data analyses and interpretation. Primary person involved in the conceptualisation and preparation of the manuscript
2.2	Sensorimotor network dynamics predicts loss of upper and lower limb function in people with multiple sclerosis	Submitted for publication to <i>NeuroImage: Clinical</i> on 09-07-2020	Project design, data analyses and interpretation. Primary person involved in the conceptualisation and preparation of the manuscript
3.1	Functional correlates of motor control impairments in multiple sclerosis: A 7 Tesla task functional MRI study	Published by <i>Human Brain Mapping</i> on 05-03-2021	Project design, participant recruitment, data collection, data analyses and interpretation. Primary person involved in the conceptualisation and preparation of the manuscript
3.2	Axonal loss in major sensorimotor tracts is associated with impaired motor performance in minimally disabled multiple sclerosis patients	Published by <i>Brain Communications</i> on 16-03-2021	Project design, participant recruitment, data collection, data analyses and interpretation. Primary person involved in the conceptualisation and preparation of the manuscript

Dr Scott Kolbe and Dr Menno Schoonheim provided intellectual support during all stages of this thesis; including, experimental designs, data analyses and reviewing/editing of this thesis. In addition, Prof Jeroen Geurts, Dr Jon Cleary and Prof Leigh Johnston contributed to the development and refinement of the ideas and conclusions by providing valuable comments and support during the completion of each experiment. Work contributed by others is duly acknowledged below.

Participants included in Chapter 2.1 and 2.2 were derived from the Amsterdam MS cohort, with the conduct of the studies supported by the Dutch MS Research Foundation [grant number 14-358e and 13-820] and the Melbourne University (Melbourne Research Scholarship). While participant recruitment and data collection were completed before the commencement of this PhD, all analyses, interpretation and publications derived were completed during candidature. Work contribution by others is acknowledged as follows;

- Dr Scott Kolbe and Dr Menno Schoonheim contributed to all stages of the manuscripts described in Chapters 2.1 and 2.2 including experimental designs, data analyses, interpretation of data and writing of the manuscripts.
- Dr Kim Meijer and Dr Anand Eijlers assisted in retrospective analyses of data and contributed to writing of the manuscript in Chapter 2.1.
- Dr Anand Eijlers, Dr Linda Douw and Tommy Broeders assisted in data analyses and co-authored in Chapter 2.2.
- Dr Iris Dekker, Dr Anand Eijlers, Dr Menno Schoonheim recruited participants and collected data for both studies described in Chapters 2.1 and 2.2.
- Dr Declan Chard assisted with the experimental design and provided valuable advice regarding interpretation and writing of the experimental Chapter 2.1.
- Dr Matteo Pardini, Prof Bernard Uitdehaag contributed to the interpretation and writing of the manuscript in Chapter 2.1 and Prof Joep Killestein of the manuscript described in Chapter 2.2.

Data for experimental Chapters 3.1 and 3.2 were derived from the ‘Biomarkers of motor performance in multiple sclerosis’ study, with funding provided by the University of Melbourne; a) Melbourne Neuroscience Institute Interdisciplinary Seed Grant to Dr Scott Kolbe and b) Melbourne International Research Scholarship to the candidate, Myrte Strik. All participant recruitment and data collection was done during candidature. Work contributed is acknowledged as follows;

- Dr Scott Kolbe and Dr Menno Schoonheim contributed to all stages including project design, analyses of data, interpretation and writing of the manuscripts described in Chapters 3.1 and 3.2.
- Dr Camille Shanahan was involved in development of the recruitment protocol and documentation of data, assisted in participant recruitment and data collection and co-authored in Chapters 3.1 and 3.2.
- Dr Jon Cleary and Rebecca Glarin assisted in the development of the 7T MRI protocol used in the studies described in Chapters 3.1 and 3.2.
- Rebecca Glarin assisted with the 7T MRI experiments and scanned all participants included in the studies described in Chapters 3.1 and 3.2.
- Dr Frederique Boonstra assisted with analyses of data and co-authored in Chapters 3.1 and 3.2.
- Prof Kilpatrick and A/Prof Anneke van der Walt screened potential study participants at the Royal Melbourne Hospital MS Clinic and provided helpful advice regarding interpretation and writing of the studies described in Chapters 3.1 and 3.2.
- Dr Eduardo Cofré Lizama analysed and co-wrote paragraphs in Chapter 3.2 pertaining to biomechanical measurements of gait and balance and provided with valuable advice on the interpretation and writing.
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Publications, presentations and awards

Published articles

Axonal loss in major sensorimotor tracts is associated with impaired motor performance in minimally disabled multiple sclerosis patients

M. Strik, L.E. Cofré Lizama, C.J. Shanahan, A. Van der Walt, F.M.C. Boonstra, R. Glarin, T.J. Kilpatrick, J.J.G. Geurts, J.O. Cleary, M.M. Schoonheim, M.P. Galea, S.C. Kolbe. *Brain Communications*, 2021.

Functional correlates of motor control impairments in multiple sclerosis: A 7 Tesla task functional MRI study

M. Strik, C.J. Shanahan, A. Van der Walt, F.M.C. Boonstra, R. Glarin, M.P. Galea, T.J. Kilpatrick, J.J.G. Geurts, J.O. Cleary, M.M. Schoonheim, S.C. Kolbe. *Human Brain Mapping*, 2021.

Increased functional sensorimotor network efficiency relates to disability in multiple sclerosis.

M. Strik, D.T. Chard, I. Dekker, M. Pardini, K.A. Meijer, A.J.C. Eijlers, B.M.J. Uitdehaag, S.C. Kolbe, J.J.G. Geurts, M.M. Schoonheim. *Multiple Sclerosis Journal*, 2020.

Technologies for advanced gait and balance assessments in people with multiple sclerosis.

C.J. Shanahan, F.M.C. Boonstra, L.E. Cofré Lizama, M. Strik, B.A. Moffat, F. Khan, T.J. Kilpatrick, A. van der Walt, M.P. Galea, S.C. Kolbe. *Frontiers in Neurology*, 2018.

Thalamus structure and function determine severity of cognitive impairment in multiple sclerosis.

M.M. Schoonheim, H.E. Hulst, R.B. Brandt, **M. Strik**, A.M. Wink, B.M.J. Uitdehaag, F. Barkhof, J.J.G. Geurts. *Neurology*, 2015.

Breinmeesters in sp : jonge neurowetenschappers over hun onderzoek naar MS.

B. Baan, R.B. Brandt, Q. van Geest, H.E. Hulst, S. Kiljan, S. Rademaker, G.J. Schenk, M.M. Schoonheim, **M. Strik**. Editors M. Zuidhof, G.J. Schenk, J.J.G. Geurts, 2015
A collection of columns from students who researched the development of MS and cognitive impairments in MS.

Journal articles under review

Sensorimotor network dynamics predicts loss of upper and lower limb function in people with multiple sclerosis.

M. Strik, A.J.C. Eijlers, I. Dekker, T.A.A. Broeders, L. Douw, J. Killestein, S.C. Kolbe, J.J.G. Geurts, M.M. Schoonheim. *NeuroImage: Clinical*, 2020.

Regional cerebellar atrophy related to disability and cognitive progression in multiple sclerosis.

Dekker, A. Ruet, **M. Strik**, A.J.C. Eijlers, H.E. Hulst, M.P. Wattjes, F. Barkhof, B.M.J. Uitdehaag, J. Killestein, J.J.G. Geurts, M.M. Schoonheim. *Multiple Sclerosis Journal*, 2020.

Evaluation of cerebellar function scores in relation to cerebellar axonal loss in multiple sclerosis.

F.M.C. Boonstra, S.I. Gajamange, G. Noffs, T. Perera, **M. Strik**, A.P. Vogel, H. Butzkueven, A. Evans, A. Van der Walt, S.C. Kolbe. *Scientific Reports*, 2020.

Invited presentations

Australian Ultra High Field Network Virtual Workshop, online, 2020 – “Axonal loss and motor performance in multiple sclerosis: a fixel-based diffusion MRI analysis at 7T”

International Women in Multiple Sclerosis Neuroimaging Interest Group Webinar, online, 2020 – *“Investigation of upper and lower limb disability progression in multiple sclerosis with longitudinal functional network dynamics”*.

Siemens Victorian Chapter User Group meeting, Melbourne, Australia, 2019 – *“The corticospinal tract in relapsing-remitting multiple sclerosis: a preliminary tractography and fixel-based MRI analysis at ultra-high field”*.

Conference oral presentation

MS Research Australia Progress in MS Research Conference, Melbourne, Australia, 2019 – *“Predicting upper limb disability progression in multiple sclerosis with functional network analysis”*.

Conference poster presentations

High resolution functional mapping of upper and lower limb sensorimotor function in minimally disabled people with multiple sclerosis using 7T MRI.

M. Strik, C.J. Shanahan, A. Van der Walt, F.M.C. Boonstra, R. Glarin, M.P. Galea, T.J. Kilpatrick, J.J.G. Geurts, J.O. Cleary, M.M. Schoonheim, S.C. Kolbe. *Joint ECTRIMS-ACRIMS meeting, online, 2020*.

Sensorimotor network dynamics predicts loss of upper and lower limb function in people with multiple sclerosis.

M. Strik, A.J.C. Eijlers., I. Dekker, T.A.A. Broeders, L. Douw, J. Killestein, S.C. Kolbe, J.J.G. Geurts, M.M. Schoonheim. *Joint ECTRIMS-ACRIMS meeting, online, 2020*.

Clinical monitoring of axonal loss in multiple sclerosis using advanced diffusion MRI. S.C. Kolbe, M. Clough, F.M.C. Boonstra, **M. Strik**, A. Van der Walt, H. Butzkueven, O.B. White, J. Fielding, M. Law. *ISMRM, Sydney, Australia, 2020*.

Predicting upper limb disability progression in multiple sclerosis with functional network analysis.

M. Strik, A.J.C Eijlers, I. Dekker, K.A. Meijer, J. Killestein, B.M.J. Uitdehaag, S.C. Kolbe, J.J.G. Geurts, M.M. Schoonheim. *OHBM, Rome, Italy, 2019; OHBM Australian Chapter, New Castle, Australia, 2019.*

Increased functional efficiency of the sensorimotor network is associated with disability in multiple sclerosis.

M. Strik, D.T. Chard, I. Dekker, M. Pardini, K.A. Meijer, A.J.C. Eijlers, B.M.J. Uitdehaag, S.C. Kolbe, J.J.G. Geurts, M.M. Schoonheim. *ECTRIMS, Berlin, Germany, 2018.*

The corticospinal tract in relapsing-remitting multiple sclerosis: a pilot tractography and fixel-based MRI analysis at ultra-high field.

M. Strik, C.J. Shanahan, S. Telianidis, A. Van der Walt, R. Glarin, R.J. Ordidge, B.A. Moffat, F. Khan, A. Bastani Jahromi, L.E. Cofré Lizama, M.P. Galea, T.J. Kilpatrick, J.O. Cleary, S.C. Kolbe. *Ultra-high field magnetic resonance symposium, Berlin, Germany, 2017; Joint ECTRIMS-ACRIMS meeting, Paris, France, 2017; ISMRM, Paris, France, 2018.*

Public display

Diffusion images displayed at Mind Gallery Exhibition located at the Melbourne Museum, Melbourne, Australia, 2018-2020

Awards and scholarships

- Best Young Investigator Poster Presentation award, 8th Joint ACTRIMS-ECTRIMS Meeting September 11-13, 2020
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- OHBM Australian chapter bursary, New Castle, Australia, 2019

Publications, presentations and awards

- White matter study group trainee competition award, ISMRM, Paris, France, 2018
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Abbreviations

9-HPT	9-hole peg test
3D	three dimensional
3DT1	three dimensional T1-weighted imaging
ADC	analogue to digital converter
ANOVA	analysis of variance
ANTs	advanced normalisation tools
AP	anterior-to-posterior
AROMA	automatic removal of motion artifacts
As Sens C	associative parietal sensory cortex
B	baseline
B	predictor specific b-value
BA	Brodmann area
BBR	boundary-based registration
BNC	bayonet neill-concelman
BOLD	blood-oxygen-level-dependent
BP	brain parenchymal
Cau	caudate nucleus
CGMV	cortical grey matter volume
CIS	clinically isolated syndrome
CoV	coefficient of variations
CSA	cross-sectional area
CSF	cerebrospinal fluid
CST	corticospinal tract
CTT	cerebello-thalamic tract
DGM	deep grey matter
DGMV	deep grey matter volume
DIR	double inversion recovery
DTI	diffusion tensor imaging
DWI	diffusion weighted imaging
EDSS	expanded disability status scale
EPI	echo planar imaging
F	female
FA	flip angle
FC	fibre bundle cross-section
FC	functional connectivity
FD	fibre bundle density
FDC	fibre bundle density and cross-section
FDR	false discovery rate
FEAT	model-based fMRI expert analysis tool
FIRST	linear registration tool

FLAIR	fluid-attenuated inversion recovery
fMRI	functional magnetic resonance imaging
FMRIB	functional magnetic resonance imaging of the brain
FNIRT	non-linear registration tool
FOD	fibre orientation distribution
FSL	FMRIB software library
FSS	functional system scores
FU	follow-up
FWE	family-wise error
GE	global efficiency
GLM	general linear model
GM	grey matter
GRAPPA	generalized autocalibrating partial parallel acquisition
HC	healthy controls
HD	high disability
ICV	intracranial volume
IHST	interhemispheric primary sensorimotor tracts
IQR	interquartile range
L	left
LCD	liquid-crystal display
LD	low disability
LE	local efficiency
LEAP	lesion automated pre-processing
LST	lesion segmentation tool
M	male
M1	primary motor cortex
M2	secondary motor area
MATLAB	computing environment and programming language
MB	multiband factor
MELODIC	model-free fMRI analysis tool using independent component analysis
MP2RAGE	magnetisation prepared 2 rapid acquisition gradient echoes
MRI	magnetic resonance imaging
MS	multiple sclerosis
MSMT-CSD	multi-shell multi-tissue constrained spherical deconvolution
MT	middle temporal visual area
MVC	maximum voluntary contraction
N	newton
NBV	normalized brain volume;
NCGMV	normalized cortical grey matter volume;
NDGMV	normalized deep grey matter volume
NP	non-progressing patients
NWMV	normalized white matter volume
P	progressing patients

Abbreviations

PAL	pallidum
PAS	posterior associative sensory cortex
PC	personal computer
PE	phase encoding direction
PF	prefrontal cortex
PFC	prefrontal cortex
PM	premotor cortex
Post assoc	posterior associative sensory cortex
PPMS	primary progressive multiple sclerosis
Premot	premotor cortex
Put	putamen
pwMS	people with MS
R	right
ROI	region of interest
RRMS	relapsing-remitting multiple sclerosis
S-M1	primary sensory motor cortex
S.E.	standard error
S1	primary somatosensory cortex
S2	secondary sensory cortex
SIENAX	structural brain analysis toolbox
SMA	supplementary motor area
SMN	sensorimotor network
SNR	signal-to-noise
SPM	statistical parametric mapping
SPMS	secondary progressive multiple sclerosis
SPSS	statistical package for the social sciences
SUSAN	noise filtering algorithm
SWI	susceptibility weighted imaging
T1	a rate constant relation to the longitudinal recovery of magnetisation
T2	a rate constant relation to the transverse recovery of magnetisation
T25FW	timed-25 foot walk
TE	echo time
Tha	thalamus
TI	inversion time
TR	repetition time
USB	universal serial bus
WM	white matter
WMV	white matter volume

Chapter 1

General introduction

Epidemiology and disease course of multiple sclerosis

Multiple sclerosis (MS) is a progressive autoimmune disorder of the central nervous system (CNS), and is the most common cause of neurological disability in young and middle-aged adults, ranging between 20 and 40 years old. The pathology of MS is caused by an unknown aetiology and is characterized by inflammation, demyelination and axonal injury and loss within the brain and spinal cord. The disease affects more women than men with ratios as high as 3:1, and has a huge impact on participation and quality of life. More than 2.5 million people world-wide suffer from MS, which makes it a major public health concern.¹ While MS can occur across the globe, prevalence varies with ethnicity and location plays a role. Particularly people in the northern parts of Europe, North America and Australia appear to be highly susceptible to develop MS.^{2,3}

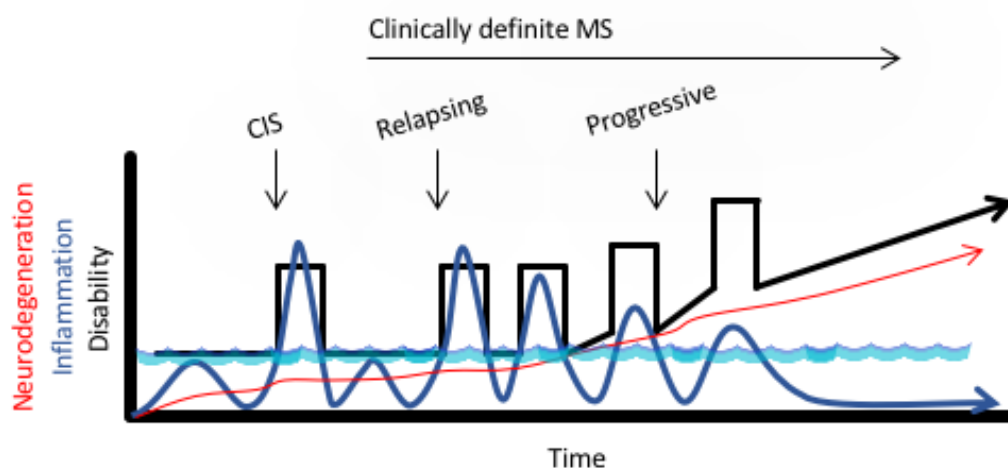


Figure 1. Example of multiple sclerosis disease course. A clinical presentation suggestive of multiple sclerosis is called a clinically isolated syndrome (CIS). Most people get diagnosed with relapsing-remitting multiple sclerosis, which is predominantly driven by relapses and characterized by high clinical and subclinical inflammatory disease activity. The relapsing-remitting disease course can transit into a secondary progressive stage, characterized by increase in neurodegeneration and worsening of disabilities.

Most people with MS (pwMS) have a biphasic disease course, starting with periods where neurological symptoms flare up (i.e. a relapse) and then mostly disappear (i.e. a remission), so called relapsing-remitting MS (RRMS) (Figure 1). Remission following an acute relapse often occurs within weeks to months and most pwMS experience complete

recovery. However recovery is incomplete in a minority of pwMS and becomes less over time leading to permanent disability. In approximately 65% of RRMS patients the disease evolves into a secondary more progressive stage characterized by irreversible, continuous neurological decline with minimal relapses and remissions (SPMS).¹ Conversion to SPMS happens after roughly 10 years in 50% of pwMS,⁴ with older age and motor symptoms at RRMS onset associated with higher risk of conversion.⁵ In a minority of pwMS, approximately 15%, MS is characterized by slow unremitting progressive deterioration from onset and irreversible disability, known as primary progressive MS (PPMS) (Figure 1).^{1,4} Despite the distinction between relapsing-remitting and progressive stages, the clinical course of MS is highly variable and largely unpredictable.

Pathology of multiple sclerosis

MS is typically characterized by damage to the myelin in the white matter (WM) and grey matter (GM), also known as “lesions” or “scars”. Myelin is a fatty sheath around axons, also known as nerve fibers, and is created by oligodendrocytes. These cells wrap myelinated sheaths around several axons with gaps in between, known as the nodes of Ranvier, which improves signal conduction in the brain. MS not only alters axonal wiring in brain and spinal cord initially by demyelination of axons, but also involves axonal injury and loss. Subsequent to axonal injury, Wallerian (distal) and retrograde (proximal) degeneration can occur, eventually leading to neuronal death in cortical⁶ and deep GM structures⁷ and disconnections within and between brain networks. Whereas both WM and GM pathology can involve focal demyelinating lesions as well as neurodegeneration, GM differs from WM pathology in that immune cell infiltration is limited and absence of blood-brain barrier breakdown,^{8,9} two typical pathological characteristics of MS.

Over the last decades extensive and comprehensive research has been done studying the aetiology of MS, but unfortunately the cause remains unknown. Suggested potential risk factors include environmental elements such as vitamin D deficiency, Epstein-Barr viral infection, smoking² and obesity in early life.¹⁰ Even though MS is not considered as an inherited disease, recent genome wide association studies have discovered a possible genetic component. More than 200 risk loci have been implicated to contribute to MS

pathogenesis.¹¹ While presumably the interplay between environmental and genetic factors play a role in the development of MS, most research evidently points towards dysregulation of the immune system.

Two major immune system hypotheses have been proposed, the “outside-in” and “inside-out” theory. With the “outside-in” theory MS is seen as a classical autoimmune disease where a primary trigger within the immune system leads to a malfunctioning system and an immune response is directed against brain and spinal cord leading to inflammatory and demyelinated lesions.^{1,12} However, one single specific event leading to pathology within the CNS has not been identified.¹³ Alternatively, the inside-out theory suggests that neuroaxonal or oligodendrocyte disturbances in the brain could be initial triggers that lead to immune response and focal areas of inflammatory lesions as secondary response.^{14,15}

MRI as a clinical tool for diagnosis and identifying multiple sclerosis pathology

Magnetic Resonance Imaging (MRI) is a powerful tool in human neuroscience and has contributed significantly in our understanding of MS. In clinic, conventional MRI plays an imperative role in diagnosis and monitoring disease progression. The focal inflammatory demyelinating lesions in the WM, the classical pathological hallmark of MS, can be visualized using multiple MR sequences including a fluid-attenuated inversion recovery (FLAIR) scan, T1-weighted and T2-weighted imaging (Figure 2). The diagnostic criteria involves dissemination of lesions in space and time on MRI, in addition to clinical observation and laboratory assessment of oligoclonal bands in the cerebrospinal fluid.^{16,17} A T1-weighted image with gadolinium enhancing contrast can be used to differentiate between active lesions (enhancing) and chronic lesions (non-enhancing) (Figure 2). Whereas enhancing lesions are an indication of ongoing inflammatory processes,¹⁸ hypointense lesions are suggestive of axonal destruction and irreversible damage, also known as black holes.¹⁹ The presence of both T1-weighted enhancing and non-enhancing lesions suggests that onset of lesions occurred at different times, which fulfils the dissemination in time criteria. To meet the dissemination of lesions in space criteria, one or more lesions must be present in at least

two areas considered typical for the disease (periventricular, juxtacortical, infratentorial and spinal cord) (Figure 2).

While visualizing focal WM lesions is essential in clinic for diagnosis, the relation between lesions and disability and cognitive dysfunction is relatively poor.^{20,21} The dissociation between volume and number of lesions and clinical performance is known as the clinico-radiological paradox.²² In other words, pwMS with different lesion loads can have similar symptomatologic presentations or the other way around. The paradox was a driver to not only focus on lesion pathology but to investigate other pathological processes including axonal injury/loss and GM pathology.

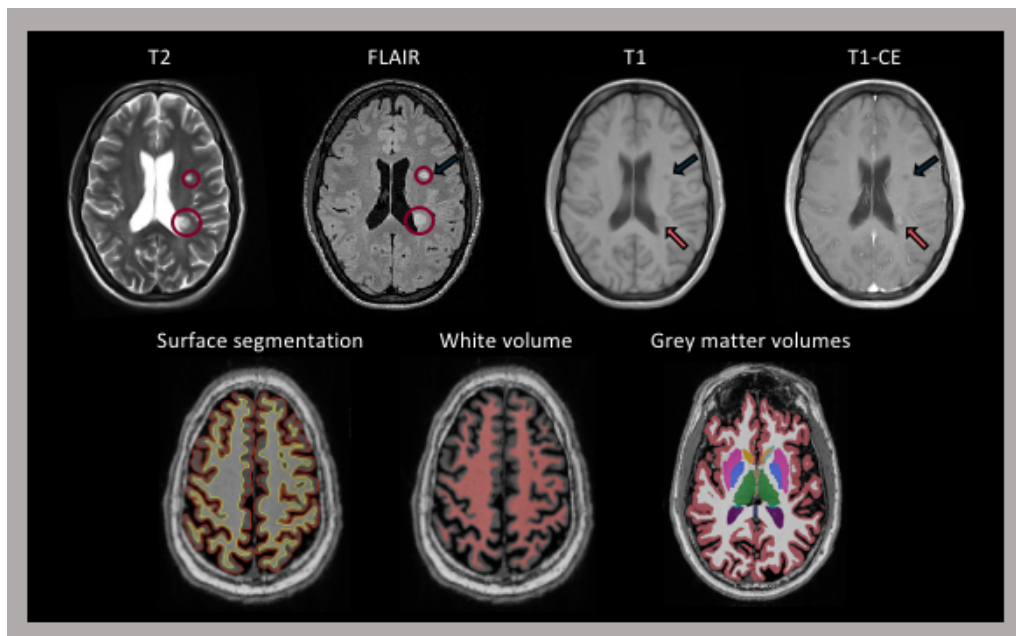


Figure 2. Current radiological practice and brain volumetrics. Four sequences that are often used in clinic for diagnosis and disease progression can include a T2-weighted image, fluid-attenuated inversion recovery (FLAIR) scan and T1-weighted scan without and with contrast enhancing gadolinium (T1-CE). White matter lesions are clearly visible on a T2-weighted MRI and FLAIR scan as bright white spots in contrast to the darker surroundings suggestive of normal appearing WM (red circles). Contrast-enhanced imaging is a sensitive method to visualize blood brain barrier breakdown and enhancing active lesions, i.e. lesions in the inflammatory phase (pink arrows). A black hole appears hypointense on T1-weighted scan and hyperintense in the corresponding T2-weighted image (dark green arrows). Brain volumetrics can be measured using 3D T1-weighted image and segmentation methods such as FreeSurfer (<https://surfer.nmr.mgh.harvard.edu/>) and can include white matter, (sub) cortical grey matter and ventricular volume.

Apart from WM and GM inflammatory lesions, a very prominent and important pathological component of MS is neurodegeneration. A marker of neurodegeneration is brain atrophy, which can be measured using 3D-T1 weighted imaging (Figure 2). Brain atrophy can be quite extensive with a yearly rate of 0.5-1.35% and proceeds substantially faster compared to normal aging.^{23,24} Whereas GM atrophy appears to predominate over WM atrophy and progressively accelerates after conversion to SPMS, the rate of change in WM atrophy remains constant over time,²⁵ suggesting partially independent pathological processes. A reduction in WM volume may at least partly reflect lesion volume reduction due to axonal degeneration.^{13,26} The histopathological substrate of GM atrophy could be neuroaxonal cell loss due to either retrograde degeneration as consequence of WM axonal transection or due to pathological processes within GM.^{26,27} The predominant pathological substrates of MRI-measured GM atrophy was suggested to involve neuronal and axonal pathology including reduced neuronal size, neuronal and axonal density.²⁸

Whereas the number and volume of lesions explain variability in disability only to a small extent,^{21,22} stronger relations have been observed with GM volume loss.^{29,30} Brain atrophy relates to clinical impairments from onset³¹ with progression of disability more closely correlated to GM atrophy than loss of WM volume.³² Regional analyses have highlighted the importance of damage to specific structures in the progression of clinical impairments. Atrophy of the deep GM structures relates strongly to disability, particularly the thalamus plays an important role in MS.³³ Thalamic atrophy precedes clinical symptoms³⁴ and progresses rapidly throughout the disease with strong correlation to disability progression.³³ Besides brain volumetrics, spinal cord damage is commonly observed in MS but often overlooked. Spinal cord damage is not always readily apparent in early RRMS patients³⁵ and is greater in those in the progressive stage of MS.³⁶

Symptomatology of multiple sclerosis

This disseminated nature of MS pathology leads to a wide range of symptomatologic presentations. The presenting signs, degree of disability and disease course are highly heterogeneous both within and between pwMS. An example of a common manifestation and presenting sign of MS is optic neuritis, characterized by vision and colour loss evolving over days and ocular pain intensified by eye movements.³⁷ However, the widespread pathology can lead to a broad range of symptoms including motor and sensory deficits, pain, bowel and bladder (incontinence) problems, sexual dysfunction, fatigue and cognitive dysfunctions.



Figure 3. Unemployment in multiple sclerosis. Many people with multiple sclerosis (MS) develop motor impairments due to pathological processes in the brain and spinal cord, which results in unemployment already early on in MS. The proportion of people with MS in employment decreases evidently as disability worsens. Image taken from Giovannoni et al. 2016.²⁴

Although MS is characterized by a range of symptomatologic manifestations, the disease is typically viewed as a motor disease. Up to 90% of people with MS experience motor impairments, which are present across all phases and forms of the disease. Motor impairments are highly disabling and have a considerable impact on the independence and quality of life.^{38,39} Given that pwMS are typically diagnosed as young adults and

impairments can occur early on, motor disabilities significantly affect personal life choices such as starting a family and employment (Figure 3).²⁴

Functionality of both upper and lower limb limbs can be affected and dysfunctions can present as tremors (postural and action tremor), coordination difficulties, altered sensations (hemisensory), motor weakness, spasticity, balance problems and gait disturbances.¹ Mobility impairments are a typical hallmark of MS and around 80% of pwMS experience difficulties with walking and balance within 10 to 15 years after onset.⁴⁰ Upper limb impairments on the other hand are less well recognised, yet disabilities can occur in approximately 80% of pwMS as well.⁴¹ Even though people with MS are highly likely to develop upper and lower limb impairments over the disease course,⁴² the degree of dysfunction between limbs correlates only moderately,⁴³ suggesting that at least partially differential brain processes might underlie.

Treatment

Unfortunately MS is a chronic disease with no known cure, but management is possible. Over the last years, an increasing number of disease modifying treatment options exist for the relapsing-remitting form of MS. These treatments are aimed to modulate or suppress the dysregulated immune system to preserve neurological function, ease symptoms and reduce relapse risk. First-line treatments are prescribed in pwMS that present with mild inflammatory disease activity (high-safety drug), but when proven to be inadequate, second-line treatments are considered (more safety risks). Second-line treatments have proven to be particularly effective in RRMS, mostly by preventing immune cell migration to the brain, but seems to be less successful in the progressive stage of the disease,¹³ which is characterized by minimal active demyelinating and neurodegeneration. Treatment options in the progressive stages of MS are limited. A treatment that not only targets the inflammatory component, but also is neuroprotective and promotes remyelination to slow and ultimately prevent disease activity remains one of the largest challenges.

Current treatment options reduce relapse frequency and slow progression of disabilities, but to develop a therapeutic option that is able to prevent disabilities, we need to better understand the pathological mechanisms that underlie impairments. To improve our understanding we need to study motor function as well as brain mechanisms underlying these functions. Tools to assess motor functioning and imaging methods to visualize pathology and to study the neuronal mechanisms of motor disability in MS will be discussed in the paragraphs below.

Measuring sensorimotor impairments

There are several ways of assessing motor signs and symptoms in MS ranging from simple tests such as a finger-to-nose test to more comprehensive measures like the Expanded Disability Status Scale (EDSS) and advanced measures of gait using motion capture systems.

The EDSS is a well validated measure of disability in MS. This assessment tool is routinely used in clinic to assess and monitor disabilities and is widely used in MS research as a reflection of overall disability. The EDSS is a rating scale ranging from 0 - 10 with steps of 0.5 that represent higher levels of disability and is based on simple clinical tests and visual evaluation.⁴⁴ PwMS with EDSS scores between 0 - 4 show no to mild physical disability, are clinically able to walk and do not require any assistance or walking aid. EDSS scores between 4.0 and 5.5 reveal mild impairment in ambulation and scores equal to or beyond 6 refer to loss of mobility. At the higher end of the scale patients require an walking aid, wheelchair or are restricted to bed (scores beyond 8.0). Even though this rating scale is widely used in clinic and research, it is limited by moderate inter-rater reliability⁴⁵ and is heavily weighted to walking ability at the higher ends of the scale.

To overcome these issues, upper and lower limb dysfunction can be examined separately using the 9-Hole Peg Test (9-HPT) and Timed-25 Foot Walk Test (T25FW) respectively. The 9-HPT is a commonly used quantitative arm and hand performance tool that requires a participant to place as quickly as possible nine pegs into nine holes, one at the time, and then to remove these pegs again. This test is done for dominant

and non-dominant hand and time in seconds to complete these tests is a reflection of dexterity. The T25FW is a quantitative test to assess leg and mobility function and involves a timed 25 feet walk with outcome measure walking speed. The T25FW is considered to be reliable across a range of disability levels and a difference in scores is considered reflective of a change in real-life activities.⁴⁶

To quantify mobility in pwMS in more detail advanced kinematic analysis technologies that allow for 3-dimensional imaging of gait can be used. Kinematics (study of motion) and kinetics (study of causes of motion) of gait can be assessed using motion capture systems, force plates and electromyography. A previous study in MS has demonstrated the added value of using these techniques to detect subclinical disease changes not evident based on clinical assessment alone.⁴⁷ In early stage MS, pwMS displayed altered timing of ankle muscle activations and ankle angles in absence of clinical disability, no acute relapse or change in EDSS.⁴⁷

Advanced MRI to investigate sensorimotor impairments in multiple sclerosis

Whereas conventional MRI methods are essential for diagnosis and monitoring disease progression in clinic, the correlation between lesions load and clinical impairments is only moderate.^{21,22} Stronger relations have been observed with more advanced measures such as brain atrophy,^{31–33} but these findings still do not explain the clinical heterogeneity fully. Instead of a focal approach, as the brain is a complex system, to understand behaviour we need to understand how brain regions interact with each other. Relatively recent imaging techniques such as diffusion weighted imaging and functional MRI have made it possible to study structural and function connectivity between regions and the brain in its entirety. The use of these techniques in understanding the complex mechanisms driving motor disability in MS will be discussed in the paragraphs below.

Structural damage within the sensorimotor system in multiple sclerosis

Over the last decade, advanced MRI imaging techniques and analysis methods have been developed and/or optimized, allowing to study the microstructural damage in the brain in great detail. Whereas conventional MRI can image pathology at a macrostructural level, to visualize the microstructural organisation of the WM, newer MRI techniques such as diffusion weighted imaging (DWI) are needed. DWI measures the motion of water molecules, with the preferable direction depended on microstructural restrictions such as cell membranes and axons (Box A). Diffusion MRI is therefore an informative tool to quantify axonal and myelin pathology in MS.

In MS, one of the first models and still a commonly used method to describe and characterize preferential displacement of water molecules to assess microstructural damage is diffusion tensor imaging (DTI). Previous research has shown that DTI measures relate more strongly to disability than conventional measures such as lesions load.⁴⁸ Diffusion tensor abnormalities are present from the onset of the disease and are not only observed in lesions, but also measured in the normal appearing WM in both cerebrum and cerebellum.⁴⁹ Within the sensorimotor system specifically, the WM integrity of important motor tracts in the brain is damaged including the corticospinal tracts (Figure 4), the main descending motor pathway,^{50–52} and the corpus callosum,^{53,54} a major WM bundle connecting hemispheres, involved in the control of movements.⁵⁵ The microstructural damage within these tracts have been related to clinical disability^{52,53}, dexterity^{51,54,56} and walking⁵¹.

Even though DTI has been used extensively to assess WM integrity, there are several limitations to consider. Firstly, the DTI model assumes that a brain voxel includes a single fibre population in one orientation, however many voxels may contain partial volume fractions of distinct fibre populations (Figure 5). Multiple fibre orientations can be detected in up to 90% of WM voxels in the brain.⁵⁷ Secondly, metrics derived from a tensor provides a suboptimal estimation of axonal injury as it is insensitive to axonal damage specifically, it contains signal from non-neuronal cells and extra-cellular fluids.⁵⁸

Axonal density, dispersion, atrophy and (de)myelination can all contribute to the value of FA, which makes interpretation difficult.⁵⁸

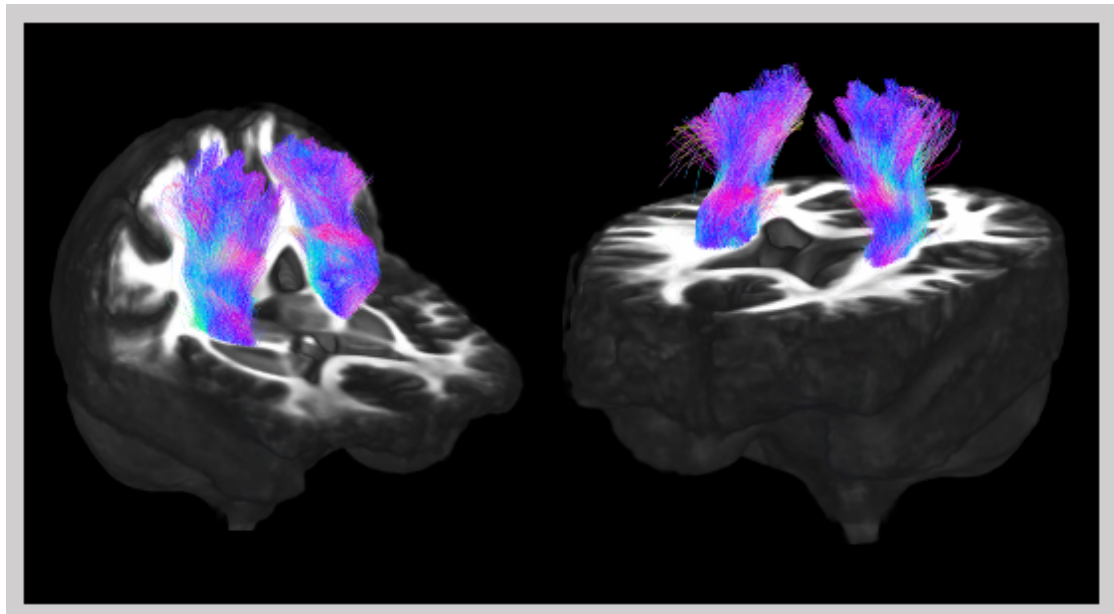


Figure 4. The corticospinal tracts. The corticospinal tract is an important motor pathway that runs from the cerebral cortex to the spinal cord and is involved in the execution of voluntary movements. The motor tracts were derived using diffusion weighted imaging and subsequent analysis method tractography, a technique to delineate white matter tracts in the brain. Whole brain probabilistic tracking was performed first, which involves sampling of diffusion orientations from a large distribution of possible orientations, followed by selection of the corticospinal tract using several inclusion and exclusion regions (described in more detail in Chapter 3.2).

In addition, as neurodegeneration is present from early stages of the disease, there is a high need for early detection with sensitive and specific markers of axonal loss for timely intervention of functional decline and treatments efficacy. To overcome these issues and to investigate sensorimotor axonal loss more specifically more advanced higher order models are needed such as constrained spherical deconvolution. With this method the fibre orientation distribution of multiple fibre bundles within a voxel can be estimated and quantitative measures of the underlying microstructure can be derived (Figure 5).

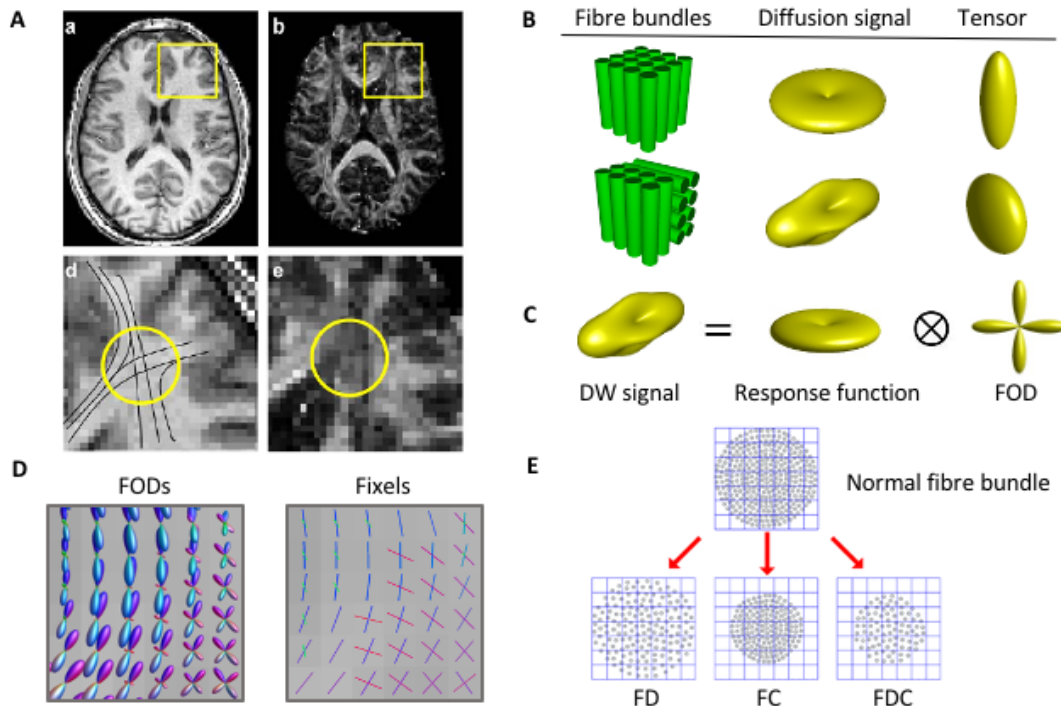
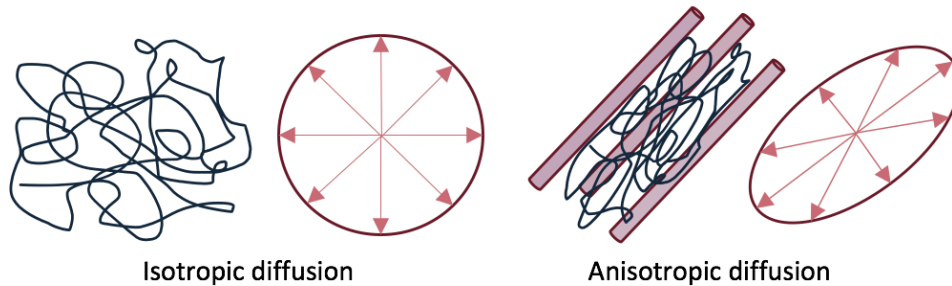


Figure 5. Crossing fibres and diffusion weighted imaging models. A) Partial volume effect and crossing fibres. Image adapted from Assaf et al. 2008.⁵⁹ B) Corresponding diffusion signal and diffusion tensor ellipsoid of a signal fibre population (top row) and two crossing fibre populations (bottom row). When fibres cross, the tensor estimated does not reflect the fibre populations and does correspond to the orientation of either population. C) Using constrained spherical deconvolution, the diffusion signal is the convolution between the estimated signal expected for a single-fibre population, i.e. response function, and the fibre orientation distribution (FOD). Image adapted from Raffelt et al 2012.⁶⁰ D) The FODs and corresponding fixels, i.e. the specific fibre bundles within a voxel. E) Fixel-specific metrics can be derived, including fibre density (FD), fibre cross-section (FC) or the combination between the two (FDC). The schematic represents the cross sections of fibre bundles. Axons are represented as grey circles and the grid represents imaging voxels.

Box A – Diffusion Weighted Imaging

Diffusion is driven by the random interaction of water molecules as they collide with one another, known as the Brownian motion. The preferable direction of motion depends on microstructural restrictions such as cell membranes and axons. Axons will effectively restrict movement of water molecules preferentially along the axis of the axon itself. This type of diffusion is known as anisotropic diffusion.



The basic diffusion weighted scheme is a spin echo pulse sequence with a gradient magnetic field be pulsed before and after 180 refocusing pulse, known as the diffusion gradients. First, the radiofrequency pulse flip protons in the transverse plane. Secondly, the diffusion gradient before the refocusing pulse results in increased frequency of the spins along the gradient axis. Once the diffusion gradient is turned off the spins will rotate at identical frequency but are phase shifted. Application of the second gradient of equal magnitude and duration but in opposite direction, results in rephasing of spins. Only when spins maintain in exact same position during application of diffusion gradients, spins will rephrase completely. Displacement of spins during dephasing and rephrasing results in incoherent phasing, spins remain out of phase compared to surrounding protons. This displacement of water molecules is the fundament for the signal intensity measured with diffusion MRI. Solid tissues give a strong signal whilst motion of water molecules lead to decrease in signal intensity.

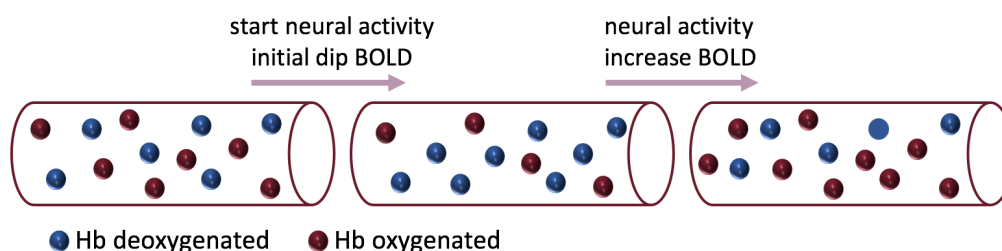
The amount of diffusion (D) is calculated with two images; one with (S) and one without diffusion gradients (S_0), with the following equation; $D = (-\ln(S/S_0))/b\text{-value}$. The factor D describes displacement of protons in all directions and is a property of tissue, but it does not reflect preferential diffusion direction. To detect diffusion accurately, multiple diffusion gradients applied along several directions with different b-values. Higher b-values lead to stronger diffusion weighting but also decrease of signal-to-noise ratio. The magnitude (G), duration (δ), and time interval (Δ) between two diffusion gradients can be described as follows: $b\text{-value} = \gamma^2 G^2 \delta^2 (\Delta - \delta/3)$.

Functional imaging perspective on sensorimotor disability in multiple sclerosis

Disruptions caused by inflammatory and neurodegenerative processes are highly likely to have consequences for brain function and integrity of the system. Brain function can be studied using functional MRI, a non-invasive tool that can assess and quantify neural activation through detection of changes in blood oxygenation (Box B). Functional brain activity can be assessed while performing a specific task (task fMRI). Also, as the brain is a complex system that is highly connected, the communication between regions can be assessed and the brain can be viewed as a network, often studied at rest (resting-state fMRI). Both rest-dependent and task-dependent neural activation has been studied in relation to disabilities in MS, described in the paragraphs below.

Box B – Functional MRI

The fMRI signal in the brain reflects changes in blood flow in response to neural activity that can be detected using the blood-oxygen-level dependent (BOLD) contrast. Oxygen is transported through the cerebral vascular network and supplied to active neurons by haemoglobin (Hb), an iron-rich protein in red blood cells with primary function to carry and transport oxygen. Upon neural activation, the cerebral blood-flow increases, what results in higher cerebral blood volume and higher oxygen levels, that is delivered to neurons by haemoglobin. Higher oxygen levels, i.e. more bound oxygen to haemoglobin, change the magnetic property of blood that can be detected with fMRI imaging, going from paramagnetic, haemoglobin without oxygen, to diamagnetic properties. The change in BOLD magnetic susceptibilities of haemoglobin and resulting change in T2/T2*-weighted signal is exploited as contrast agent in functional BOLD MRI. Brain regions with more oxy-haemoglobin will have a higher signals and therefore appear brighter on a MRI scan than areas with more deoxy-haemoglobin. Important to note is that the hemodynamic response is located near the location of neural activity and results from blood flow changes directly modulated by neuronal activity, but it is an indirect measure of neural activity.



Functional activation patterns during movements in multiple sclerosis

The functional disturbances within the sensorimotor system underlying motor dysfunctions in MS have been assessed using a task fMRI experiment, which typically involves alternating blocks of the task or stimulus and a control condition, i.e. a period of rest or a task unrelated to the function or behaviour of interest. These alternating blocks of task and control conditions are used to identify activated areas specifically related to the task and to study different activation patterns between groups.

In MS, during a motor task experiment two commonly phenomena are observed, either increased activation in the same regions as healthy controls and/or recruitment of addition regions.⁶¹ In the earlier functional task studies increased brain activity was interpreted as adaptive functional processes in response to structural damage to maintain clinical functions, particularly early on in the disease. One of the earlier motor task fMRI studies showed higher activation in the motor cortices during flexion and extension of the fingers in pwMS and interpreted this change in activation as an adaptive mechanism to limit motor impairment.^{62,63} These changes in activation were referred to as functional reorganisation. The functional counteract against pathological processes was based on the direction of activation, i.e. increased activation was interpreted as adaptive and decreased activation as maladaptive. However, as most studies were based on a cross-sectional design, it is difficult to underpin when changes occur and reoccur and whether cortical reorganisation takes place to delay developments of motor impairments. In addition, the optimal curve and a possible tipping point where functional activation changes are no longer beneficial in sustaining motor disability are not completely understood. Longitudinal studies are limited but warranted to actually comprehend whether cortical plasticity occurs to preserve motor function.

Since these early motor task studies, very little research has emerged on the role of altered brain activation in relation to motor disabilities in MS with the majority of research focussed on either healthy controls^{64,65} or used resting-state fMRI to study the motor system.^{66,67} While of value in understanding how functional brain networks change in context of sensorimotor progression, resting-state studies are limited by the

fact that they do not involve performance of sensorimotor behaviours, and therefore could be difficult to interpret in terms of differences in motor performance. In addition, only a limited number of task fMRI studies have been published investigating lower limb sensorimotor processing,^{68,69} possibly due to the complexity of the experimental set-up. Moving your fingers results in less motion than for example tapping your foot and to stabilize the lower limbs to minimize head motion a more complex set-up is needed. In addition, most previous studies used a very simple motor task such as finger tapping or extension and flexion of the fingers^{61–63}, which is a poor reflection of daily motor functioning. As such, current literature does not adequately recapitulate the underlying functional activity patterns of upper and lower limb impairments in MS.

Sensorimotor resting-state network in multiple sclerosis

In absence of a task or external input, spontaneous BOLD signal fluctuations can be observed in distinct brain regions and across structurally distributed networks.⁷⁰ The significance and motivation to study spontaneous neural activity came from a study showing functional correlation in regions association with motor function, i.e. primary sensorimotor cortices and SMA, in absence of movements.⁷¹ Subsequently, studies have shown correlated temporal patterns of areas involved in motor function, auditory processing and memory, consistent across healthy subjects.⁷² Interestingly, these major brain networks at rest closely match with brain activity during activation,⁷³ suggesting an “active” brain at rest.

Besides mapping brain activity, the connectivity between brain regions is often assessed in absence of a task. The connectivity between two regions reflects how strongly two regions are communicating (Figure 6). In MS, early resting-state studies investigating brain connectivity and plasticity reported altered functional connectivity within several brain networks including the sensorimotor system.^{74,75} In early stages of the disease higher functional connectivity was observed and suggestions were made in favour of adaptive brain plasticity in response to increasing structural damage to sustain clinical functions. Whereas similar suggestions have been made for brain activation in presence of a task or stimuli, different mechanisms are measured, i.e. regional activation strength

versus how well brain regions are connected. However, similar to brain activity, over the years it became clear that it was more complex than initially thought. Studies demonstrated not only increased^{75,76}, but also reduced connectivity in early disease stages.⁷⁷ Looking at the sensorimotor network and physical disability specifically, complex patterns of increased^{74,78,79} and decreased^{77,80,81} connectivity has been observed, with limited evidence for clinical correlates.^{79,80}

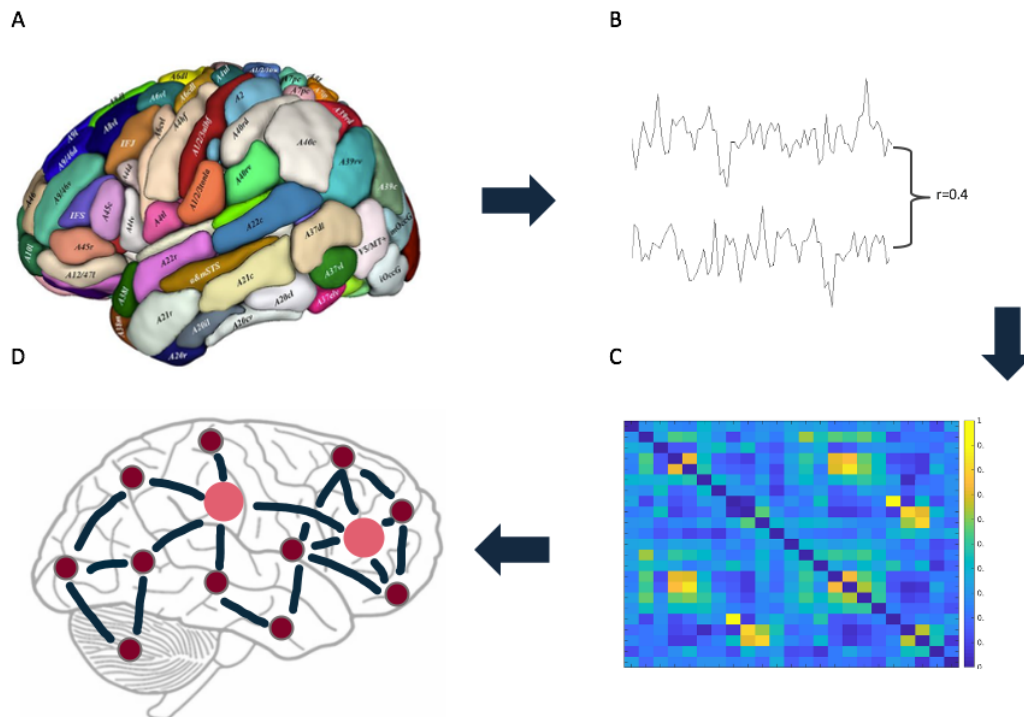


Figure 6. Functional connectivity and network topology. A) The brain can be parcellated in multiple regions of interest. Image adapted from Fan et al. 2016.⁸² B) Time series can be extracted from regions of interest and functional connectivity can be calculated using correlation. The correlation strength is a reflection of how strongly two areas are connected. C) Connectivity can be calculated between all regions of interest resulting in a connectivity matrix. The colour indicates the connectivity strength with in blue weak connections and in yellow strongly connected regions. D) A network consist of nodes, representing brain regions, and edges, the functional connections between nodes. From the connectivity matrix in C different network measures can be derived such as efficiency, a measure of information processing in the brain.

The complex patterns of functional connectivity and limited correlates of disability might be explained by variety of reasons including the heterogeneity in terms of group

sizes and/or the acquisition and analyses methods. In addition, the conflicting results might be explained by the type of patients investigated, ranging from a specific disease stage, often with high variability in disease duration, or all disease stages. Presumably the type of MS does not explain the complex patterns observed, but motor disability can be better understood by grouping based on level of impairment. Further, most previous functional connectivity studies did not focus on the entire sensorimotor (Figure 7), rather a specific region or on whole brain network connectivity was used which potentially could overlook relevant areas or include functionally irrelevant areas. Besides differences in study designs, the significant between-subject variability in functional connectivity observed in healthy participants adds to the complexity.^{83,84} To be able to compare functional connectivity across subjects the variability should be taken into account.

	R PFC	R S2	R M2	R As Sens C	R S-M1	R deep GM	R cerebellum	L PFC	L S2	L M2	L As Sens C	L S-M1	L deep GM	L cerebellum
R PFC														
R S2														
R M2														
R As Sens C														
R S-M1														
R deep GM														
R cerebellum														
L PFC														
L S2														
L M2														
L As Sens C														
L S-M1														
L deep GM														
L cerebellum														

Figure 7. The sensorimotor network. The sensorimotor network contains of multiple cortical and subcortical regions. To delineate white matter tracts and derive the structural connections between these regions a technique called fibre tractography was used. The light blue represents when two regions were structurally connected, in dark blue regions when no tracts between the regions were delineated. The secondary motor area (M2) consists of the supplementary motor regions and premotor area. PFC=prefrontal cortex; S2=secondary sensory area; As Sens C=associative parietal sensory cortex; S-M1=primary sensory motor cortex; deep GM=deep gray matter. This image was copied from Pardini et al. 2015.⁸⁵

In addition to investigating the connectivity of spatially separated brain regions, the brain can be studied in its entity. Investigating the system as a whole allows to study the network as a complex system. Simple intuitions from highly complex systems can be derived using a network approach (Figure 6). In MS, previous research has demonstrated altered communication between regions on a network level. Reduced efficiency, a measure of the ability of a region to propagate information to other regions, and decreased local and global efficiency, measures of integration and segregation, were suggested to be clear identifiers of a disrupted network organisation.⁸⁶ These changes on a network level emerge from the earliest stage of MS and is consistent with findings from studies using structural imaging modalities.^{85,87,88} This evidence for a disrupted network organisation highlights the value of using a network approach in understanding MS pathology. Rather than investigating the connectivity between regions, a network approach could potentially give new insight into the functional mechanisms underlying motor disability in MS.

Ultra-high field imaging

Like network science, over the last years significant progress has been made in ultra-high field imaging (7T). The high signal-to-noise ratio that can be achieved using ultra-high field can be invested in higher spatial resolutions and better contrasts, allowing finer structures to be visualized. In MS, compared to clinical field strengths, previous studies shown the added value of high-field imaging as it improved diagnostic confidence⁸⁹ and detection of cortical lesion pathology (Figure 8).^{90,91} Besides the ability to image at higher spatial resolutions, a major advantage of high field MRI is functional imaging (Figure 8). The spatial specificity and sensitivity at clinical field strengths is relatively poor. This could possibly impair the detection of subtle changes underlying complex mechanisms underlying impairments such as motor disabilities. Ultra-high field MRI allows to map activations more accurately with minimal spatial smoothing⁹² at near anatomical MRI resolution even at reasonable temporal resolution.⁹³ Functional high-field imaging in MS is still limited used⁹², presumably due to the limited availability, however the rich spatiotemporal content has the potential to detect subtle changes with higher accuracy, potentially improving our understanding of more nuanced motor

compensatory mechanisms underlying upper and lower limb motor disability progression in MS.

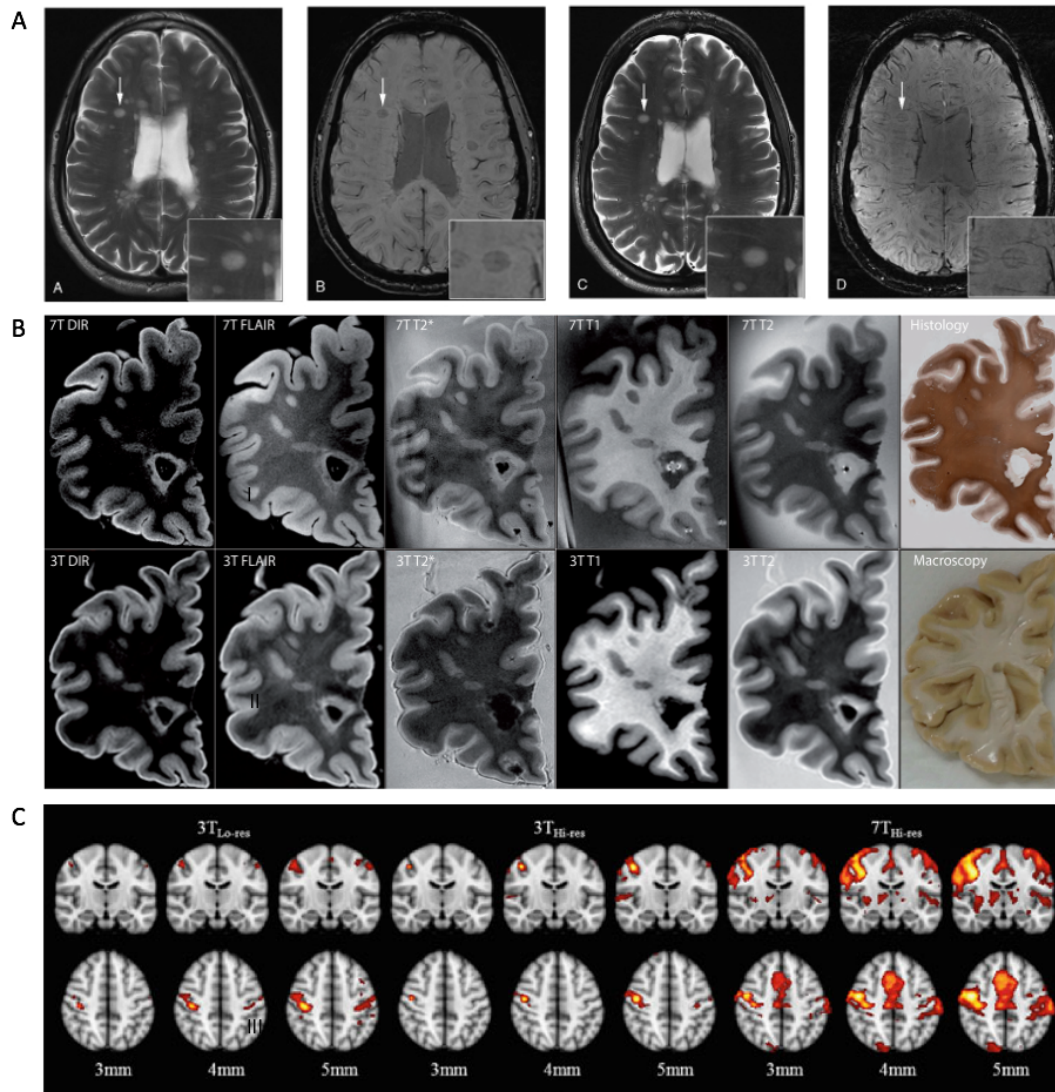


Figure 8. Functional and structural imaging at 3T and 7T in multiple sclerosis. A) Comparison of routine T2-weighted imaging at 3T (A.A) and 7T (A.B) and corresponding axial susceptibility weighted images (SWI) at 3T (A.C) and 7T (A.D).⁸⁹ The white arrow indicates the central vessels in MS white matter lesions which are easier and more clearly depicted on 7T compared to 3T SWI. Image adapted and copied from Springer et al. 2016.⁸⁹ B) A single brain slice of an MS patient acquired at 7T and 3T using multiple sequences including double inversion recovery (DIR), fluid attenuation inversion recovery (FLAIR), T2*, T1-weighted and T2-weighted.⁹⁴ Compared to 3T, higher field imaging resulted in improved quality and grey matter lesion detection. Detected lesion were verified post-mortem. Image adapted from Kilsdonk et al. 2016.⁹⁴ C) Compared to 3T, a higher field strength and lower spatial smoothing improves the spatial specificity of connectivity maps and results in the identification of motor cortices. Image adapted from Hale et al. 2010.⁹²

This thesis: aims and outline

The overarching aim of this thesis was to expand our knowledge of the underlying structural and functional neuronal mechanisms of sensorimotor impairments in pwMS. Despite the evidence of structural damage and functional connectivity disturbances within the sensorimotor system, the clinical heterogeneity is not fully understood. Whereas previous studies often focused on general MS effects, we aimed to study neuronal damage and dysfunction underlying upper and lower limb impairments separately in minimal disabled patients, prior to overt loss of dexterity and mobility, and in patients with more serious disabilities. More sensitive measures of disability as well as more optimal imaging methods were employed to elucidate the association between microstructural damage and brain function and disability in MS.

The overarching research questions of this thesis were

- 1. Which functional network changes underlie sensorimotor impairments in patients with more severe disabilities, and are these predictive of subsequent progression?**
- 2. In early disability stages, prior to more severe impairments, which functional activation pattern underlie subtle deficits in the upper and lower limbs?**
- 3. In people with MS with minimal physical disabilities, to what extent is axonal loss prevalent in of the major motor pathways of the brain, and does it relate to subtle changes in hand function and walking?**

In the first part of this thesis the functional disturbances within the sensorimotor system in a large cohort of pwMS was investigated using a network approach and functional resting-state MRI. First, to get a better understanding of the functional network mechanisms underlying disability severity, in **Chapter 2.1** we aimed to directly compare the functional sensorimotor network efficiency in the presence or absence of overt motor disability. Next, to get a better understanding of disability progression and the

mechanisms underlying upper and lower limb specifically, in **Chapter 2.2** baseline network measures were explored as possible predictors of progression of upper and lower limb impairments.

In the second part of this thesis we performed two more focused studies in minimally disabled patients using ultra-high field MRI (7 Tesla) and sensitive measures of motor behaviour derived from force tracking and spatiotemporal measures of gait. With this dataset we were able to explore the subtle functional changes in brain activity during a complex visuomotor task in relation to subtle changes hand and foot motor performance, addressed in **Chapter 3.1**. In addition, to understand to what extent axonal loss is a feature in pwMS with minimal physical disability, and whether the degree of axon loss relates to subtle changes in upper and lower limb performance, we assessed the white matter integrity within major motor tracts in the brain using novel measures of axonal damage and precise kinematic measures of dexterity and gait, described in **Chapter 3.2**. The key findings of these studies are summarized, integrated and discussed in **Chapter 4**.

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Chapter 2

Sensorimotor network efficiency in patients with more severe disability

An introduction to Chapter 2

This project involved a large data set including RRMS, PPMS, and SPMS patients, examined at two time points with 5 years in between. With this data set I aimed to get a more comprehensive understanding of the functional mechanisms underpinning disability severity and progression of upper and lower limb disabilities.

1. Project design

Data collection

Baseline data was collected between 2008 and 2012 and the follow up assessments were done between 2014 and 2017. At baseline the study comprised of a total 332 multiple sclerosis patients and 96 healthy participants of which 70% (234/332) MS patients and 63% (60/96) healthy controls returned for the second assessment 5 years after baseline.

Inclusion criteria

- Clinically definite MS diagnosed using the McDonald criteria¹
- 18 years of age or over
- Availability of disability measurements (Expanded Disability Status Scale (EDSS)) and either high or low disability severity (see below) (Chapter 2.1)
- Upper limb (9-Hole Peg Test (9HPT)) and or lower limb (Timed-25 Foot Walk Test (T25FW)) functional tests at both time points (Chapter 2.2)
- Whole brain and cerebellar resting-state fMRI coverage (Chapter 2.1 and 2.2)

Exclusion criteria

- MS relapse in the two months prior to MRI
- History or presence of psychiatric and/or neurological disease other than MS

Disability assessments

The EDSS was used as a reflection of overall disability and used in Chapter 2.1 to divide patients into low disability ($EDSS \leq 3.5$, no or minimal walking disability) and high disability ($EDSS \geq 6$, unable to walk without aid or assistance). The EDSS is part of routine clinical

practice and the scale ranges from 0 (normal function) to 10 (death).² Disability is based on simple clinical tests and visual evaluation. Sensory and motor impairments are quantified by 3 out of the 8 functional system scores (FSS) the EDSS involves, i.e. the sensory, pyramidal and cerebellar FSS. The main advantage of the EDSS is the widely use in clinical practice and in research, however the EDSS varies between levels of disability with less reliability when more disabled. The rating scale is heavily weighted towards mobility at the higher end of the scale and is not sufficiently sensitive to detect subtle changes in minimal disabled patients.

To get a more precise measure of upper and lower limb function separately, the 9HPT and T25FW were also performed. In Chapter 2.2, these tests were used to assess dexterity and mobility and patients were divided into progressing and non-progressing patients based on the change in performance over time (5 years). Progression was defined as more than 20% decline in performance on the respective assessment tools, all remaining patients were considered stable and classified as non-progressing.

MRI acquisition

MRI data was acquired using a 3 Tesla MRI scanner (GE Signa HDxt, Milwaukee, WI) with an 8-channel phased-array head coil. In Chapters 2.1 and 2.2 we used the 3D-T1 weighted fast spoiled gradient-echo imaging for brain volumetrics, the fluid attenuated T2-weighted images for white matter lesion detection and functional imaging during resting-state using an echo planar imaging sequence to assess the functional network connectivity and efficiency.

Contributions Chapter 2.1

Study conceptualisation and design: M. Strik, S.C. Kolbe, J.J.G Geurts, M.M. Schoonheim.

Acquisition of data: A.J.C. Eijlers, I. Dekker, M.M. Schoonheim.

Data analysis: M. Strik, A.J.C. Eijlers, K.A. Meijer, M.M. Schoonheim.

Statistical analysis: M. Strik, M.M. Schoonheim.

Drafting of a significant portion of the manuscripts: M. Strik, D.T. Chard, I. Dekker, A.J.C. Eijlers, K.A. Meijer, B.M.J. Uitdehaag, S.C. Kolbe, J.J.G. Geurts, M.M. Schoonheim.

Contributions Chapter 2.2

Study conceptualisation and design: M. Strik, S.C. Kolbe, M.M. Schoonheim, J.J.G. Geurts.

Acquisition of data: A.J.C. Eijlers, I. Dekker, M.M. Schoonheim.

Data analysis: M. Strik, A.J.C. Eijlers, I. Dekker, T.A.A. Broeders, L. Douw, M.M. Schoonheim.

Statistical analysis: M. Strik, M.M. Schoonheim.

Drafting of a significant portion of the manuscripts: M. Strik, I. Dekker, A.J.C. Eijlers, T.A.A. Broeders, L. Douw, J. Killestein, S.C. Kolbe, J.J.G. Geurts, M.M. Schoonheim.

2. Background information on a network perspective on disability in MS

The brain is an intricate system and to understand motor behaviour we need to understand how different regions in the brain interact with each other. The study of brain connectivity has been made possible with the emergence of advanced imaging techniques such as diffusion weighted imaging and functional MRI. Brain connectivity can be distinguished into three classes including structural, functional and effective connectivity. Structural connectivity is defined as the existence of the physical or anatomical connections between brain areas and it reflects the integrity of tracts between regions and the transmission of information through axons. The second class, functional connectivity, is defined as the statistical dependence between physiological signals between spatially separated brain regions (used in Chapters 2.1 and 2.2). When two regions in the brain show correlated signal fluctuations over time they are defined as functionally connected, indicating information is exchanged. Functional connectivity is commonly quantified with the correlation coefficient. Whereas the structure connectivity is intrinsically directed as each axon has a source and a target, the directionality cannot be resolved with functional MRI, but with effective connectivity the causality of neuronal connectivity could be determined. Dynamic causal modelling for example is a Bayesian framework for inferring the influence of one region exerts over another.

Network science has been increasingly applied over the last years and revealed meaningful information about the effects of pathology on the organisation of complex networks in the brain and the dynamic nature and adaptivity of these systems. In neuroscience, the mathematical approach of graph theory is commonly used to study the architecture of brain as a graph which is comprised of nodes representing brain regions and edges interposed between nodes which reflect the structural or functional connections (Figure 1).³ By building a graph, from a highly complex system very simple intuitions can be derived, what makes network science an intriguing approach to study not only the healthy human brain but also intricate multifaceted diseases such as MS.

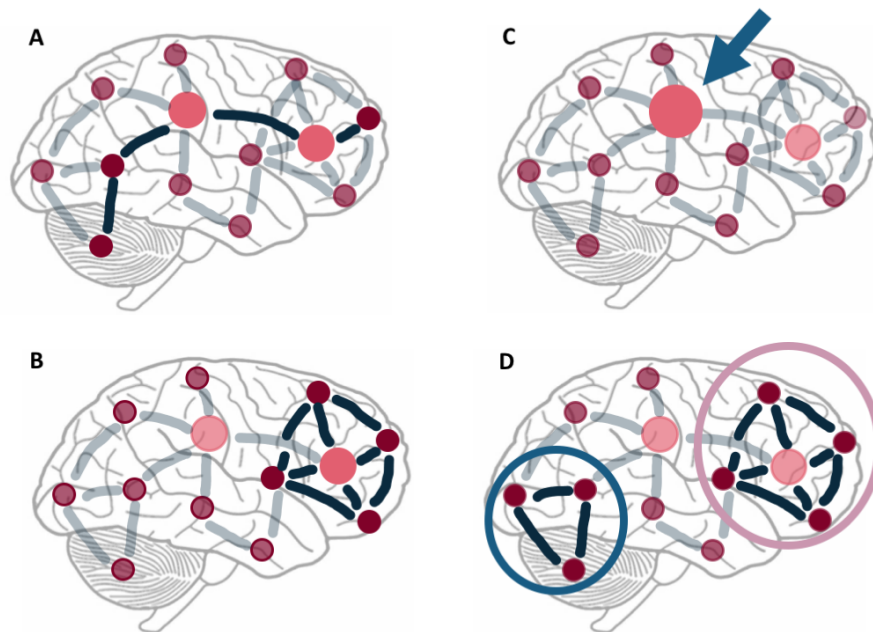


Figure 1. Brain network as a graph. The brain consists of around 100 billion neurons and 100 trillion connections and to simplify this complexity the brain can be viewed as a graph with nodes (coloured in red) and connections between nodes (coloured in blue). Several measures can be derived from a graph such as path length (X), the number of steps going from one node to the other (A), and clustering coefficient (B), a measure of the connectiveness between neighbour nodes. To have an efficient information flow a graph should not be random but comprised of short path lengths and high clustering, i.e. small-world network. Other network features observed in human brain networks are highly connected central hub regions (C) and many networks divide naturally into modules (sub networks) (D) that are characterized by densely interconnected nodes within a module and few connections between modules.

Despite the evidence of connectivity changes in MS, changes have been difficult to relate to clinical impairment. Instead of looking at the connectivity between sensorimotor regions, studying the sensorimotor system as a whole might elucidate on the functional processes underlying motor deficits in MS. To investigate the functional disturbances within the sensorimotor system underlying disability severity and progression of upper and lower limb impairments in MS, we used the graph metric network efficiency (Chapters 2.1 and 2.2). We choose network efficiency as it is a well validated and informative measure about the integration and segregation of information processing in the brain.

Whereas until recently most imaging studies assumed that functional connectivity is static and therefore connectivity was measured over long periods of time (Figure 2). However, evidence is growing that connectivity dynamically changes during task and at rest (Figure 2).^{4,5} Recent advances have shown the added value of a more comprehensive and dynamic perspective on the brain, especially when studying cognition.^{6,7} Investigating continuously changing patterns of network features over the course of the functional scan, might be highly relevant for motor disability as well (investigated and discussed in Chapter 2.2).

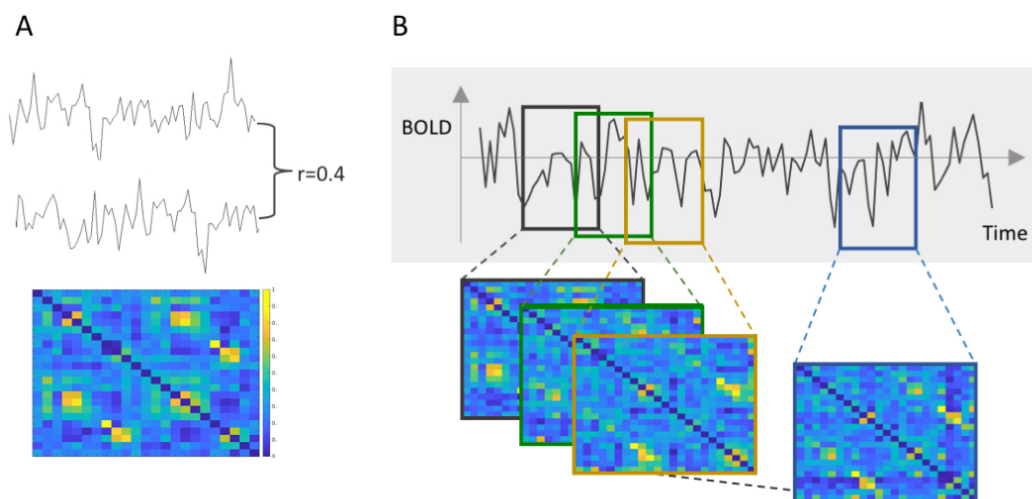


Figure 2. Static and dynamic functional network connectivity. A) With static connectivity the connectivity over the entire scan is calculated and B) with dynamic connectivity the variations over time is estimated.

The sensorimotor system

As simple as walking or lifting your arm may sound, these movements are intricate activities with complex underlying neuronal brain mechanisms. Today's understanding of the neural brain mechanisms of control is still relatively sparse, but clinical observations, pathological findings and imaging modalities have shown that the sensorimotor system is comprised of several cortical and subcortical regions and multiple pathways with high degree of interconnectivity.

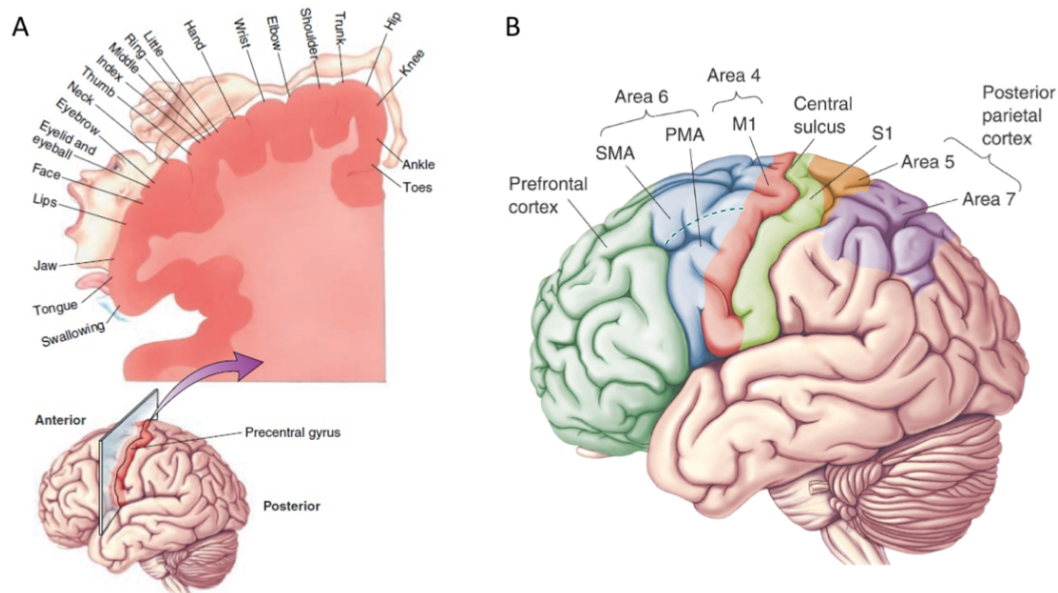


Figure 3. The homunculus and cortical sensorimotor regions. Images copied and adapted from *Neuroscience: Exploring The Brain* ⁸.

The primary motor cortex (M1) is a key structure in motor function and is located anterior to the central sulcus. Research dating back from the 1800s showed that M1 functions roughly as a map of the body's musculature. Penfield and Boldet illustrated a map of the human body in the brain, referred to a 'homunculus', a very small distorted man with the legs located medial and hands and face more lateral (Figure 3).⁹ The concept of the body lying within cortex is too simplistic as not one single cortical site controls a single muscle, but is involved in range of muscles across several joints to control individual movements. The overlapping and complex nature of M1 could be

explained by the fact that a simple movement involves complex coordination of several muscles and joints. Up till today the exact mechanisms of M1 are not fully understood, but researches still refer to, get inspired by or research the homunculus.¹⁰ Whereas M1 neurons encode the parameters of individual and or simple movements including force of the actual movements, directionality and speed,^{8,11,12} the secondary motor areas, the premotor cortex and SMA, are thought to be involved in more complex movements and motor planning.^{8,13} Input to the premotor cortex and SMA comes from areas in prefrontal and posterior parietal cortices. These areas represent the highest level of motor control and are suggested to be involved in decision making and visuomotor coordination.⁸

The human subcortex consists of hundreds of grey matter (GM) nuclei that makes up approximately a quarter of total brain volume. A large group of those subcortical GM nuclei is called the basal ganglia, which is widely connected with the rest of the brain and includes the caudate nucleus, putamen, nucleus accumbens and globus pallidus. A closely related GM nucleus is the thalamus, an important relay centre in the brain. The basal ganglia is best known for its role in facilitating movements. The basal ganglia does not cause movements independently, but promotes and inhibits motor output by influencing activity in other parts of the brain, particularly M1. An example of motor dysfunctions clearly caused by an unbalanced communication between these pathways is such as tremor and postural instability are also often observed in MS patients.^{14,15}

The cerebellum is anatomically and functionally a remarkable and powerful but complex structure in the CNS. It contains roughly more than half of all neurons in the brain, approximately 69 billion neurons.¹⁶ The dentate nucleus is considered to be the main output with nerve bundles leaving via the superior peduncle connecting to a wide variety of areas including spinal cord, brainstem, deep GM structures and cerebral cortex. The cerebellum is known to be as varied in its function as the cerebral cortex and is involved in both cognitive processes and motor function including ataxia (problems with coordination) and tremor (involuntary rhythmic oscillatory), both common manifestations in MS.

3. Experiments and hypothesis

Experiment 1 | Chapter 2.1

Investigation of the functional sensorimotor network disturbances in the presence or absence of overt motor disability in patients with MS.

- Compute the global efficiency (entire sensorimotor network) and local measures of efficiency for each sensorimotor region and compare between MS patients and healthy controls.
- Assess the main predictors of disability severity including functional efficiency measures, structural measures (brain and lesion volumes) and demographics.
- Assess relations between areas important to disability and clinical functional system scores and functional connectivity patterns with other sensorimotor regions.

Experiment 2 | Chapter 2.2

Investigation of functional drivers of upper and lower limb disability progression in MS using a static and dynamic network approach.

- Classification of patients into progressors or non-progressors for both 9-HPT and T25FW (> or < 20% decline).
- Assess the differences in static and dynamic sensorimotor network efficiency between progressing and non-progressing patients.
- Determine the primary predictors of upper and lower limb disability progression.
- Investigate the connectivity patterns of significant predictors of functional with other sensorimotor regions.

Hypothesis

- Inflammation and neurodegeneration alters the functional sensorimotor network efficiency and dynamics, which subsequently drives dexterity and walking impairment in MS.

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Chapter 2.1

Increased functional sensorimotor network efficiency relates to disability in multiple sclerosis

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Abstract

Background: Network abnormalities could help explain physical disability in multiple sclerosis (MS), which remains poorly understood.

Objective: This study investigates functional network efficiency changes in the sensorimotor system.

Methods: We included 222 MS patients, divided into low disability (LD, Expanded Disability Status Scale (EDSS) ≤ 3.5 , $n = 185$) and high disability (HD, EDSS ≥ 6 , $n = 37$), and 82 healthy controls (HC). Functional connectivity was assessed between 23 sensorimotor regions. Measures of efficiency were computed and compared between groups using general linear models corrected for age and sex. Binary logistic regression models related disability status to local functional network efficiency (LE), brain volumes and demographics. Functional connectivity patterns of regions important for disability were explored.

Results: HD patients demonstrated significantly higher LE of the left primary somatosensory cortex (S1) and right pallidum compared to LD and HC, and left premotor cortex compared to HC only. The logistic regression model for disability ($R^2 = 0.38$) included age, deep grey matter volume and left S1 LE. S1 functional connectivity was increased with prefrontal and secondary sensory areas in HD patients, compared to LD and HC.

Conclusion: Clinical disability in MS associates with functional sensorimotor increases in efficiency and connectivity, centred around S1, independent of structural damage.

Introduction

Multiple sclerosis (MS) is a demyelinating and neurodegenerative disorder of the central nervous system leading to disabling sensorimotor impairments.¹ The pathophysiology of neurological dysfunction is complex and not fully understood. Conventional magnetic resonance imaging (MRI) measures of lesion load in brain and spinal cord have shown only modest relations with disability.^{2,3} More advanced measures, such as spinal cord⁴ and (sub)cortical grey matter (GM) atrophy,⁵ relate more strongly to disability, but still do not fully explain clinical heterogeneity, suggesting that additional processes may also be important determinants of disability.

In addition to structural brain changes, functional alterations might be relevant to disability progression. Resting-state functional magnetic resonance imaging (fMRI) has been used to identify changes within sensorimotor network (SMN) from the earliest stages of MS. However, results have been difficult to interpret due to conflicting observations with increased^{6–10} and decreased^{11–15} functional connectivity (FC), local¹⁴ and widespread connectivity changes^{6,7,12}, and limited or conflicting evidence of clinical correlations.^{6–9,11–15} In part, differences between studies are likely to reflect differences in cohorts, and limited correlations with outcomes to reflect the inclusion of networks that are not relevant to clinical outcomes that have been assessed.

In this study we assessed the motor system using the concept of network efficiency,¹⁶ a well validated measure of the integration and segregation of information processing in the brain. We assessed functional efficiency in the presence or absence of overt motor disability in a large cohort and investigated the most important correlates of sensorimotor disability in MS.

Methods

Participants

For this study we included 222 patients (age 45.35 ± 10.47 , 165 females (74%)) with MS (disease duration 13.60 ± 8.22), part of the Amsterdam MS Cohort,¹⁷ and 82 healthy controls (HC, age 45.93 ± 10.79 , 52 females (63%)). Inclusion criteria included the presence of relapse-onset MS, availability of disability measurements, either high or low disability severity (see below) and whole brain and cerebellar functional coverage. All patients included in this study were diagnosed with clinically definite MS according to the 2010 revised McDonald criteria,¹⁸ did not experience a relapse in the 2 months prior to the scanning session. This study was approved by the local institutional ethics review board and all participants provided written consent before participation.

Motor disability assessments and group definition

Disability was assessed in patients using the Expanded Disability Status Scale (EDSS).¹⁹ We divided patients into two groups based on walking impairments; that is, low disability (LD, $EDSS \leq 3.5$ (no or minimal walking impairment), $n = 185$, age 43.83 ± 10.12 , 135 females) and high disability (HD, $EDSS \geq 6$ (unable to walk without aid or assistance), $n=37$, age 52.94 ± 8.87 , 30 females). Subsequently, the low disability group was reduced into only patients with very minimal disability, $EDSS \leq 2$, after which general linear model (GLM) and regression analyses (see below) were repeated.

Imaging data acquisition

All participants were scanned using a 3T-MRI (GE Signa HDxt, Milwaukee, WI) with an 8-channel phased-array head coil. Functional whole-brain resting-state MRI data were acquired with an echo planar imaging sequence (repetition time (TR) = 2200 ms, echo time (TE) = 35 ms, flip angle (FA) = 80° , 3 mm contiguous axial slices, in-plane resolution $3.3 \times 3.3 \text{ mm}^2$). For brain volumetric calculations, a three-dimensional (3D) T1-weighted fast spoiled gradient-echo sequence was used (TR = 7.8 ms, TE = 3.0 ms, FA = 12° , inversion time (TI) = 450 ms, 1.0 mm sagittal slices, $0.9 \times 0.9 \text{ mm}^2$ in-plane resolution), and a 3D fluid-attenuated inversion-recovery (FLAIR) was acquired to identify white

matter (WM) lesions (TR = 8000 ms, TE = 125 ms, TI = 2350 ms, 1.2 mm sagittal slices, 0.98 x 0.98 mm² in-plane resolution).

White matter lesion segmentation and brain volume calculations

WM lesions were automatically segmented on FLAIR images using k-nearest neighbor classification with tissue type priors.²⁰ These images were registered to T1 weighted images and lesions were filled using Lesion Automated Pre-processing.²¹ Lesion-filled images were subsequently used to calculate total brain volumes and subcortical volumes using SIENAX and FIRST respectively (<https://fsl.fmrib.ox.ac.uk/fsl/>). Deep GM (DGM) volumes were subtracted from the total GM volume to calculate cortical GM volume specifically. To account for differences in head size, all brain volumes were normalized using V-scaling factor derived from SIENAX.

Resting-state fMRI pre-processing

Resting-state fMRI pre-processing involved removal of the first two volumes, brain extraction, head motion correction, spatial smoothing with a 5-mm full width at half-maximum Gaussian kernel and high-pass temporal filtering (100 seconds cut off) using the MELODIC pipeline (FSL5). Registration parameters were calculated between fMRI and 3DT1 sequences, using boundary-based registration (BBR), and between 3DT1 and the standard brain using non-linear registration, both of which were inverted to co-register regions of interest (ROIs) to the fMRI sequence (see below). Images were checked for head motion, artefacts and registration errors. Level of motion was calculated based on the average frame-to-frame head motion, as reported previously.²² The average frame-to-frame head motion did not exceed more than one voxel (3 mm) and did not differ between MS patients and HC ($p = 0.36$). In addition, voxels without reliable signal caused by echo-planar imaging distortions artefacts or non-brain tissue were excluded using a robust-range based threshold.

Regions of interest

The cortex was segmented using the Brainnetome atlas (<http://atlas.brainnetome.org>), the cerebellum using the Harvard-Oxford atlas and DGM structures using FIRST. All ROIs were combined to form one atlas, subsequently registered to individual functional scans

using inverted BBR parameters and nearest-neighbour interpolation and. assessed on sufficient reliable signal, that is, at least 30% of voxels in at least 90% of all subjects after removing unreliable voxels. This resulted in the removal of orbitofrontal, inferior temporal cortices and nucleus accumbens and a final atlas containing 193 ROIs. Signal intensities within each individual atlas region were averaged for each time-point to form 193 time series and FC was calculated with Pearson correlations, generating a 193 by 193 weighted undirected connectivity matrix. People have very distinct FC profiles,²³ for which we corrected by dividing each connection with average whole-brain FC, enabling us to find disease-related changes in network patterns with relative FC values (Supplementary Figure 1). All negative correlations were set to zero.²²

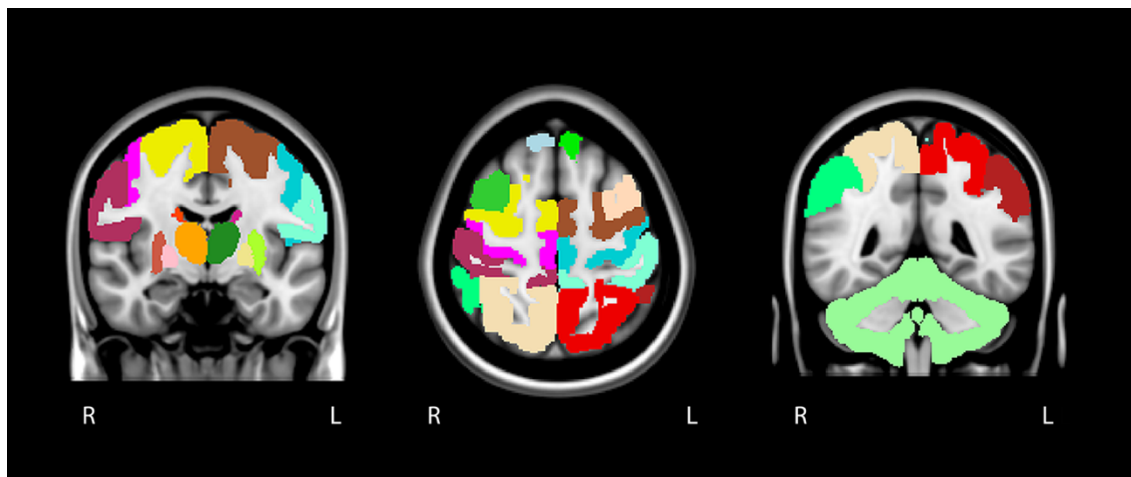


Figure 1. The sensorimotor network. *The sensorimotor network was defined based on previous literature and we included 23 cortical and subcortical grey matter areas. The cortical components of the motor network are the primary motor cortex, premotor cortex, supplementary motor area, prefrontal cortex, primary somatosensory cortex, secondary sensory cortex and posterior associative sensory cortex. Each cortical area was subdivided into a right and left region of interest. As for sub-cortical regions, we included the cerebellum and left and right thalamus, caudate nucleus, putamen, and pallidum.*

The sensorimotor system

We chose to include a broad range of regions beyond conventional ‘typical’ motor areas such as primary motor cortex (M1) and somatosensory cortex (S1), based on a previously published approach.²⁴ This consisted of left and right frontal, parietal and cortical motor regions, deep grey matter areas and the cerebellum, forming a 23 x 23 FC matrix (Figure

1). The cerebellum was included as whole because of limited scan coverage, difficulties in parcellating only motor regions accurately on fMRI and to limit the total number of ROIs. Global efficiency (GE) of the entire SMN and local efficiency (LE) for each ROI was calculated using the Brain Connectivity Toolbox (<https://sites.google.com/site/bctnet/>). The global efficiency is based on the inverse of the average shortest path length of all individual network links, representing how efficient information flows throughout the entire SMN. The LE quantifies efficiency on a smaller scale and is related to the clustering coefficient, a network topology characteristic that reflects local processing of information.¹⁶

Statistical data analyses

All statistical analyses were performed in SPSS 22 (IBM, USA). All measures were checked for normality using the Kolmogorov-Smirnov test and visual inspection of histograms. Lesion volumes were non-normally distributed and log-transformed. Variables with a non-normal distribution were compared with nonparametric tests (Mann-Whitney *U* test). For normally distributed variables general linear models were used correcting sex and age with Bonferroni-correction over groups; significance after correcting for the number of variables is also reported. To assess the most important correlates of disability, a binary logistic regression model with backward elimination was used including significant network measures, brain and lesion volumes and demographics. GLM and regression analyses repeated including LD patients ($EDSS \leq 2$) and HD patients. Areas where LE significantly contributed to the regression model were further explored by examining FC to other sensorimotor areas between groups using GLMs and Spearman correlations were performed between LE and functional system scores (FSS).

Results

Demographics, clinical data, and brain volumes

Demographics, clinical variables and brain volumes are shown in Table 1. The highly disabled group had a significant longer disease duration ($p < 0.001$) and consisted of relatively more secondary progressive MS patients ($p < 0.001$) than the group with low disability. HD patients were significantly older compared to both LD patients ($p < 0.001$) and HC ($p < 0.01$). No differences in handedness were found between patients with high and low disability.

Compared to HC, both MS groups displayed significantly lower whole brain, WM, cortical and DGM volumes ($p < 0.05$). HD patients exhibited more pronounced cortical GM atrophy ($p = 0.001$), DGM atrophy ($p < 0.001$), and whole brain ($p < 0.001$) and WM volume loss ($p < 0.05$) compared to LD patients. In addition, HD patients demonstrated a higher lesion volume compared to LD patients ($p < 0.001$).

Global and local efficiency of the SMN

Global efficiency was not statistically significant. Local efficiency (see Table 2) was higher in the HD group compared to HC in left premotor cortex ($p = 0.011$), S1 ($p = 0.001$) and right pallidum ($p = 0.044$). HD patients showed higher LE in left S1 ($p = 0.013$) and right pallidum ($p = 0.040$) compared to LD patients. No differences in efficiency were seen between LD patients and controls. The local efficiency of S1 remains significant after also correcting for the number of variables. These comparisons were not significant when using uncorrected FC matrices.

In addition, when compared to the low disability group based on EDSS ≤ 2 , highly disabled patients showed higher GE ($p = 0.026$) and LE of the left premotor cortex ($p = 0.035$), M1 ($p = 0.011$), S1 ($p = 0.001$), pallidum ($p = 0.016$) and right M1 ($p = 0.033$), S1 ($p = 0.007$), putamen ($p = 0.040$) and pallidum ($p = 0.005$). Also here, S1 remains significant after additional correction.

	Healthy controls	Low disability	High disability
Demographics			
• Sex, F/M	52/30	135/50	30/7
• Age, y	45.93 (10.79)	43.83 (10.12)	52.94 (8.87) • □
• Disease duration		12.07 (7.27)	21.23 (8.55) □
• Phenotypes (RRMS/SPMS), n		178/7	11/26 □
• EDSS Total ^a		2.5 (0-3.5)	6.5 (6-8) □
• FSS Cerebellar ^a		1 (0-3)	3 (0-4) □
• FSS Pyramidal ^a		1 (0-3)	3 (2-5) □
• FSS Sensory ^a		2 (0-3)	2.5 (1-5) □
• FSS Brainstem ^a		0 (0-3)	1 (0-3) □
• FSS Visual ^a		0 (0-3)	1 (0-5) □
• FSS Bowel and Bladder ^a		0 (0-3)	2 (0-4) □
• FSS Cerebral (mental) ^a		1 (0-3)	2 (0-3) □
Brain volumes			
• NBV, L	1.51 (0.07)	1.48 (0.07) •	1.40 (0.07) • □
• NWMV, L	0.69 (0.03)	0.67 (0.03) •	0.65 (0.03) • □
• NCGMV, L	0.78 (0.05)	0.77 (0.05) •	0.72 (0.06) • □
• NDGMV, mL	62.85 (3.75)	58.31 (5.80) •	51.71 (7.10) • □
• Lesion volume (log), ml		3.88 (0.38)	4.25 (0.40) □

Table 1. Demographics, clinical, and MRI characteristics. Abbreviations: F = females; M = males; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; EDSS = Expanded Disability Status Scale; FSS = Functional Systems Scores; NBV = normalized brain volume; NWMV = normalized white matter volume; NCGMV = normalized cortical grey matter volume; NDGMV = normalized deep grey matter volume. All values represent means and standard deviations unless denotes otherwise. ^aMedian and range. Significant difference compared to healthy controls • and patients with low disability □.

Sensorimotor regions	HC	LD	HD	p-values
Left prefrontal cortex	0.95 (0.11)	0.96 (0.12)	1.00 (0.13)	0.059
Left supplementary motor area	1.09 (0.16)	1.11 (0.20)	1.16 (0.28)	0.391
Left premotor cortex	1.04 (0.15)	1.07 (0.18)	1.16 (0.28)*	0.015
Left primary motor cortex	1.05 (0.14)	1.08 (0.16)	1.14 (0.20)	0.099
Left primary somatosensory cortex	1.07 (0.12)	1.09 (0.16)	1.20 (0.23)*□	0.002
Left secondary sensory cortex	1.06 (0.12)	1.08 (0.14)	1.15 (0.24)	0.051
Left posterior associative sensory cortex	1.07 (0.14)	1.09 (0.16)	1.16 (0.23)	0.172
Left thalamus	1.08 (0.23)	1.11 (0.24)	1.20 (0.37)	0.352
Left caudate nucleus	1.05 (0.21)	1.03 (0.25)	1.12 (0.29)	0.272
Left putamen	1.07 (0.19)	1.05 (0.22)	1.14 (0.28)	0.297
Left pallidum	0.96 (0.20)	0.95 (0.20)	1.08 (0.41)	0.105
Right prefrontal cortex	0.95 (0.16)	0.97 (0.12)	1.01 (0.15)	0.084
Right supplementary motor area	1.10 (0.17)	1.12 (0.19)	1.16 (0.24)	0.556
Right premotor cortex	1.06 (0.15)	1.07 (0.17)	1.14 (0.23)	0.269
Right primary motor cortex	1.05 (0.14)	1.08 (0.16)	1.13 (0.19)	0.188
Right primary somatosensory cortex	1.08 (0.13)	1.10 (0.17)	1.18 (0.21)	0.064
Right secondary sensory cortex	1.07 (0.15)	1.07 (0.15)	1.14 (0.20)	0.395
Right posterior associative sensory	1.07 (0.13)	1.08 (0.17)	1.15 (0.24)	0.257
Right thalamus	1.08 (0.22)	1.10 (0.25)	1.20 (0.34)	0.384
Right caudate nucleus	1.06 (0.20)	1.06 (0.23)	1.11 (0.36)	0.747
Right putamen	1.07 (0.20)	1.06 (0.24)	1.14 (0.27)	0.453
Right pallidum	0.95 (0.18)	0.96 (0.22)	1.10 (0.46)*□	0.033
Cerebellum	1.02 (0.20)	1.05 (0.21)	0.96 (0.23)	0.095

Table 2. The mean local efficiency values of each sensorimotor region for each group. General linear model was used to compare local efficiency differences between groups and main effects were significant for the left premotor cortex, left primary somatosensory cortex and right pallidum (in bold). After post-hoc Bonferroni-correction, significant differences were found compared to healthy controls* and patients with low disability□. Abbreviations: HC = healthy controls; HD = patients with high disability; LD = patients with low disability.

Predictors of disability in MS

A final backward binary logistic regression model was created to identify the most important correlates of higher disability (Nagelkerke $R^2 = 0.36$, Chi-square = 53.56, $p < 0.001$), which included a higher age (Wald = 9.71, $p = 0.002$), lower DGM volume (Wald = 22.19, $p < 0.001$) and higher LE of left S1 (Wald = 6.26, $p = 0.012$). Repeating this regression model using only right-handed patients did not change results. In addition, when repeating this regression model after redefining 'low disability' to EDSS ≤ 2 , the same predictors were identified.

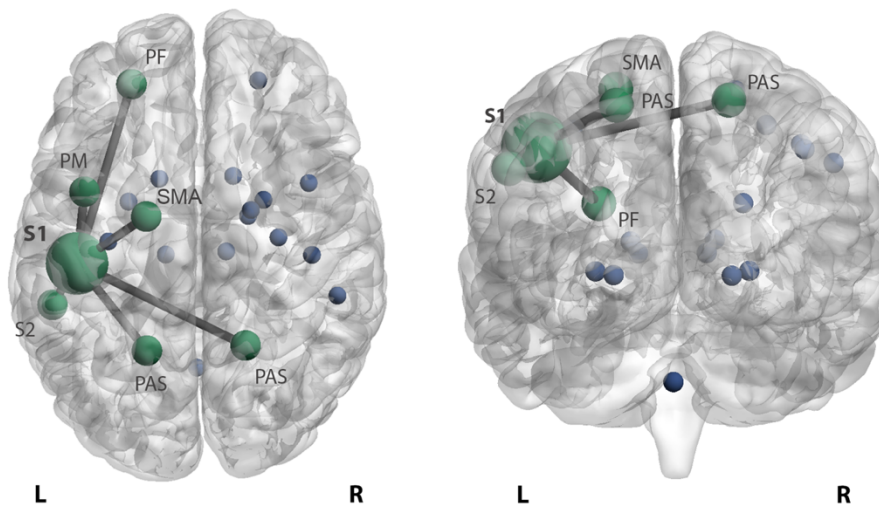


Figure 2. Increased functional connectivity of the somatosensory cortex in highly disabled MS patients. Patients with high disability display higher primary left somatosensory connectivity with the left prefrontal cortex (PF), premotor cortex (PM), secondary sensory cortex (S2) and right and left posterior associative sensory cortex (PAS) compared to patients with low disability and HC. In addition, stronger connectivity with the left supplementary motor area (SMA) was seen in highly disabled patients compared to HC. The stronger connectivity between these areas is reflected by edges between the bigger nodes in green. The blue dots reflect the remaining sensorimotor network regions. Abbreviations: L = left; R = right.

Changes in primary somatosensory FC

HD patients displayed significantly higher FC between left S1 and left prefrontal cortex ($p = 0.001$ versus HC and $p = 0.004$ versus LD), premotor cortex ($p < 0.001$ and $p = 0.023$), secondary sensory cortex ($p = 0.002$ and $p = 0.011$) and right ($p = 0.002$ and $p = 0.026$) and left ($p < 0.001$ and $p = 0.007$) posterior associative sensory cortex compared to HC and LD patients (Figure 2). In addition, compared only to HC, HD patients displayed

higher left S1 connectivity with the left supplementary motor area (SMA) ($p = 0.031$). In patients with LD, no connectivity changes of the left S1 cortex were found compared to HC. After additional correction, prefrontal, premotor and posterior associative sensory cortices remained significant.

Relations between left S1 changes and clinical functional subsystems

In MS, higher S1 LE significantly correlated with worse pyramidal ($r = 0.239$, $p < 0.001$), brainstem ($r = 0.210$, $p = 0.002$), sensory ($r = 0.211$, $p = 0.002$) FSS and EDSS ($r = 0.256$, $p < 0.001$) (Figure 3).

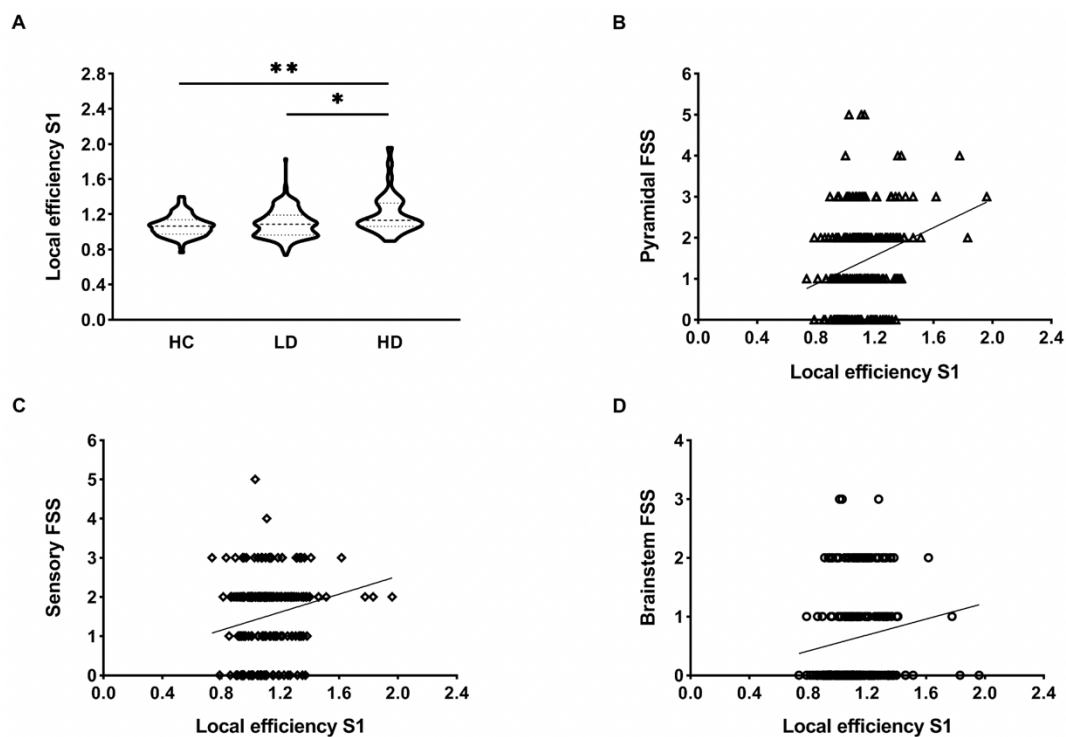


Figure 3. Efficiency of the left primary somatosensory cortex and relations with functional systems scores. (a) Highly disabled MS patients demonstrated significantly higher local efficiency (LE) of S1 compared to both patients with low disability (*, $p < 0.05$) and control subjects (**, $p < 0.01$). The violin plots show the data distributions, that is, the kernel density reflects an estimation of underlying distributed data, and the dashed lines represent the median and the quartiles. (b-d) The scatter plots display significant correlations between LE of left S1 and clinical functional system scores including pyramidal ($r = 0.239$, $p < 0.001$) (b), sensory ($r = 0.211$, $p = 0.002$) (c), and brainstem ($r = 0.210$, $p = 0.002$) (d). Higher LE of S1 significantly correlated with worse pyramidal, brainstem and sensory FSS. HC = healthy controls; LD = patients with low disability; HD = highly disabled patients; S1 = primary somatosensory cortex; FSS = Functional System Scores.

Discussion

In this study, we investigated whether FC and efficiency within the SMN could explain disability in MS and whether specific regions might be particularly affected. Patients with high disability showed extensive SMN changes, mostly centred around S1 as well as the premotor area and pallidum. Patients with minimal disability showed no changes. Increased network efficiency and connectivity of S1 was related to worse disability, even after correcting for structural damage.

S1 and disability in MS

We explicitly identified network changes of S1 that correlated with disability and worse sensory, pyramidal and brainstem FSS (see Figure 3). Impairments in S1 function has shown to be a significant contributor to ineffective motor output and function in other neurological conditions like stroke,²⁵ but in MS this has not been demonstrated previously. Previous MS literature investigating functional changes has been heterogeneous, identifying global changes that were typically not directly related to disability.^{6-8,12,14} One of the first fMRI studies looking at the sensorimotor system investigated connectivity of M1 and showed decreased interhemispheric connectivity but without any clinical correlates.¹⁴ Instead of one specific area, more recent studies investigated the sensorimotor system using independent component analyses (ICA) and reported either only subcortical changes⁸ or global alterations,^{6,7,12} but again no significant clinical relations were found.

Previous literature did not clearly link SMN changes to clinical scores, which could possibly be explained by the common exclusion of cerebellar, subcortical, and prefrontal GM structures. These areas were often not included by independent component analysis as ‘motor network regions’, but considered as a separate network^{10,11,13} or part of other networks investigated^{6,12}, despite their critical role in sensorimotor functioning and processing. This omission of previous sensorimotor connectivity findings within the framework of motor disability may therefore be the result of an overly simplistic view of network functioning, which may be counteracted by applying whole-network measures such as efficiency. Nonetheless, a connectivity approach was valuable post-

hoc, as seen in our data, to show that secondary processing areas, that is, prefrontal cortex, premotor cortex, SMA and secondary sensory cortex, had higher connectivity with S1 (Figure 2), the primary predictor of disability in MS, while primary motor areas (such as M1) did not. These supplementary motor areas play an essential role in the fine balance between somatosensory processing and motor production and altered cortical connectivity might therefore reflect disturbed sensorimotor processing.

Complex patterns of increases versus decreases

Overall, both efficiency and connectivity measures in our study showed increases in disabled patients compared to those with lower disability and HC. Higher sensorimotor connectivity has been found previously in early MS patients with either no or minimal disability,^{6,7} frequently interpreted as beneficial functional reorganisation to limit clinical impairment although this remains to be proven.²⁶ In later stages of MS, increased sensorimotor connectivity has also been observed previously but without clinical relations.^{8,10} In our study, no FC changes were observed in patients with low disability, while patients with more severe disability only showed increased FC compared to controls. Decreased connectivity was not seen in our data, but has been observed previously, related to worse disability.^{11,13} It was previously suggested that a decrease in connectivity could follow from an initial increase in FC, which remains difficult to prove given the strong lack of longitudinal data.

Our findings indicate that increases in connectivity that are sufficient to alter global motor network efficiency might actually not be related to favourable disability outcomes at all. This is supported by previous studies demonstrating association between increased connectivity and worse cognitive functioning,²⁷ and disability.²⁸ In our study, S1 also showed higher FC with several sensorimotor regions, which might be driven by a disruption of mechanisms designed to guide meaningful ascending and/or descending sensorimotor information due to pathological processes in the corticospinal tract. This disruption could result in a loss of inhibition of input reaching S1, resulting in higher connectivity, despite the fact that the information contained within these signals could essentially be noise. However, such causal claims remain speculative, as the opposite may also be true, that is, that the efficiency and connectivity change could reflect a

beneficial mechanism present in patients with severe damage only. As our analyses are limited to cross-sectional, non-directional connectivity measures, future studies should pinpoint how such network changes come to be. The use of empirical computational models might give insight into the change and interaction of functional and structural processes over time.²⁹

Structure and function

Our study showed that functional measures provide added value beyond structural damage. Previous studies have shown that higher levels of disability correlate moderately to WM damage, measured by lesion load.² Recently, structural network efficiency was shown to explain 58% of disability variation, much more than simply averaging damage.²⁴ In addition, using an empirical informed model, WM damage in the form of loss of diffusion-based tracts, was shown to drive increased connectivity and network efficiency changes.²⁹ As such, it would be of high interest to combine structural and functional network measures in the future.

GM damage has also been related to disability, especially DGM and thalamic atrophy.³⁰ The thalamus has gained considerable interest in MS research as atrophy may precede clinical symptoms,³¹ and thalamic atrophy and function strongly correlate to both cognitive decline²⁷ and disability progression.^{5,32} While DGM volume was also a significant correlate of disability in our model, we did not observe significant functional efficiency changes in the thalamus, which is in line with a previous study showing no correlation between thalamic atrophy and thalamic connectivity.²⁷ We did find an increased efficiency of the pallidum and S1, both areas strongly connected to the thalamus and important in the regulation of movement and sensory processing. The pallidum as a correlate of disability was supported by another recent study.³³ Together, these studies highlight the added value of regional and network-based information.

Future directions

We included areas beyond ‘typical’ motor regions such as M1 and S1, based on a previous study,²⁴ but not cognitive regions that could still influence disability or cerebellar subregions. Furthermore, we used relative FC scores due to inter-participant

variabilities, that warrant some caution.²³ How best to correct for this variability requires future studies to determine. Even though a recent study showed that cerebral network changes can explain disability beyond spinal cord atrophy,²⁴ the latter was not included in our study. Furthermore, other graph analytical concepts such as network centrality could provide additional information. Including neurological tests such as the nine-hole peg test could detect other aspects of sensorimotor dysfunction, enabling a more comprehensive way of defining disability. Finally, our approach was based on commonly used 'static' connectivity, that is, efficiency across the entire scan, but unique information may reside in dynamic fluctuations and stability of connectivity patterns.^{34,35} Finally, future multimodal longitudinal studies are needed to investigate the order of events leading to the accumulation of disability.

Conclusion

Using an advanced network imaging approach, this study shows that functional changes in the sensorimotor network centred around S1 are associated with high disability, independently from structural damage. Patients with severe disability (aid or assistance required to walk) showed increased local efficiency and connectivity of S1, suggesting that increases in brain network efficiency may be a marker of poorer clinical function.

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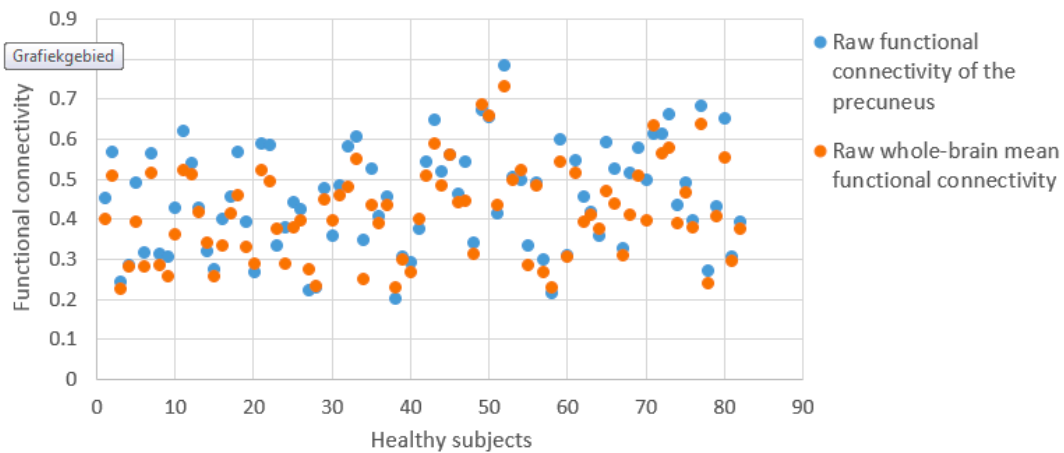
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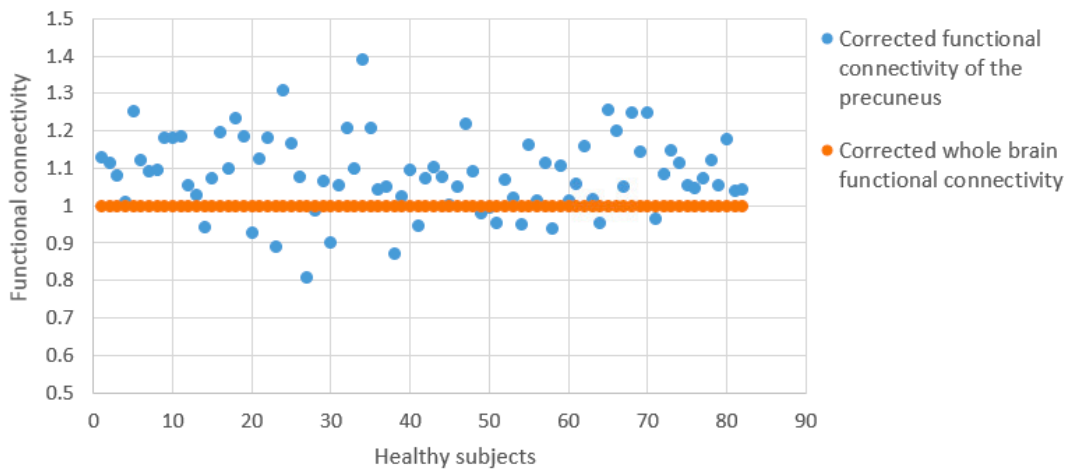
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Supplementary material

A. Uncorrected functional connectivity



B. Corrected functional connectivity



Supplementary Figure 1. Inter-subject functional connectivity variability. A) To visualize the variability in functional connectivity (FC) profiles between subjects, for each healthy subject (x-axis) the average whole-brain FC (orange) and the uncorrected FC values (blue) of the precuneus, a major hub in the brain, are plotted on the y-axis. This plot suggests that the precuneus is not a major hub as it is often not higher than the average connectivity of the population. However, compared to each individual whole brain FC, the FC of the precuneus is usually higher. B) By dividing each connection by each individual average whole-brain FC, the variability of the hubness of the precuneus is less and can be more readily compared between subjects and groups.

Chapter 2.2

Sensorimotor network dynamics predicts loss of upper and lower limb function in people with multiple sclerosis

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Abstract

Objective: Upper and lower limb disability is common in multiple sclerosis (MS), but seem to have partially independent underlying (network) mechanisms. This paper investigated the relationship between upper and lower limb progression and functional sensorimotor network changes in MS.

Methods: Magnetic resonance imaging (MRI), dexterity (9-Hole Peg Test) and mobility (Timed 25-Foot Walk) measurements were included from the Amsterdam MS cohort at baseline and after 5 years. Patients were stratified into progressors (>20% decline) or non-progressors for both tests. Measures of network efficiency were calculated from resting-state functional MRI data using static (i.e. calculated on the entire scan) and dynamic (i.e. fluctuations during the scan) approaches, which were compared between groups.

Results: This study included 214 MS patients (age 47 ± 11 ; 149 women) and 58 healthy controls (age 46 ± 10 ; 31 women). Compared to respective non-progressors, upper limb progression ($n=24$) was related to higher dynamic efficiency of the right premotor cortex, somatosensory cortex and thalamus, while lower limb progression ($n=37$) was related to higher dynamic efficiency of the right supplementary motor area (SMA) at baseline ($p < 0.05$). Dynamic efficiency of the thalamus and SMA best predicted upper and lower limb progression respectively, independent of structural damage ($p < 0.01$). Both areas displayed widespread higher dynamic connectivity in progressing compared to non-progressing patients at baseline ($p < 0.05$).

Conclusion: Disability progression can be predicted by the severity of fluctuations (i.e. higher dynamics) in the sensorimotor network efficiency. Differential effects for upper and lower limb progression were observed, possibly indicating different mechanisms underlying these types of progression in MS.

Introduction

Loss of upper and lower limb function are two of the most common and disabling symptoms of multiple sclerosis (MS), an inflammatory neurodegenerative disease of the central nervous system.^{1,2} The lower limbs are often affected early in the disease and more severely than the upper limbs,^{1,3} yet more than 50% of patients with MS will also experience upper limb difficulties including tremor, clumsiness and muscle weakness.² Upper and lower limb function can be assessed using dexterity and walking tests. Interestingly, the severity of upper and lower limb impairments in individual patients correlate only modestly,² suggesting at least partially different underlying processes and highlighting the need for personalized monitoring strategies.

Due to the known importance of the sensorimotor network underlying normal motor function, disability in MS can be appreciated in terms of both structural and functional damage to this network. Previous studies in MS have indicated that functional network measures may provide additional insights beyond common radiological assessments of lesions load or atrophy.^{4,5} Such studies have shown complex patterns of connectivity in people with MS, including higher^{4,5} and lower⁶ connectivity between sensorimotor regions. However, it remains unclear whether such functional changes indicate ameliorative or maladaptive neuroplasticity and whether these findings are predictive, as longitudinal studies are rare.⁷ In addition, studies investigating correlates of upper and lower limb dysfunction specifically using advanced imaging are lacking.

In this study we therefore aimed to investigate functional changes to the sensorimotor network that are specifically related to upper or lower motor impairments and their predictive value. We hypothesized that functional network measures would identify specific patterns for upper and lower limb disability progression, that provided additional information beyond the severity of structural damage. To investigate our hypothesis, we assessed sensorimotor network “efficiency”,⁸ a graph theoretic measure of a network node’s connectedness to other nodes that has previously been applied to the study of cognitive dysfunction⁹ and overall disability⁷ in MS. In addition to measurements of network efficiency across the entire resting-state functional MRI

(fMRI) scan, we also measured temporal fluctuations (i.e. dynamics) in efficiency within the scan as a measure of network stability which was recently validated in previous studies.^{10,11}

Methods

Participants

For this retrospective analysis of longitudinal data of the prospectively acquired cohort, approval was obtained from the institutional ethics review board and all participants provided written consent prior to participation. This paper included data from the Amsterdam MS cohort,¹⁰ which consists of 332 patients with MS and 96 healthy controls, recruited between 2008 and 2012. All patients were diagnosed with clinically definite MS according to the 2010 revised McDonald criteria.¹² Exclusion criteria included no MS relapse in the two months prior to MRI and no history or presence of psychiatric and/or neurological disease other than MS.¹³ 70% of patients (234/332) and 63% of controls (60/96) returned for a follow-up assessment after five years between 2014 and 2017. This study focused specifically on motor impairments so patients were included if they: (a) had whole brain and cerebellar resting-state fMRI coverage and (b) upper and or lower limb functional tests at both time points. This resulted in 214 patients (47±11 years; 149 women) and 58 healthy controls (46±10 years; 31 women).

Upper and lower limb disability assessments and classification of patients

Overall disability was assessed using the Expanded Disability Status Scale (EDSS).¹⁴ Upper limb disability (i.e. dexterity) was assessed using the 9-Hole Peg Test.¹⁵ Performance of dominant and non-dominant hands was averaged. Lower limb disability (i.e. mobility) was assessed using the Timed 25-Foot Walk¹⁶ Test with the averaged time of two walks. Upper and lower limb disability progression was defined as at least a 20% decline in performance on the respective tests, as previously reported.¹⁷

Magnetic Resonance Imaging acquisition

Imaging was performed on a 3-Tesla GE MRI system (Signa HDxt, Milwaukee, WI) with an 8-channel phased-array head coil. A hardware upgrade (gradient system amongst others) took place between scan sessions, which was corrected for using a procedure previously published using the same dataset.¹³ The image acquisition protocol included resting-state fMRI, acquired with an echo planar imaging sequence covering the whole brain TR=2200ms, TE=35ms, FA=80°, 3mm contiguous axial slices, in-plane 3.3×3.3mm²,

202 volumes (first two discarded). For brain volumetric measurements, a 3DT1 FSPGR was used (TR=7.8ms, TE=3.0ms, FA=12°, TI=450ms, 1.0mm sagittal slices, 0.9x0.9mm² in-plane resolution) and fluid-attenuated inversion recovery (FLAIR) for lesion detection (TR=8000ms, TE=125ms, TI=2350ms, 1.2mm sagittal slices, 0.98x0.98mm² in-plane resolution).

Brain measures: white matter lesion and brain volumes

Lesions were semi-automatically detected and segmented on FLAIR and filled on 3-dimensional T1 scans using Lesion Automated Preprocessing (LEAP).¹⁸ Total brain, cortical and deep grey matter volumes were calculated using SIENAX and FIRST (FSL5, Oxford, UK, <https://fsl.fmrib.ox.ac.uk/fsl/>) and corrected for head size using V-scaling from SIENAX.

Resting-state fMRI pre-processing

The resting-state fMRI data was pre-processed using the MELODIC pipeline (included in FSL5, FMRIB 2012, Oxford, UK, <https://fsl.fmrib.ox.ac.uk/fsl/>, using standard settings) and involved removal of the first two volumes, brain extraction, head motion correction, spatial smoothing with a 5-mm full width at half-maximum Gaussian kernel. In addition, pre-processing involved white matter and cerebrospinal fluid signal regression and images were checked for artefacts and registration errors. In addition to the motion correction included in the MELODIC pipeline (FSL5, FMRIB 2012, Oxford, UK), for each subject, motion effects were further minimized using an independent component analysis-based strategy for Automatic Removal of Motion Artifacts (ICA-AROMA, v0.4-beta 2017, Nijmegen, the Netherlands).¹⁹ Subsequently, voxels without reliable signal caused by EPI-distortions artefacts or non-brain tissue were excluded using a robust-range based threshold and a high-pass temporal filtering equivalent to 0.01 Hz (100 seconds cut-off) was applied, as previously reported.¹⁰ Registration parameters between fMRI and lesion-filled 3DT1 were calculated with boundary-based registration and the standard brain using FNIRT, both of which were inverted (FSL5).

Regions of interest

The cortical regions of interest (ROIs) were segmented with the Brainnetome atlas (Beijing, China, <http://atlas.brainnetome.org>).²⁰ First the atlas was registered to each individual T1-weighted scan using the inverted non-linear registration parameters and nearest-neighbour interpolation. Next, to ensure only cortical grey matter voxels were included, the cortical atlas was multiplied with a grey matter mask derived from the SIENAX analysis (FSL5, FMRIB 2012, Oxford, UK). A cerebellar region of interest was created from the Harvard-Oxford atlas (part of FSL5, FMRIB 2012, Oxford, UK) and using the same pipeline as mentioned above. Deep grey matter structures were outlined on each individual T1-weighted scan using FIRST (FSL5, FMRIB 2012, Oxford, UK) and included the thalamus, caudate nucleus, pallidum, putamen, hippocampus, nucleus accumbens and amygdala. Cortical and subcortical ROIs were combined to form one atlas, which was then registered to each individual functional resting-state fMRI scan using inverted boundary-based registration matrix and nearest neighbour interpolation to optimize registration. Finally, areas were assessed on sufficient reliable signal, i.e. each ROI required at least 30% voxels remaining in at least 90% of all subjects after removing unreliable voxels. This approach led to the removal of inferior temporal, orbitofrontal and nucleus accumbens areas at baseline and follow-up, resulting in an atlas comprising of 193 ROIs across the entire brain. The sensorimotor system was defined based on an approach used previously,²¹ including 23 ROIs.

Functional connectivity (FC) and efficiency of the sensorimotor system

For each subject, signal intensities were averaged for each volume within each individual ROI to form 193 time series. All subsequent analyses were performed in MATLAB (MathWorks, R2018b, Natick, Massachusetts) using custom scripts. Stationary FC was calculated using correlations between all ROIs. Next the 23x23 sub-matrix of the sensorimotor system was extracted and each connection was corrected for whole-brain connectivity, to reduce inter-individual differences. To assess dynamic FC, partially overlapping sliding windows of 27 time points (59.4s) and a shift length of 5 volumes (11s) was applied, resulting in 34 windows per scan (illustrated in Figure 1), comparable to previously suggested settings.²² For each window FC was calculated, resulting in a 23x23x34 matrix. Dynamic FC was defined as the coefficient of variation over time for

each connection, i.e. the standard deviation over time divided by the mean over time. For both dynamic and static connectivity, absolute Pearson correlation values were used.

Next, graph analysis was applied on the static connectivity matrix to derive the global efficiency of the entire sensorimotor network and local efficiency for each ROI using the Brain Connectivity Toolbox (<https://sites.google.com/site/bctnet/>). The global efficiency is inversely related to average shortest path length of the network, i.e. how many steps are required to connect regions. The local efficiency is efficiency on a smaller scale and is related to clustering coefficient, i.e. a network measure of the degree to which neighbour nodes cluster together.²³ To derive dynamic efficiency measures, this process was repeated for each window and the coefficient of variation was calculated, comparable to the dynamic connectivity approach.

Null models

Dynamic FC and dynamic efficiency measures were compared with null-models to assess their deviation from random noise. These randomized data were acquired using phase-randomisation of the data after Fourier-transformation, averaging connectivity and efficiency measures across 50 randomisation runs.²⁴ All dynamic functional efficiency measures used in this manuscript were significantly different compared to measures calculated using null-models, indicating non-random data (all $p < 0.001$).

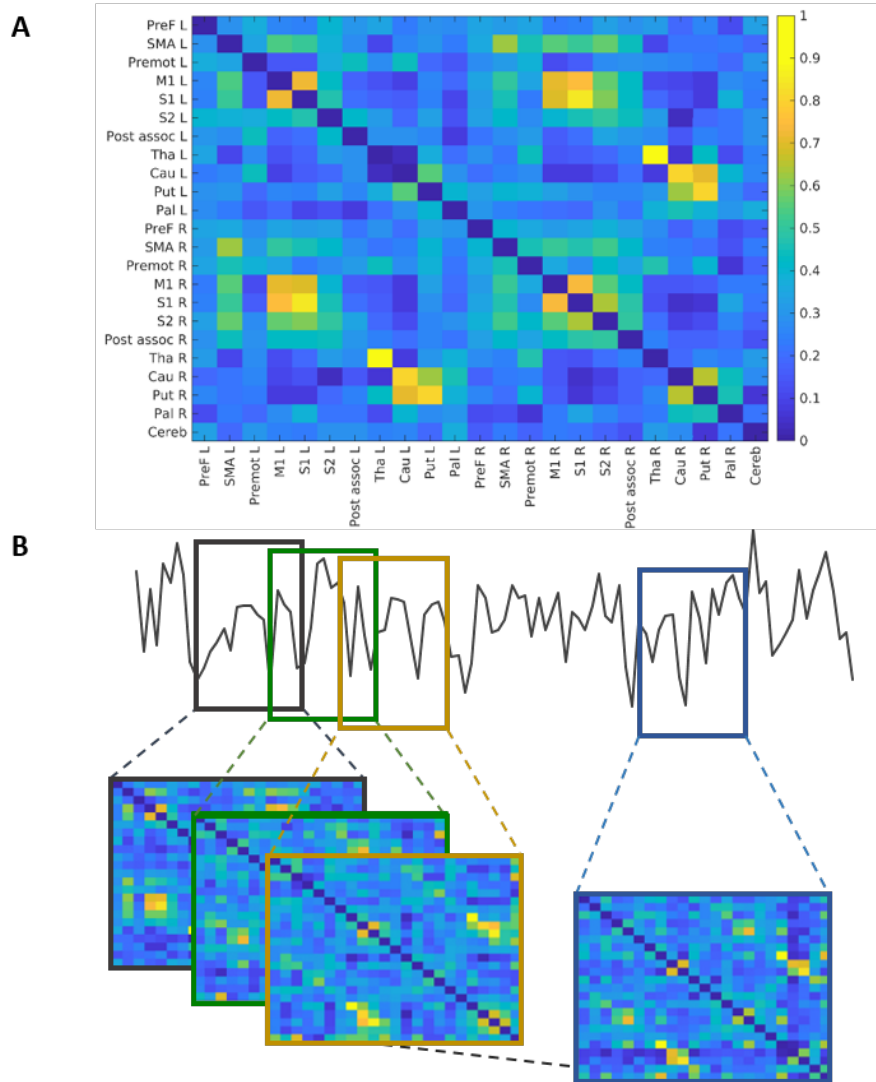


Figure 1. Dynamic connectivity and efficiency of the sensorimotor network. A) Matrix of the sensorimotor network connectivity during one window of a single subject. The colour bar reflects the Pearson correlation, i.e. the connectivity strength. **B)** To compute the dynamic efficiency a shifting window approach was used (shift of 5 volumes), resulting in partially overlapping windows. For each window the connectivity was calculated and network efficiency was computed. Next, to quantify the dynamics, the relative standard deviation over all windows for each sensorimotor region was calculated. Abbreviations: L = Left; R = Right; PreF = Prefrontal cortex; SMA = Supplementary Motor Area; Premot = Premotor cortex; M1 = Primary motor cortex; S1 = Primary somatosensory cortex; S2 = Primary sensory cortex; Post assoc = Posterior associative sensory cortex; Tha = Thalamus; Cau = Caudate nucleus; Put = Putamen; Pal = Pallidum.

Statistical analysis

All statistical analyses were performed using SPSS 26 (IBM, Chicago, USA). First, all demographic, clinical and imaging variables were checked for normality using the Kolmogorov-Smirnov test and visual inspection of histograms. All connectivity and efficiency variables were converted into Z-scores by subtracting the healthy controls mean and dividing by the healthy control standard deviation; lesion volumes were log-transformed. Non-normally distributed variables were analysed with Mann-Whitney U tests. Clinical and MRI measures at baseline were assessed using general linear models with sex, age and time interval between scan sessions as covariates. Significant functional measures at baseline were assessed over time using a repeated measures general linear model. Binary logistic regression with backward selection was performed to predict disability including results from baseline analyses as candidate predictors, demographics and volumetric variables. Significant predictors were explored further by investigating the dynamic and static FC to other areas between groups using general linear models. In addition, relations with brain and lesion volumes were assessed using Pearson correlations. P-values < 0.05 were considered statistically significant and were Bonferroni-corrected, if applicable. All reported p-values are after correction.

Results

Demographic and clinical characteristics

Baseline demographics, clinical characteristics and brain volumes are shown in Table 1. Median EDSS progressed over time (from 3.0 to 3.5, $p < 0.001$) and 21/172 (12%) patients converted to secondary progressive MS. Patients were divided (Figure 2) into upper limb non-progressing ($n=186$, age 47 ± 11) and progressing ($n=24$, age 49 ± 13) and lower limb non-progressing ($n=124$, age 47 ± 11) and progressing ($n=67$, age 46 ± 11). 13 patients progressed on both tests. Lower limb progressors had a higher EDSS ($p=0.001$) at follow-up and were more commonly man (43.3% (29/67) vs 24.2% (30/124), $p=0.007$) compared to non-progressing patients. No differences were seen between upper limb progressing and non-progressing patients on demographic variables, nor on baseline upper and lower limb functioning.

Brain volumes in progressing and non-progressing patients

At baseline, compared to control subjects, all four MS groups showed significantly lower brain volumes (Table 1, $p < 0.05$). For the upper limb MS groups, patients with progression compared to non-progressing patients displayed a lower cortical grey matter volume at baseline ($p=0.002$), while no differences were found between lower limb groups for any volumetric measure.

	Upper limb non-progressing (n = 186)	Upper limb progressing (n = 24)	Lower limb non-progressing (n = 124)	Lower limb progressing (n = 67)	Healthy controls (n = 58)
Demographics					
Sex, W/M	128/58	18/6	94/30	38/29□	31/27
B age, y	47 (11)	49 (13)	47 (11)	46 (11)	46 (10)
B symptom duration, y	15 (9)	14 (7)	15 (8)	13 (7)	
Time interval B and FU, y	5 (1)	5 (1)□	5 (1)	5 (1)	6 (1)
B phenotypes (RR/SP/PP), n	150/26/10	20/2/2	109/12/3	53/8/6	
Converted to SPMS at FU, n	15 (10%)	6 (30%)	14 (12.8)	6 (11.3%)	
B EDSS ^a	3.0 (8.0)	3.5 (5.5)	3.0 (6.5)	3.0 (6.5)	
FU EDSS ^a	3.0 (8.5)	4.0 (6.0)	3.0 (6.0)	4.0 (7.5)□	
Dominant hand (right/left), n	164/22	21/3	109/15	61/6	
B can walk (yes/no/missing), n	166/3/17	23/0/1	112/0/12	62/0/5	
FU can walk (yes/no/missing), n	167/5/14	20/0/4	116/0/8	57/1/9	
B 9-HPT, s ^a	19.4 (155.3)	19.2 (23.3)	19.25 (23.3)	19.33 (146.1)	
FU 9-HPT, s ^a	20.03 (80)	24.95 (29.93)	19.95 (32.8)	21.13 (30.75)	
B T25FW, s ^a	4.3 (177.7)	4.25 (21.9)	4.3 (12.3)	4.1 (13.8)	
FU T25FW, s ^a	4.8 (177.1)	5.35 (176.5)	4.4 (12.5)	5.94 (177.1)	
Brain volumes					
NWMV, L	0.67 (0.03)●	0.67 (0.05)●	0.67 (0.03)●	0.68 (0.04)●	0.7 (0.03)
NCGMV, L	0.76 (0.05)●	0.72 (0.05)●,□	0.76 (0.05)●	0.75 (0.05)●	0.78 (0.05)
NDGMV, L	0.06 (0.01)●	0.06 (0.01)●	0.06 (0.01)●	0.06 (0.01)●	0.06 (0)
Lesion volume (log), ml	3.93 (0.39)	4.1 (0.33)	3.93 (0.38)	3.94 (0.4)	

Table 1. Demographics, clinical variables, and MRI characteristics. Abbreviations: W=women; M=men; B=baseline; FU=follow-up; RR=relapsing-remitting multiple sclerosis; SP=secondary progressive multiple sclerosis; PP=primary progressive multiple sclerosis; EDSS=Expanded Disability Status Scale; 9-HPT=9-Hole Peg Test; T25FW=Timed 25-Foot Walk test; NWMV=normalized white matter volume; NCGMV=normalized cortical grey matter volume; NDGMV=normalized deep grey matter volume. All values represent means and standard deviations unless denoted otherwise. ^aMedian and range. Significant difference compared to healthy controls● and non-progressing patients□.

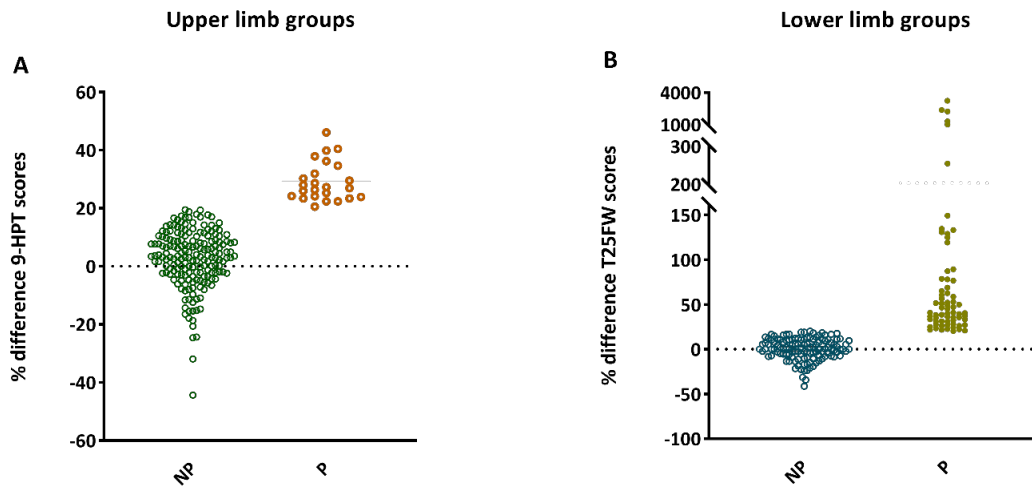


Figure 2. Percentage change in upper and lower limb performance over time. Percentage change in performance scores on the Timed 25-Foot Walk (A) and 9-Hole Peg (B) tests, quantitative tests for lower and upper limb function respectively. **A)** We included 186 patients in the upper limb non-progressing group and 24 patients were defined as upper limb progressing. Of the non-progressing patients, 62 out of 186 (33%) scored lower at follow-up, which means a better performance, with the remainder scoring <20% worse. **B)** Looking at lower limb function, 67 patients were defined as progressing and 124 as non-progressing patients of which 52 patients (42%) improved and all remainder patients performed slightly worse over time (<20%). Abbreviations: T25FW = Timed 25-Foot Walk test, 9-HPT = 9-Hole Peg Test; NP = non-progressing patients; P = progressing patients.

Global and local efficiency differences at baseline

Patients with upper limb progression, compared to non-progressors, displayed higher dynamic efficiency at baseline in the right premotor cortex ($p=0.04$), primary somatosensory cortex ($p=0.04$) and thalamus ($p=0.04$) (Figure 3). Patients with lower limb progression, compared to non-progressors, displayed higher baseline dynamic efficiency of the right supplementary motor area (SMA) ($p=0.03$) (Figure 3). At baseline, neither global efficiency (both static and dynamic), nor static local efficiency significantly differed between progressing and non-progression groups for upper and lower limbs.

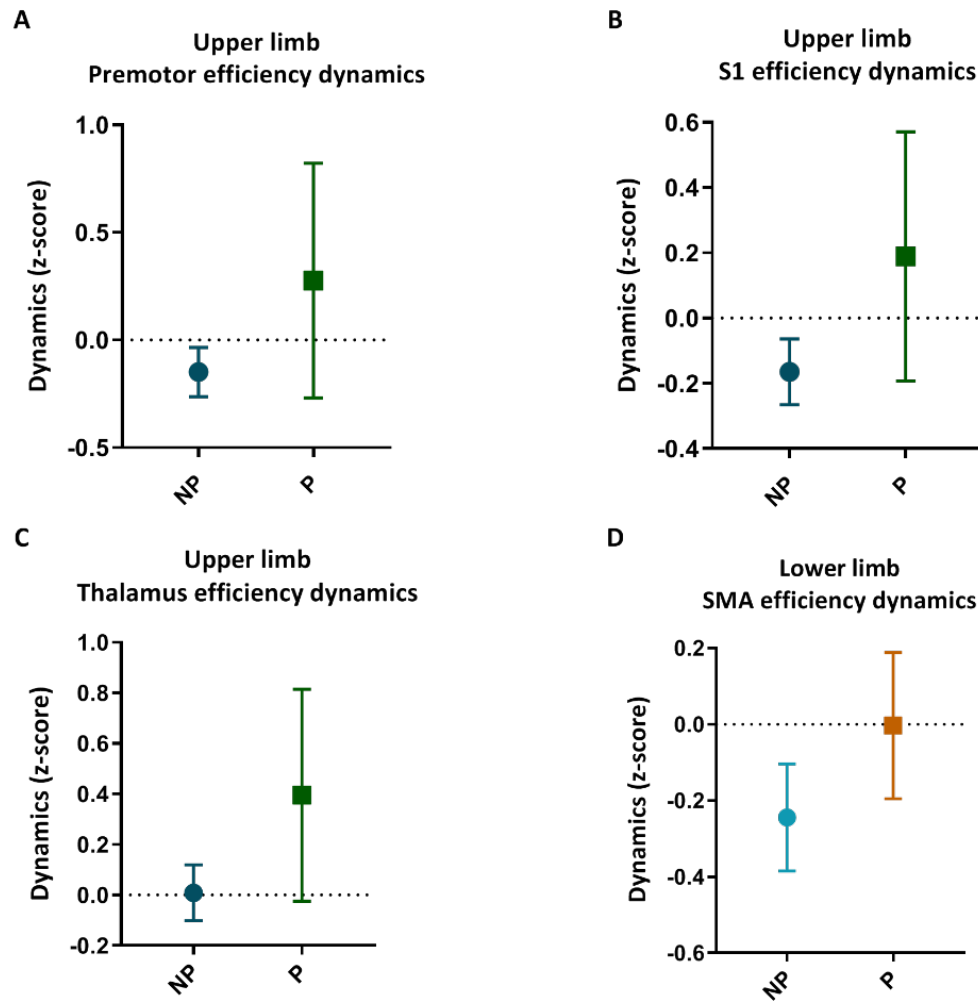


Figure 3. Differences in baseline efficiency dynamics in upper and lower limb progressing versus non-progressing patients. We observed significantly increased baseline dynamic network efficiency in patients with progressive decline in upper and lower limb function compared to patients who were categorized as “stable”. Higher dynamic efficiency was seen in the **A)** right premotor cortex ($p=0.04$), **B)** primary somatosensory (S1) ($p=0.04$) and **C)** thalamus ($p=0.04$) in patients with upper limb progression and in the **D)** supplementary motor area (SMA) ($p=0.03$) in lower limb progressors. The colours reflect either the non-progressing (blue) or progressing (green/orange) patients. Abbreviations: T25FW = Timed 25-Foot Walk test, 9-HPT = 9-Hole Peg Test; NP = non-progressing patients; P = progressing patients.

Longitudinal network changes

The longitudinal behaviour of the significant baseline variables was assessed and of the three regions related to upper limb progression, only the dynamic network efficiency of the premotor cortex showed a significant interaction between group and time ($p=0.04$, Figure 4), driven by a trend of an increase in the progressing group ($p=0.09$, post-hoc paired t-test), which was not seen in the non-progressing patients ($p=0.89$). The change in the dynamic efficiency of the SMA over time did not significantly differ between lower limb groups.

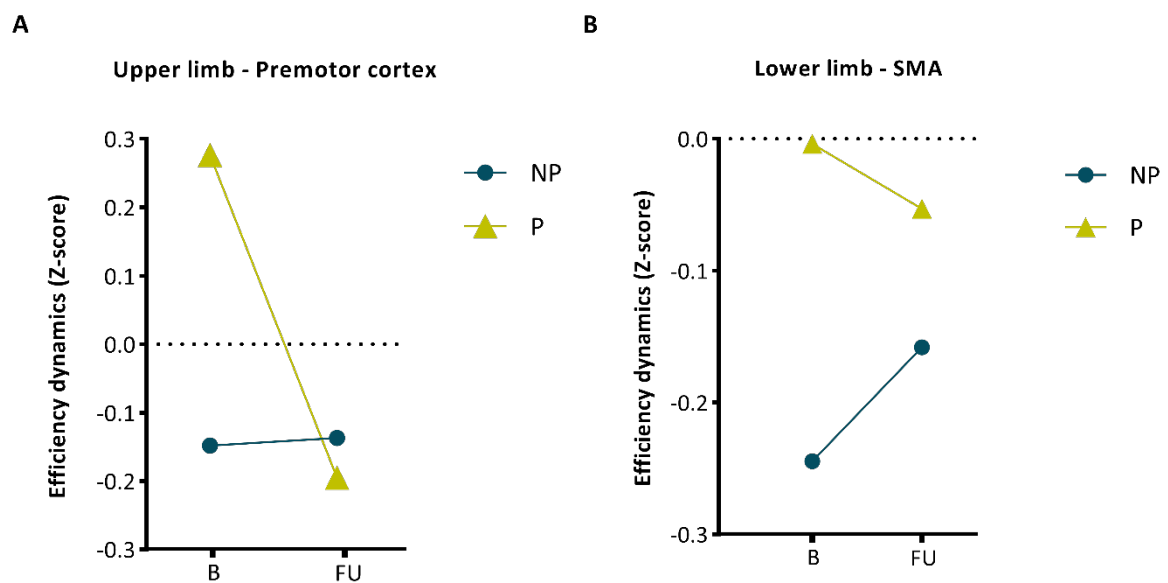


Figure 4. The dynamics of the significant predictors of upper and lower limb disability over time. We investigated the stability or changeableness of the significant network areas from baseline GLM analyses and found significant result for upper limb groups only. **A)** The change of the efficiency dynamics of the right premotor cortex over time was significantly different between non-progressing and progressing patients ($p=0.04$). At baseline the premotor network dynamics was significantly higher but progressively declines, whereas the non-progressing patients remain reasonably stable over time. **B)** The change in dynamics over time for the main predictor of lower limb progression, the SMA, did not significantly differ between groups, but the progressing patients show a small decline whereas the non-progressing patients slightly increase over time. The plots show the average efficiency dynamics at baseline (B) and follow-up (FU) for patients with (P) and without (NP) decline in upper and lower limb function.

Predictors of upper and lower disability progression in MS

Binary logistic regression (with backward selection) was used to identify the most important predictors of upper and lower limb progression. The final model predicting upper limb progression (Nagelkerke $R^2=0.22$, $p<0.001$) included lower cortical grey matter volume at baseline (Wald=13.65, $p<0.001$), longer time interval (Wald=4.73, $p=0.03$) and higher dynamic efficiency of the right thalamus at baseline (Wald=4.27, $p=0.04$) (Table 2). The final model predicting lower limb progression (Nagelkerke $R^2=0.08$, $p=0.003$) included sex (Wald=7.80 $p=0.01$) and higher dynamic efficiency of the right SMA (Wald=4.45, $p=0.04$) (Table 2). The dynamic efficiency of the thalamus and SMA was not related to their respective volumes, nor EDSS.

	Model			Predictor			
	Nagelkerke R^2	Chi-square	P	B	S.E.	Wald	P
Predicting upper limb progression	0.22	25.28	<0.001				
Right thalamus LE dynamics				0.60	0.29	4.27	0.04
NCGM (l)				-27.00	7.20	13.65	<0.001
Time interval				0.61	0.28	4.73	0.03
NDGM (l)				92.49	53.13	3.03	0.08
Predicting lower limb progression	0.08	11.81	0.003				
Right SMA LE dynamics				0.41	0.20	4.45	0.04
Sex				0.92	0.33	7.80	0.01

Table 2. Significant predictors of upper and lower limb progression. Logistic regression analyses with backward elimination were performed to predict upper and lower limb progression during follow-up using significant baseline efficiency variables from the general linear model analyses (group comparisons), demographics and measures of structural damage. Predictors were considered statistically significant when $p<0.50$. Abbreviations: B=predictor specific b-value; S.E.=standard error; LE=local efficiency; NCGM=normalized cortical grey matter volume; NDGM=normalized deep grey matter volume; SMA=supplementary motor area.

Baseline connectivity profile of the thalamus and SMA

Upper limb progressors showed higher dynamic connectivity between the right thalamus and left prefrontal cortex ($p=0.02$), premotor cortex ($p=0.02$), primary motor cortex ($p=0.02$) and right premotor cortex ($p=0.01$), primary sensory cortex ($p=0.045$),

secondary sensory cortex ($p=0.046$), posterior associative sensory cortex ($p=0.02$) and pallidum ($p=0.03$) compared to non-progressing patients (Figure 5). Lower limb progressors displayed higher dynamic connectivity between the right SMA and left premotor cortex ($p=0.01$), S1 ($p=0.02$), posterior associative sensory cortex ($p=0.03$) and right M1 ($p=0.02$) and right posterior associative cortex ($p=0.004$) (Figure 5).

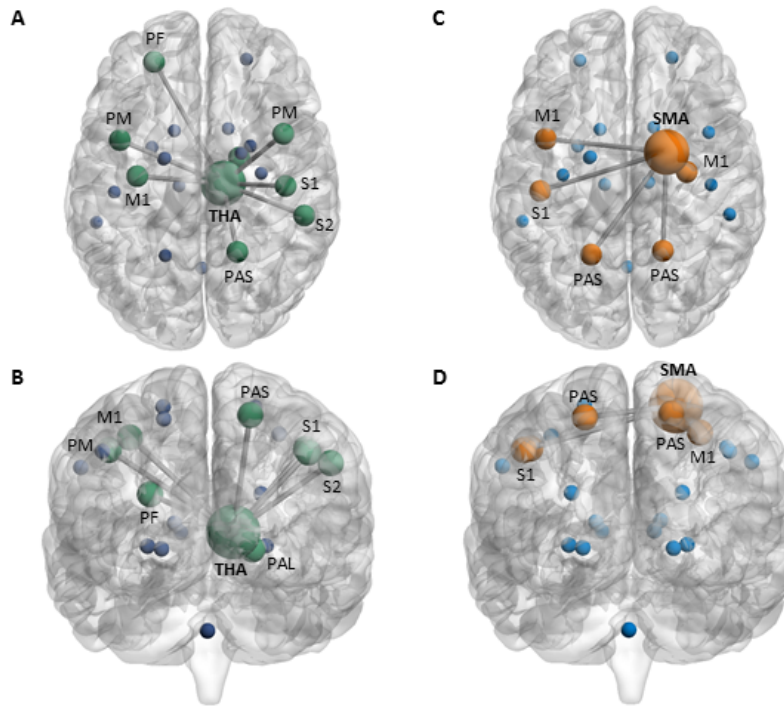


Figure 5. Higher dynamic functional connectivity of the thalamus and supplementary motor area in patients with upper and lower limb progression. The right thalamus and supplementary motor area (SMA), the main predictors of upper and lower limb progression respectively, showed higher dynamic functional connectivity with several sensorimotor regions at baseline. **A-B)** The thalamus showed higher dynamic connectivity with left prefrontal cortex (PF) ($p=0.02$), premotor cortex (PM) ($p=0.02$), primary motor cortex (M1) ($p=0.02$) and right premotor cortex (PM) ($p=0.01$), primary sensory cortex (S1) ($p=0.045$), secondary sensory cortex (S2) ($p=0.046$), posterior associative sensory cortex (PAS) ($p=0.02$) and pallidum (PAL) ($p=0.03$) compared to non-progressing patients. **C-D)** Patients with lower limb progression compared to non-progressing patients showed significantly higher dynamic connectivity between the right SMA and left premotor cortex ($p=0.01$), S1 ($p=0.02$), posterior associative sensory cortex ($p=0.03$) and right M1 ($p=0.02$) and right posterior associative cortex ($p=0.004$). Higher dynamic functional connectivity is reflected by the bigger nodes in green (upper limb disability progression) and orange (lower limb disability progression) and the dark edges between nodes. The smaller dots in blue reflect the remaining sensorimotor areas.

Discussion

This study investigated functional brain network changes related to upper and lower limb disability progression in a cohort of 214 people with multiple sclerosis, showing distinct functional network patterns for the upper and lower limbs. Higher temporal fluctuations in network efficiency of thalamus and supplementary motor area at baseline were precursor events of upper and lower limb progression respectively and both areas displayed widespread higher baseline dynamic functional connectivity with mostly cortical sensorimotor regions.

The role of the thalamus and SMA in disability progression

In this study the thalamus and the SMA showed the most substantial effects on disability progression. The thalamus is an important hub in the brain as it relays sensory information from the periphery to the cortical sensorimotor areas. Previous research showed higher thalamic FC²⁵ and centrality²⁶ in relation to clinical symptoms in MS. In addition, thalamic atrophy is present prior to clinical symptoms,²⁷ progresses rapidly throughout the disease course,²⁸ and strongly correlates with both disability progression^{29,30} and cognitive decline.²⁵ A recent study showed that thalamic atrophy was most severe in cognitively declining patients compared to patients who remained stable over time.³¹ Our study added to this existing knowledge by showing that disturbed functional network dynamics of the thalamus provided additional information beyond atrophy.

The SMA is involved in the control and planning of voluntary movement and is connected to cortical motor regions but axons also innervate distal motor units directly in the spinal cord. Our results showed that functional changes in the SMA, specifically higher dynamic network efficiency, are associated with disability progression of the lower limbs. Previous research has demonstrated connectivity³² and network efficiency⁷ alterations in the SMA in MS,^{7,32} related to overall disability. Interestingly, in this study, thalamic network dynamics were not identified as a predictor lower limb progression. Possibly lower limb function relies more on normal functioning of primary motor structures as well as the spinal cord. Upper limb functioning could then relate more strongly to more complex network structures like the thalamus, which might explain

why upper limb dysfunction has been difficult to grasp. Such pure motor structures like the primary sensorimotor cortices were also implicated in this study as we observed that primary somatosensory cortex dynamic network efficiency was disrupted at baseline for upper limb progressing patients, but was not implicated as a primary predictor of progression. Functional changes of the primary somatosensory cortices have been indicated by previous research, but these studies did not find relations with disability,⁶ which might be explained by the static instead of dynamic network approach used.

Dynamic sensorimotor network efficiency

We observed increases in dynamic network efficiency and connectivity in patients with disability progression at baseline, with no change in static measures, indicating that a more dynamic and perhaps unstable functional network topology may be a sign of imminent progression. In MS, the body of literature using dynamic connectivity and/or network approaches is still very small, and mostly focuses on cognition.^{10,11,26} A recent study on network dynamics related to cognition in MS identified that patients with cognitive impairment spent less time in a specific connectivity state, i.e. reoccurring functional network connectivity patterns over time, and switched less between states compared to preserved patients.¹¹ Similarly, another recent study reported reduced dynamic centrality of several brain RS networks in cognitively impaired patients with MS including the default mode network and sensorimotor network. It was hypothesized that a less dynamic network could perhaps be 'stuck' and therefore unable to perform as needed.¹⁰ We only observed higher dynamic network efficiency within the motor system at baseline, but focused on network efficiency and disability instead of centrality and cognition. How dynamic changes in connectivity, efficiency and centrality relate in the same subjects remains to be evaluated in future studies.

The specific role of the higher dynamics observed in this study could represent a compensatory mechanism in response to more severe structural damage. This compensation could falter at a certain point resulting in a destabilisation or even collapse of the network, after which a less dynamic network is visible, as observed in previous studies.^{10,11} Alternatively, the higher dynamics could merely represent a more unstable network, which could directly affect clinical progression. Specific patterns

evident in different stages of this disease was also seen in a static FC study showing increased FC in minimally disabled but decreased FC in highly disabled patients,⁷ similar to our finding of higher dynamic efficiency of the premotor cortex at baseline followed by a decline in dynamic efficiency over time. Interestingly, regions identified as the primary predictors of decline did not show additional changes over time, which might suggest that different anatomical regions could be at play at different stages of disability progression.

Limitations

This study has several key limitations to be considered. Firstly, the time interval between scan sessions varied minimally between subjects, but was included as a covariate in relevant analyses. Secondly, dynamic efficiency was calculated using a sliding window approach and not using methods without specific choices such as Markov models. Thankfully, previous work has shown limited influence of specific variations in window length.¹⁰ Finally, we chose efficiency as the primary network marker in this study, while many other graph metrics have been used previously suggested to indicate “importance” of regions, such as node centrality and network modularity, which might be explored in future studies.

Conclusions

This study showed that measures of dynamic network efficiency of key sensorimotor brain regions (thalamus and supplementary motor area) can predict the progression of upper and lower limb disabilities, respectively, in multiple sclerosis. This predictive value was not driven by the severity of structural damage to the brain. Future studies are now required to further study which functional network measures can improve patient specific prognosis and treatment decisions.

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Chapter 3

Functional activation and structural integrity in patients with minimal disability

An introduction to Chapter 3

The studies in second part of this thesis are part of the project 'Biomarkers of motor performance in multiple sclerosis'. The goal of this project was to characterize changes in gait and balance in multiple sclerosis (MS) patients with either minimal or no clinically identified lower limb dysfunction and to assess the neuronal processes at baseline using ultra-high field MRI. The combination of high quality 7T data and sensitive measures of motor behaviour derived from force tracking and spatiotemporal measures of gait provides with the opportunity to detect subtle changes in brain function and microstructure in relation to subtle changes in motor performance in patients with minimal motor disabilities.

1. Project design

Participant recruitment

In this project, 31 patients with MS (age 40.9 ± 9.6 ; 25 females) were recruited from the Royal Melbourne Hospital, Melbourne, Australia and 24 healthy controls (age 39.0 ± 7.2 ; 15 females). We excluded 3 healthy controls due to incidental findings, 1 patient due to suboptimal imaging quality and 2 patients were scanned using a previous protocol, which was optimized in 2016. Recruitment started in 2016 and was completed in 2019.

Inclusion criteria

- Clinically definite MS diagnosed using the McDonald criteria ¹
- Relapsing-remitting MS
- 18 years of age or over
- EDSS less than 4, and Kurtzke Functional Scale for pyramidal and cerebellar function less or equal 2

Exclusion criteria

- Contraindications to MRI such as claustrophobia or any metal in body from operations (e.g. screws, plates, staples or clips) or injury (e.g. metal fragments in eyes or body parts)
- Neurological condition other than MS

- Coexisting unstable cardiovascular disease
- Orthopaedic conditions causing disability of the lower limbs (including painful osteoarthritis)
- Recent laryngeal infection or laryngeal surgery
- Relapse in past 6 months

MRI and gait sessions

Participation involved an ultra-high field MRI session at the Melbourne Brain Centre Imaging Unit at the University of Melbourne, Melbourne and attendance at the MOVElab in Royal Park Rehabilitation Centre in Parkville, Melbourne.

Spatiotemporal gait assessment

Laboratory gait analysis involved over ground walking. To collect kinematic data of 37 spherical reflective markers placed on body landmarks an 8 infrared-camera VICON-MX system (Oxford, UK) was used. During the experiment participants were asked to walk on a 12 meter walkway while motion data was recorded. Participant were instructed to walk at a speed of 1.4 m/s with a tolerance of $\pm 5\%$ and verbal feedback was given if the walking speed needed to be modified. A fixed speed was chosen to better compare MS patients with healthy controls since most gait parameters are greatly affected by speed, which will introduce a bias in the analysis.(Cofré Lizama et al. 2011) From the kinematic data collected, spatiotemporal measures of gait were calculated using custom scripts including step length, stride length, step width, stance, swing, single and double support.

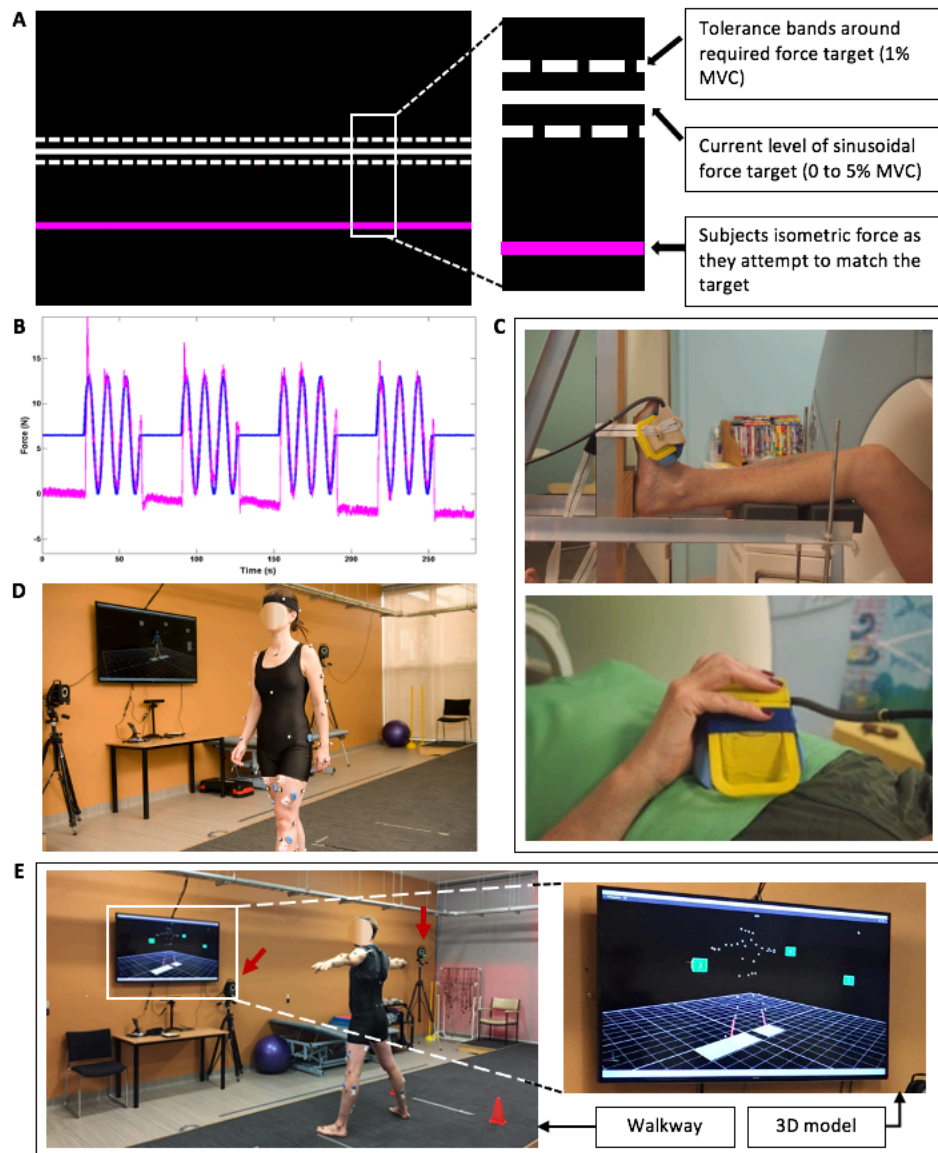


Figure 1. The visually guided force-matching task and spatiotemporal gait assessment. A) The task presented to the participants. Subjects were asked to place the pink line on the white line as accurately as possible by either squeezing and releasing their fingers gently or pulling their foot up and down slightly and to relax completely when the white line stops moving. B) A block design was used with 4 (45 seconds) contraction blocks interleaved with 5 (27 seconds) periods of rest. The blue line reflects the target force and the pink line the force produced by participant. C) MR compatible rig used to stabilize the foot and lower leg during lower limb movements. The cuff was either placed over the dorsum of the foot or held in the hand between the thumb and fingers while resting on the chest. D) Reflective markers were placed on specific landmarks and E) 8 infrared-camera VICON-MX system was used to track motion (red arrows) to assess the gait cycle, a repetitive pattern which starts from when the heel of one foot touches the ground and ends at the heel-strike of the same foot. The cycle involves steps and strides and can also be separated into different phases, including stance, swing, single and double support.

High field MRI session

MRI was conducted on a whole body Magnetom ultra-high field (7 Tesla) MRI system (Siemens Healthcare, Erlangen, Germany) with a 32-channel head coil (Nova Medical, Wilmington MA, USA). The 7 Tesla MRI session involved structural brain MRI including a high resolution MP2RAGE T1-weighted image (0.9mm isotropic) and 2D fluid attenuated T2-weighted images for volumetric metrics and lesion identification. High quality diffusion weighted imaging was acquired using multiple b-shells, 103 directions and a high spatial resolution of 1.24 mm isotropic, allowing to study brain microstructural pathology in detail. Functional data was acquired at high spatiotemporal resolution (1.2 mm isotropic, TR = 1700 ms) while participants performing a visually guided force-matching task, which involved a controlled, low force contraction of either the right foot or hand muscles (Figure 1).

Due to the complexity of the force tracking task, a practice session immediately prior to MRI scan session was held to explain and practice the task. This was done outside the scanner using a similar experimental setup as in the scanner (Figure 1).

Contributions to the project

I was involved in the optimisation of the ultra-high field MRI protocol using the 7 Tesla Human Connectome Project Lifespan protocol and improvement of the timing of the force-matching task. In addition, I was involved in the recruitment process for the project 'Biomarkers of motor performance in multiple sclerosis':

- Worked with neurologists at the Royal Melbourne Hospital to identify patients suitable for the study.
- Screened potential MS participants at the MS clinic.
- Recruited and consented eligible participants to the study.
- Healthy participant recruitment involved advertising (staff newsletter University of Melbourne) and word-of-mouth.
- If agreed to participate, an extensive MRI screening was done and discussed with the radiographer from the Melbourne Brain Centre Imaging Unit. If needed, medical notes were followed up.

- If eligible for MRI, the last step of recruitment involved scheduling appointments for an ultra-high field MRI session at the Melbourne Brain Centre Imaging Unit and attendance of the MOVElab Royal Park Rehabilitation Centre where one underwent a series of test including gait, dexterity and speech.

Contributions to Chapters 3.1 and 3.2

Study conceptualisation and design: M. Strik, J.J.G. Geurts, M.M. Schoonheim, S.C. Kolbe.

Acquisition of data: M. Strik, S.C. Kolbe, R. Glarin.

Data analysis: M. Strik, F.M.C. Boonstra, L.E. Cofré Lizama (Chapter 3.2), S.C. Kolbe.

Statistical analysis: M. Strik, S.C. Kolbe.

Drafting of a significant portion of the manuscripts: M. Strik, C.J. Shanahan, L.E. Cofré Lizama (Chapter 3.2), F.M.C. Boonstra, R. Glarin, T.J. Kilpatrick, A. van der Walt, M.P. Galea, J.J.G. Geurts, M.M. Schoonheim, J.O. Cleary, S.C. Kolbe.

2. Background information on ultra-high field MRI

The field of advanced imaging and methods is changing fast and over the last years remarkable progress has been made in ultra-high field imaging. While 1.5T and 3T MRI scanners are widely available, allowing us to investigate the human brain in both a clinical and research settings around the world, ultra-high field MRI availability is limited, but offers the opportunity to image the brain and spinal cord in-vivo in greater detail. Ultra-high field MRI allows to image the microstructure of the brain in great detail, allowing to improve detection of pathology^{2,3} and diagnosis in MS⁴.

A major advantage of ultra-high field MRI is the functional imaging. BOLD signals measured with functional MRI are an indirect measure of neural activity with poor spatial specificity and sensitivity at 1.5 Tesla and 3 Tesla scanners. Higher field strengths enable higher spatiotemporal resolutions, which improves the spatial specificity of BOLD activations and sensitivity to detect signal fluctuations, resulting in increased localizability and robustness. Imaging at faster temporal resolutions, i.e. shorter TR, improve the signal to noise ratio efficiency (SNR per unit time) as more temporal

information can be acquired and therefore improves the statistical power and modelling of the signal and thus detecting activation changes.^{5,6} The higher BOLD sensitivity as result of higher temporal resolutions was still granted at an adequately high spatial resolution with minimal physiological noise.⁶

While there are significant advantages of using ultra high field to investigate the functional activation in the brain, it also comes with some challenges including worse magnetic field inhomogeneities. Disturbances of the main magnetic field (B0) homogeneity can be caused by the scanner itself, susceptibility of the imaging subject or scanning at the edge of the field, whereas B1 inhomogeneities refers to the radiofrequency field non-uniformity, leading to image distortion and signal loss. The increased and decreased signal intensities and warping in the image are often seen around inferior parts of the brain including cerebellar area and inferior parts of the temporal lobes and frontal lobes. For the experiments undertaken in Chapter 3, these regions were not of major interest and the study specific templates used show high signal-to-noise ratio across the brain with limited dropout in motor areas and the connections between the regions of interest.

3. Experiments and hypothesis

Experiment 1 | Chapter 3.2

The aim of this study was to investigate functional brain changes related to upper and lower limb force tracking in minimally disabled MS patients using ultra-high field MRI.

- Assess task-based functional MRI activation patterns in MS patients compared to healthy controls.
- Assess motor performance during upper and lower limb force tracking (force error and lag).
- Investigate the relation between task activation and behavioural performance.

We hypothesized that MS patients with minimal loss of dexterity and mobility will display subtle changes in task activation and subtle impairments in motor performance.

Experiment 2 | Chapter 3.2

In this study we aimed to investigate axonal loss in sensorimotor pathways in minimally disabled multiple sclerosis patients in relation to precise kinematic measures of hand and foot control and gait.

- Compare the degree of white matter damage within the corticospinal tracts, interhemispheric primary sensorimotor connections and cerebellar-thalamic tracts using novel axonal markers of fibre density, fibre bundle morphology, and the combination of micro- and macrostructural fibre loss between MS patients and healthy controls.
- Assess the relation between the degree axonal loss and sensitive measures of upper and lower limb motor behaviour including measures derived from force tracking fMRI task and spatiotemporal measures of walking.

We hypothesized that axonal loss within the motor tracts will be evident early in the disease, prior to overt upper and lower limb disabilities, and associates with worse hand and foot task performance and altered patterns of gait.

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Chapter 3.1

Functional correlates of motor control impairments in multiple sclerosis: a 7 Tesla task functional MRI study

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Abstract

Upper and lower limb impairments are common in people with multiple sclerosis (pwMS), yet difficult to clinically identify in early stages of disease progression. Tasks involving complex motor control can potentially reveal more subtle deficits in early stages, and can be performed during functional MRI acquisition, to investigate underlying neural mechanisms, providing markers for early motor progression. We investigated brain activation during visually guided force-matching of hand or foot in 28 minimally disabled pwMS (Expanded Disability Status Scale (EDSS) < 4 and pyramidal and cerebellar Kurtzke Functional Systems Scores ≤ 2) and 17 healthy controls (HC) using ultra-high field 7-Tesla fMRI, allowing us to visualise sensorimotor network activity in high detail. Task activations and performance (tracking lag and error) were compared between groups, and correlations were performed. PwMS showed delayed (+124 s, $p=0.002$) and more erroneous (+0.15 N, $p=0.001$) lower limb tracking, together with lower cerebellar, occipital and superior parietal cortical activation compared to HC. Lower activity within these regions correlated with worse EDSS ($p=0.034$), lower force error ($p=0.006$) and higher lesion load ($p<0.05$). Despite no differences in upper limb task performance, pwMS displayed lower inferior occipital cortical activation. These results demonstrate that ultra-high field fMRI during complex hand and foot tracking can identify subtle impairments in lower limb movements and upper and lower limb brain activity, and differentiates upper and lower limb impairments in minimally disabled pwMS.

Introduction

Multiple sclerosis (MS) is a progressive disease of the central nervous system characterized by neuroaxonal inflammation, demyelination and degeneration. Motor impairments are common and disabling, and include tremor, muscle weakness, loss of fine motor control, ataxia and loss of mobility.¹ Walking difficulties are a typical hallmark of MS and occur in most people with MS (pwMS) (80%) within 10 to 15 years,² and upper limb impairments also eventually occur in up to 80% as well,³ yet are less well recognised and studied. Also, the degree of impairment of upper and lower limbs correlates only moderately,¹ suggesting at least partially divergent mechanisms of progression. Elucidating such mechanisms is important for formulating personalised treatment strategies, especially early in the disease when clinical signs of disability are minimal yet efficacious treatment has the best chance of avoiding significant neurological decline.

Functional MRI (fMRI) provides a useful means of studying changes in brain activity and putative neural compensations that contribute to the rate of progression of motor impairments. Task-based fMRI reveals regional brain activation indirectly via local changes in blood flow in the cortex driven by neural activity. Such studies have shown that changes in activation can predate changes in sensorimotor impairments and could provide early indicators of subsequent neurological decline.⁴ Multiple studies in MS have reported altered patterns of brain activation with increased activation seen in regions typically devoted to simple⁴⁻⁷ and more complex motor tasks^{4,5}.

However, key shortcomings of the extant literature lies in the type of task and the stronger focus on hand function⁵⁻⁷, which can easily be performed during scanning with simple tasks involving flexion and extension of the hand or fingers⁵⁻⁷. Lower limb studies are more difficult to perform and rare, mostly including clinically isolated syndrome (CIS)⁴ or primary progressive MS⁸. A recent study investigating lower limb position sense in early RRMS demonstrated functional activation changes with unchanged ipsilateral proprioception performance, suggesting potential cortical reorganization to perform correctly. Bilateral position sense as well as balance was altered and damage to corpus callosum was suggested to play a role.⁹ The motor system is an intricate system, besides

interhemispheric connections it is comprised of multiple pathways including the corticospinal tract and non-typical motor regions such as visual attention regions located in parietal and occipital cortices. As such, there is little known about the neural changes accompanying tasks involving complex motor control that provide better models of day-to-day motor functions.

This study aimed to map brain activations associated with the performance of a complex motor control task between hand and foot in healthy control subjects and pwMS and minimal clinical disability. The motor task involved a controlled visually-guided contraction of the ankle dorsiflexors (tibialis anterior) or finger/thumb flexors muscles^{10,11}, chosen to activate a broad network involved in integrating complex proprioceptive and visual inputs to accurately track a moving target. In addition, we used ultra-high field 7 Tesla MRI to perform simultaneous multi-slice fMRI at near anatomical MRI resolution (1.24mm isotropic) with high temporal resolution (1.7 sec) to detect subtle and focal activation changes that are undetectable by clinical field strength imaging systems. We hypothesized that in early disability stages, prior to more severe impairments, patients would display subtle deficits in the upper and lower limbs and altered functional brain activity patterns.

Methods

Participants

Twenty-eight participants with relapsing-remitting MS (RRMS) (mean age = 41.9 ± 10.0 years; 23 women) were recruited from the Royal Melbourne Hospital, Melbourne, Australia. Inclusion criteria at time of recruitment were: diagnosis with clinically definite MS according to the revised 2010 McDonald criteria¹², no relapses during the 6 months prior and no to minimal clinical disability, i.e. Expanded Disability Status Scale (EDSS) < 4, and Kurtzke Functional Systems Scale (FSS) for pyramidal and cerebellar function ≤ 2 .¹³ Exclusion criteria were: any neurological condition other than MS, orthopaedic conditions causing disability of the lower limbs (including painful osteoarthritis), and coexisting cardiovascular disease. Seventeen healthy controls (HC) (mean age = 39.3 ± 7.3 years; 10 women) with no reported history of neurological disorders were recruited for comparisons. PwMS received standard of care, were on disease modifying treatments and did not undergo a relapse at least 6 months prior testing. No treatment information was recorded for a single patient. Approval was obtained from the Melbourne Health Human Research Ethics Committee and all participants provided a voluntary written consent prior to participation.

MRI acquisition

Image acquisition was conducted using a whole body Magnetom 7T MRI system (Siemens Healthcare, Erlangen, Germany) with a single-channel transmit and 32-channel receive head coil (Nova Medical, Wilmington MA, USA). Two runs of functional MRI (fMRI) were acquired (upper and lower limb motor tasks performed in separate runs) with the following parameters: repetition time (TR)=1700 ms, echo time (TE)=34.4 ms, flip angle (FA)=65 degrees, multiband slice acceleration factor=6, Fat suppression, GRAPPA phase acceleration factor=2, 120 slices, 1.24 mm isotropic resolution, 165 volumes, image matrix = 168x168). In addition, a 3 dimensional T1-weighted structural image (MP2RAGE: TR=4900 ms, TE=2.89 ms, inversion time (TI)=700/2700 ms, FA=5/6 degrees, 192 slices, GRAPPA phase acceleration factor = 4, phase encoding direction = AP, voxel size = 0.9 mm isotropic, image matrix 256x256) was used for brain volumetric measurements. A fluid-attenuated inversion recovery (FLAIR) scan was acquired for

lesion identification (TR=10000 ms, TE=96 ms, TI=2600 ms, flip angle=145 degrees, 45 axial slices, GRAPPA=3, voxel size = 1.2x1.2x3.0 mm, image matrix 192x192).

Anatomical MRI processing

Anatomical MRI processing involved lesion detection and brain segmentation. White matter (WM) lesions were detected and marked on the MP2RAGE image using a semi-automated thresholding technique in MRICron (<https://www.nitrc.org/projects/mricron>) with the FLAIR image used as a reference to avoid inclusion of CSF. Next, lesion maps were used to lesion fill the MP2RAGE images using SLF software (<http://atc.udg.edu/nic/slfToolbox/index.html>) in Statistical Parametric Mapping (SPM, version 8), which were subsequently used as input for brain segmentation analyses using FreeSurfer version 6.0-patch (<https://surfer.nmr.mgh.harvard.edu>). From FreeSurfer analysis, the total white matter, cortical grey matter, deep grey matter and ventricular volumes were used for statistical analysis and normalised by total intracranial volume. We chose to also include spinal cord cross-sectional area as an important marker of spinal cord damage that could affect motor control. Spinal cord cross-sectional area at the level of the superior margin of the odontoid peg was marked manually and measured on MP2RAGE images using Horos (v4.0, www.horosproject.org) (Supplementary Figure 1).

Pre-MRI testing

Immediately prior to the MRI scan session, the upper and lower limb tasks were explained and practiced in a room outside the 7T scanner using a similar experimental setup as in the scanner. During this practice session, the maximum voluntary contraction (MVC) was performed to tailor contraction intensity to each participant's strength. The participants were asked to squeeze a sphygmomanometer cuff, used to measure force production, with their fingers or by isometric dorsiflexion against a constraint of their foot as strongly as possible for several seconds. All participants performed the task once at a maximum of 5% of MVC as it was found to activate the movement related network activation with minimal head motion.¹⁰

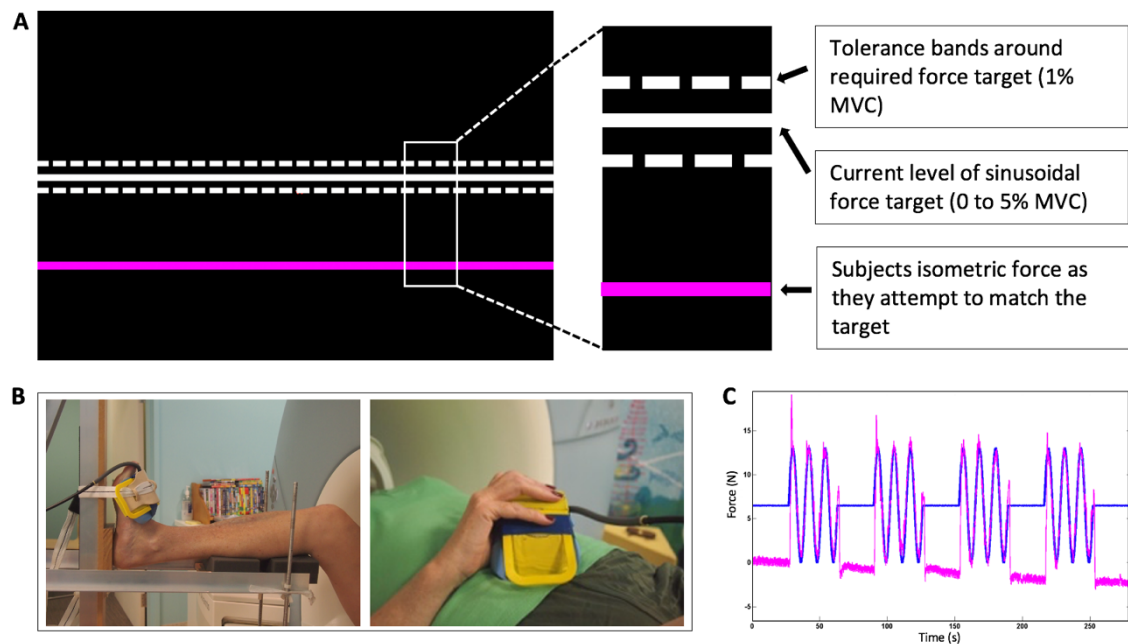


Figure 1. Force-matching task during functional MRI acquisition. **A)** The task presented to the participants. The white line slowly moved up and down during a task block and the participants were asked to follow the white line as accurately as possible with the pink by squeezing and releasing their fingers or pulling their foot up and down to make the pink line hit the white line. **B)** The MR compatible rig used to stabilise the foot and lower leg during the ankle motor task, i.e. ankle dorsiflexion to match a force indicator displayed to the participant, is shown on the left. The sphygmomanometer cuff was either positioned over the dorsum of foot close as possible to metatarsal heads without covering toes or held in the right hand between the thumb and fingers (shown on the right image). **C)** The blue line reflects the force in 4 contraction blocks interleaved with 5 periods of rest. The pink line is an example of the force produced by the participant to match the force indicator displayed on the black screen (white line).

Force matching task participant and apparatus setup

Participants performed a visually guided force-matching task adapted from a previous study and using same apparatus.¹⁰ A flowchart showing connectivity of the various apparatus used in the experiment is shown in Supplementary Figure 2. Setup of the participant in the scanner for upper limb task was that the cuff was held in the right hand between the thumb and fingers with the hand resting on the chest for support and to limit head movement (Figure 1). The lower limb task setup involved the following: the participant's right thigh was supported by the scanner bed, lower leg supported by a pillow and the plantar surface of foot resting against the footplate of a MRI compatible

rig, all to restrict movement around the ankle with minimal head motion. A sphygmomanometer cuff was positioned over the dorsum of the right foot as close as possible to the metatarsal heads without covering the toes (Figure 1). Between the upper and lower limb fMRI runs, an experimenter entered the MRI room and removed the cuff from the participant's right hand and positioned it over the foot. The hand run always preceded the foot run in order to minimise repositioning of the subject between runs when removing the cuff from the foot, which we found to cause head movement.

Visually guided force-matching motor task

The task involved a controlled, low force contraction of either the right ankle dorsiflexors (tibialis anterior) or the right finger/thumb flexor (hand) muscles in independent fMRI runs (Figure 1). Task design involved 4 (45 sec) contraction blocks interleaved with 5 (27 sec) periods of rest. During the contraction block, the force target moved up and down in a simple harmonic motion with a frequency of 0.125 Hz indicating a target contraction intensity from 0 to 5% of MVC. Participants were instructed to match the sinusoidal target force as accurately as possible. The onset of each contraction block was initiated by an MRI trigger to ensure accurate timing between the experiment and MRI data collection. Both target force and the force produced by the participant were displayed (Figure 1) at the rear of the scanner bore using an LCD projector and viewed through a head-coil mounted mirror. During the rest block, the target force did not move, and participants were asked to relax completely with their eyes open.

Upper and lower limb behavioural task parameters

Two behavioural task parameters (lag and error) were computed from the force data acquired during MRI testing using a custom MATLAB script. Lag was defined as the delay between the task cue and the response produced by the participants. Lag was calculated as the maximum cross-correlation between the cue and response time series, expressed in milliseconds (ms). The error was corrected for MVC and lag and was defined as the root mean square difference between the cue and the lag-corrected response time series, expressed in Newtons (N). The root mean square error is commonly used to

calculate the overall difference of measured signal (tracking) from an ideal signal (stimulus waveform).

Functional MRI pre-processing

Due to the cortical specificity and detailed activation patterns evident at 7T, we chose to create a study-specific template script using Advanced Neuroimaging Tool (ANTs, v.2.3.1, <http://stnava.github.io/ANTs/>) (Figure 2). Using 7 Tesla imaging and simultaneous multi-slice imaging allowed us to collect fMRI data at near anatomical resolution (1.25mm isotropic). Therefore, to avoid an unnecessary registration step and therefore avoid additional registration error, we chose to make a template directly from the fMRI data. This also allowed us to identify brain regions in the template that were affected by magnetic field distortions and signal loss (i.e. inferior temporal and orbitofrontal cortices) and to interpret our statistical maps in terms of these distortions. The template creation involves 4 iterative co-registrations of an example fMRI volume from each participant (HC and MS). Each co-registration iteration involved rigid body (n=20), affine (n=50) and deformable non-linear (n=20) co-registration calculations followed by averaging of the resultant warped images to create an updated target template. The final template was linearly registered to MNI-152 space for use in fMRI results overlays and coordinate reporting.

fMRI data were pre-processed with the following procedures using FEAT (FSL, FMRIB, Oxford, UK, v.6.0.3): rigid body head motion correction (FMRIB Linear Registration Tool), high pass filtering (cut off 100 s) and nonlinear spatial smoothing (SUSAN, extent threshold 2.5 mm) (Figure 2). Volumes affected by excessive motion (mean relative displacement from previous volume > 0.5 mm) were censored from subsequent analyses. The number of volumes deleted during lower and upper limb movements did not differ between HC (average upper = 4.2, lower = 4.5) and pwMS (average upper = 3.0, lower = 3.4).

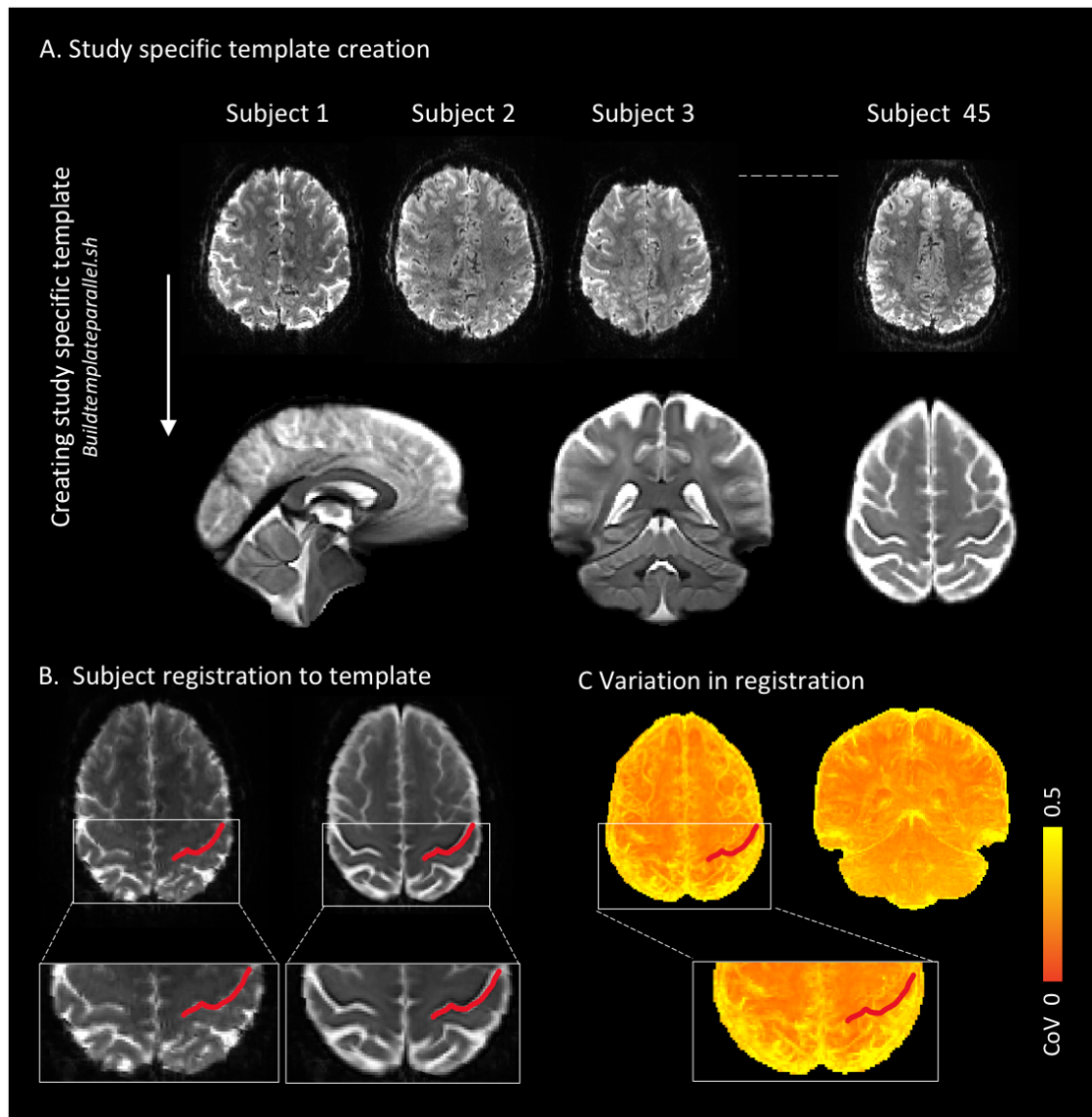


Figure 2. Study-specific template creation and fMRI processing steps. **A)** A study-specific template was created using Advanced Neuroimaging Tools (v.2.3.1, <http://stnava.github.io/ANTs/>) buildtemplateparallel script and involved 4 iterative co-registrations of an example fMRI volume from each participant (45 participants in total). **B)** An example of a participant to template registration with the red line indicating the central sulcus. **C)** The accuracy of template registrations of all subjects is visualised here. The coefficient of variations (CoV) of the fourth iteration was calculated with yellow reflecting high variability between participants, and towards orange more accurate overlap in registrations.

Functional MRI statistical analyses

We performed a three-level analysis (run, subject, group) using FEAT, model-based fMRI data toolbox based on general linear modelling. Three group-level analyses were

performed. Firstly, the main effect of the task was compared between HC and pwMS for upper and lower limbs separately including hand dominance as covariate. Where significant clusters were detected, post-hoc correlations were performed in SPSS to investigate the relationship between the max z-stat values extracted from the clusters and task performance (lag and force error), volumetrics using and clinical disability (EDSS) using partial (rank) correlation, including hand dominance, sex and age as covariates. To contextualize the effects of hand dominance, the group analyses were repeated without hand dominance as a covariate.

Secondly, differences in activation associated specifically with upper or lower limb task performance were investigated. For each participant, subject level analyses were performed on unthresholded run-level activation maps to calculate activation maps specific to either upper or lower limb movements using the following contrasts: upper > lower and lower > upper. Higher level analyses including hand dominance as covariate were subsequently performed to calculate significant mean activation maps for upper and lower limbs specifically, and to compare these activation patterns between groups.

All group level statistical analyses employed FLAME 1 mixed effects analysis, and significant voxels were identified using family-wise error correction with a threshold of $z\text{-stat} > 2.3$ and cluster wise significance of $p < 0.05$. A mask was used for all analyses to ensure that only brain regions found to be significantly active during the upper or lower limb tasks were included.

Statistical Analyses

Statistical analyses were performed using SPSS (version 26; IBM Corp., Armonk, NY, USA). Demographics, brain volumetrics and task parameters were compared between HC and pwMS with independent sample t-tests, or Mann-Whitney U tests when violations of the assumption of normality occurred (based on Kolmogorov-Smirnov testing and histogram inspection). Partial correlations were performed to investigate relations between the lag and force error of the upper limb and lower limb and between upper and lower limb task performance. In addition, partial correlations were performed between task performance measures, brain volumetrics and clinical

disability. If non-normally distributed, partial rank correlations were performed. For all correlation analyses, age, sex and hand dominance were included as covariates. P-values were corrected using False Discovery Rate (FDR) and were considered statistically significant < 0.05 .

Results

Demographics and clinical characteristics

The demographics, clinical measures and brain and lesion volumes are presented in Table 1. PwMS were all diagnosed with RRMS and had a mean disease duration of 6.4 years (SD=3.9), median EDSS of 1.5 (interquartile range (IQR)=1, 1.5). No differences between groups were observed for age, sex or hand dominance. Compared to HC, pwMS displayed significantly reduced white matter volume ($p=0.002$), cortical grey matter volume ($p=0.014$) and deep grey matter volume ($p=0.005$). No differences were observed for ventricular volume and spinal cord cross-sectional area.

Motor task performance

PwMS displayed lower hand ($p=0.013$) and foot ($p=0.001$) MVC and a higher force error (+0.15 N, $p=0.001$) and a longer lag (+124 s, $p=0.002$) during the lower limb task but not during the upper limb task (Table 1, Figure 3). In both pwMS and HC, no correlations between force error and lag were found for either upper or lower limbs. Upper and lower limb force error did significantly correlate in pwMS ($p=0.028$, $r=0.440$), but not in HC ($p=0.351$, $r=0.270$). Upper limb lag was significantly associated with lower limb lag in HC only ($p<0.001$, $r=0.902$), but not in pwMS ($p=0.332$, $r=0.202$) (Figure 3). No correlations were observed between lag and force error and task parameters and EDSS.

	Healthy controls (n=17)	MS patients (n=28)	p-value	p-value FDR _{correc}
Demographics				
Sex, F/M●	10/7	23/5	0.086	0.151
Age	39.29 (7.34)	41.75 (10.01)	0.385	0.415
Disease duration		6.50 (3.94)		
Dominant hand, R/L●	14/3	27/1	0.108	0.168
MVC hand	74.14 (16.53)	61.09 (13.51)	0.006	0.014*
MVC foot	73.80 (18.71)	51.69 (20.42)	0.001	0.014*
Disability scores				
EDSS ^a		1.5 (1.0, 1.5)		
Pyramidal FSS ^a		1.0 (0.0, 1.0)		
Cerebellar FSS ^a		0.0 (0.0, 1.0)		
Volumetric data				
WMVb, % ICV	33.48 (3.78)	29.74 (3.70)*	0.002*	0.007*
CGMVb, % ICV	31.55 (3.09)	29.45 (2.30)*	0.014*	0.028*
DGMVb, % ICV	5.94 (0.81)	5.29 (0.64)*	0.005*	0.014*
Ventriclesb, % ICV	2.06 (1.17)	2.65 (1.28)*	0.130	0.182
Lesion volume, log mm ³		3.10 (0.64)		
Spinal cord C1/C2 CSA, mm ²	69.81 (6.82)	70.71 (10.27)	0.752	0.752
Task parameters				
Upper limb lag, ms	184.71 (113.75)	216.07 (90.08)	0.201	0.256
Upper limb force error, N	0.31 (0.07)	0.34 (0.10)	0.296	0.345
Lower limb lag, ms	142.35 (116.49)	266.43 (120.68)*	0.002*	0.009*
Lower limb force error, N	0.30 (0.05)	0.45 (0.16)*	0.001*	0.007*

Table 1. Demographic, clinical, MRI and task performance characteristics. All values represent means and standard deviations, unless denoted otherwise. MS = multiple sclerosis; F = females; M = males; R = right; L = left; MVC = maximum voluntary contraction; EDSS = Expanded Disability Status Scale; FSS = Functional System Score; WMV = white matter volume; ICV = intracranial volume; CGMV = cortical grey matter volume; DGMV = deep grey matter volume. All variables were tested using independent samples t-test and values represent means and standard deviations unless denotes otherwise. ^aMedian and interquartile range. ^bBrain volumes were normalised for intracranial volume. *Chi-Square test. *Significant difference between people with multiple sclerosis and healthy controls at $p < 0.05$.

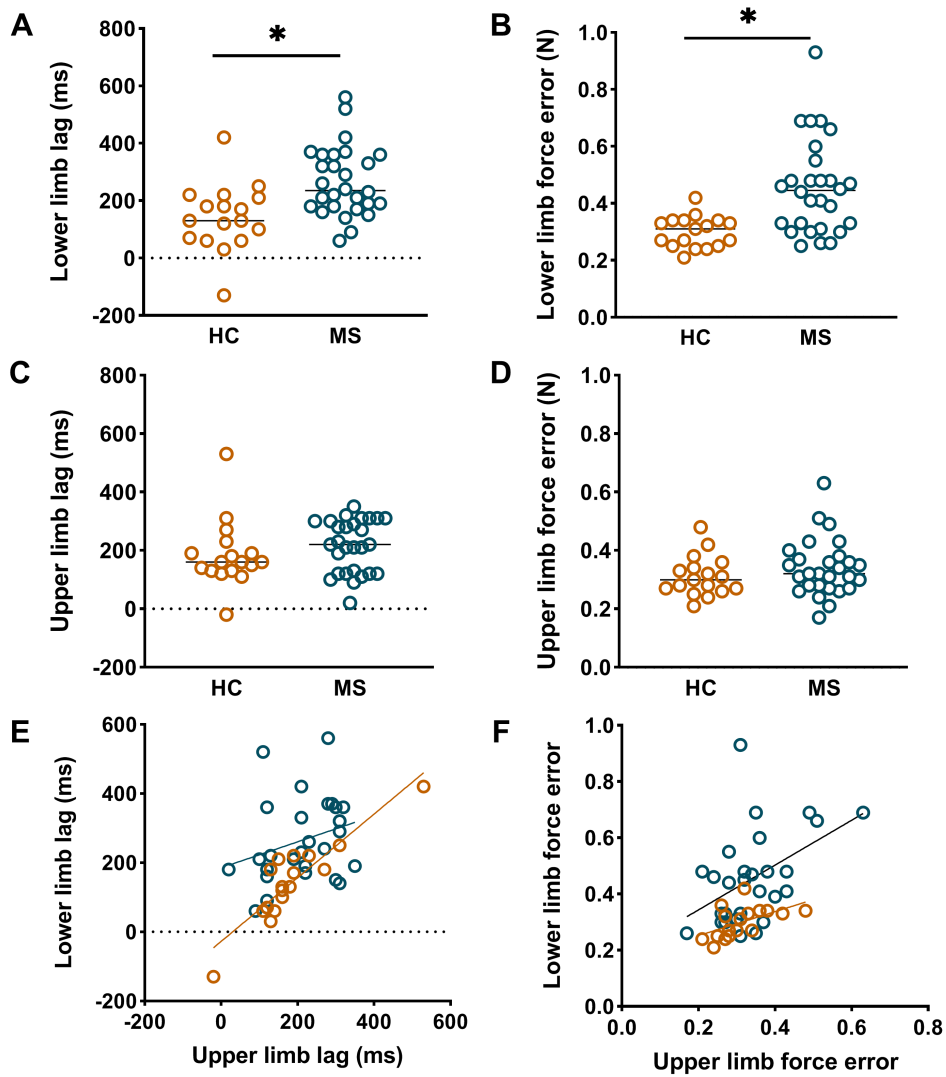


Figure 3. The functional force tracking task parameters. These plots demonstrate the group differences in task performance (lag and force error) and the correlations between the upper and lower limb performance. Each circle reflects a participant and the healthy controls (HC) are visualised in orange and people with multiple sclerosis (MS) in blue. **A-B)** People with MS showed significantly longer lag (+124 s, $p=0.002$) and higher force error (+0.15 N, $p=0.001$) during lower limb force tracking, compared to HC. **C-D)** No differences in performance were observed during upper limb movements. **E-F)** Upper and lower limb lag correlated significantly in HC ($p<0.001$, $r=0.902$), but not in MS ($p=0.322$, $r=0.202$). Force error of the upper and lower limb did significantly correlate in MS ($p=0.028$, $r=0.440$), but not in HC ($p=0.351$, $r=0.270$).

Disease related functional activation changes

Main patterns of activation are shown in Figure 4. During the lower limb task, pwMS displayed reduced activation in three ipsilateral (right) cerebellar clusters including I-IV, V, VI, Crus I/II, (Vermis) VI and brainstem, compared to controls (Figure 5, Table 2). In addition, hypoactivation was observed in three ipsilateral (right) cerebrum clusters within the superior parietal lobule (Brodmann Area (BA) 7 (objects in space in relation to body) and BA 5 (somatosensory processes and movement)), inferior temporal gyrus (BA 37 (processing colour, face/body recognition), BA 39 (body image) and V5/MT+ (visual motion)) and inferior occipital gyrus (object recognition, BA 37, V5/MT+) in pwMS compared to HC (Figure 5, Table 2). During the upper limb task hypoactivation was observed within the inferior occipital gyrus (visual processing area) in pwMS compared to controls.

Several significant post hoc correlations were identified between peak z-stat values extracted from significant clusters and task performance variables, lesion volume and EDSS (Figure 5). Lesion volume correlated negatively with peak z-stat values from the clusters within inferior temporal gyrus (BA 37, BA 39 and V5/MT+) ($r=-0.476$, $p=0.019$), superior parietal lobule (BA 5 and 7) ($r=-0.460$, $p=0.024$) and brainstem and cerebellar right I-IV and V ($r=-0.474$, $p=0.019$). A negative relation was found between clinical disability (EDSS) and peak z-stat values from the brainstem and cerebellar right I-IV and V cluster ($r=-0.417$, $p=0.034$). Lower limb force error correlated positively with the inferior occipital gyrus (V5/MT+ and BA 37) cluster peak z-stat values ($r=0.541$, $p=0.006$).

We observed a range of moderate correlations between brain volumetrics, motor performance and clinical disability (Supplementary Table 1).

Group analysis without hand dominance included as covariate resulted in lower cerebellar Crus I/II activation and increased cortical M1/premotor activation during lower limb movements, but no significant change was observed during upper force tracking (Supplementary Figure 3).

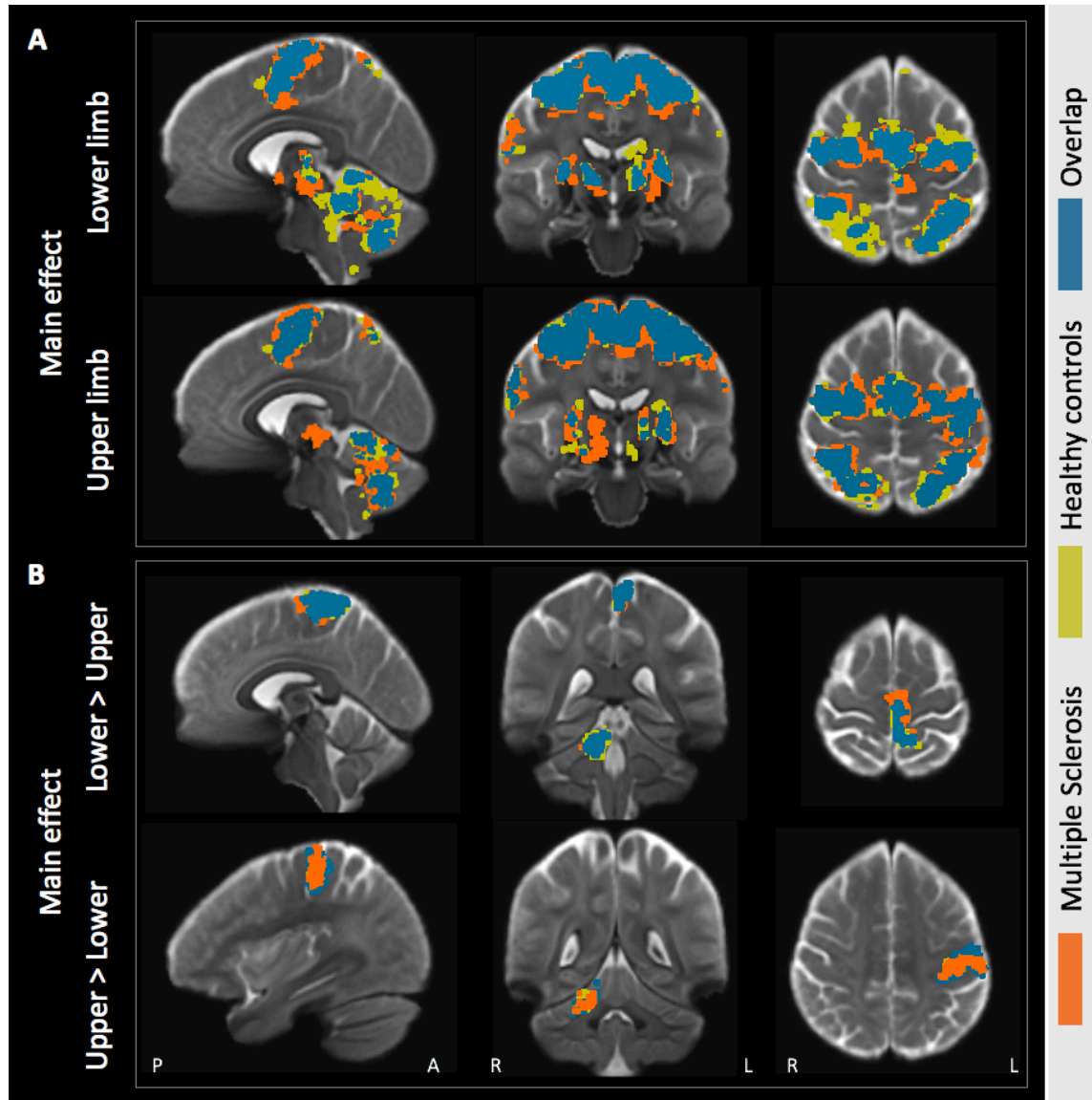


Figure 4. Average activation maps. A) The mean activation maps during the upper and lower limb visually guided force-matching tasks. **B)** Activation patterns specifically related to either lower limb (lower > upper) or upper limb (upper > lower) movements. The main effects for healthy controls (mustard yellow) and people with multiple sclerosis (orange) are presented in an overlaid manner with the overlap between groups presented in petroleum blue.

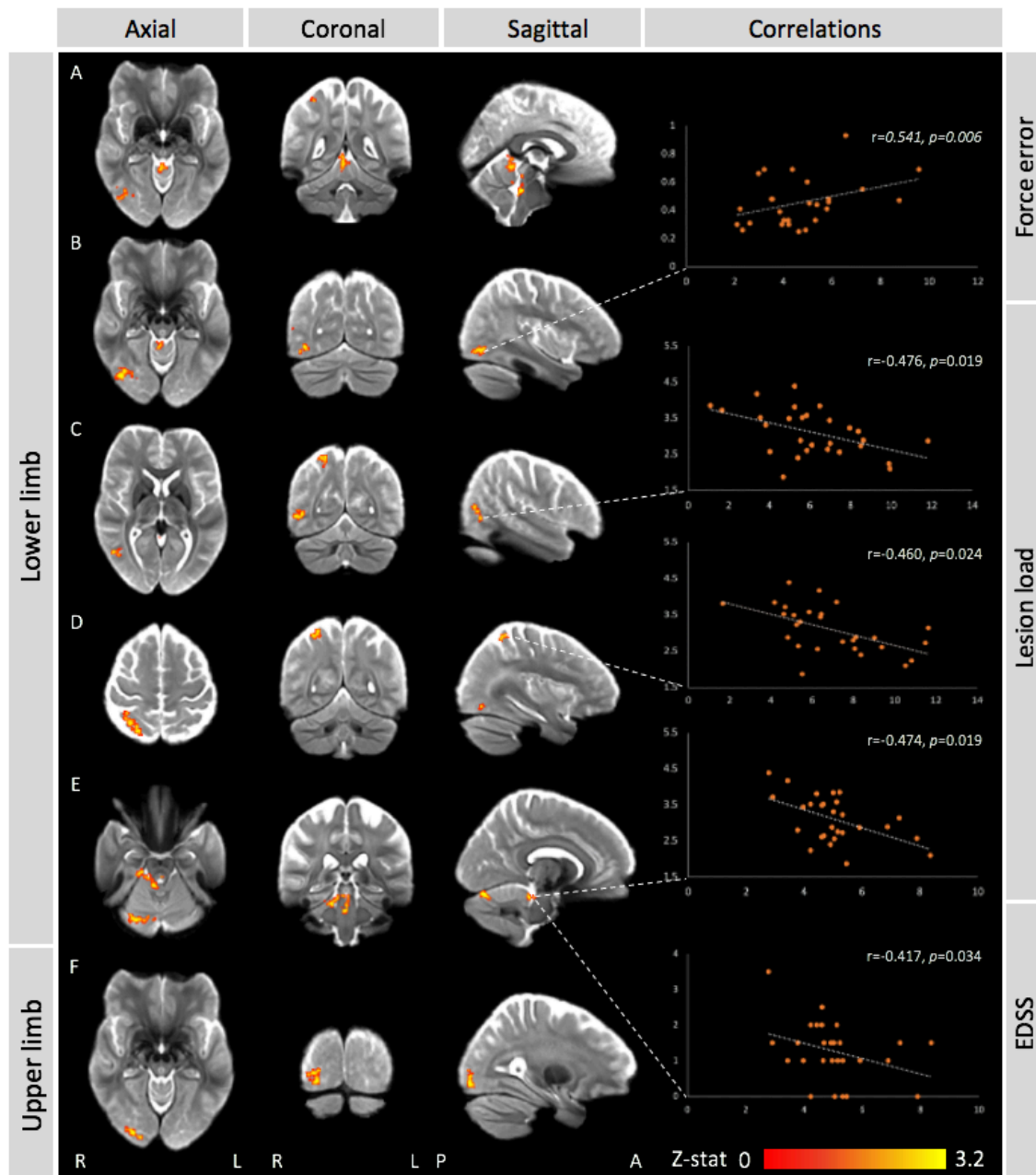


Figure 5. Functional brain activity changes in minimally disabled people with multiple sclerosis.

A-E) During the lower limb force-matching task, compared to healthy controls, people with multiple sclerosis (pwMS) displayed hypoactivation within several clusters located in right: **A)** cerebellum (I-IV), **B)** inferior occipital gyrus (V5/MT+ and Brodmann Area (BA) 37), **C)** inferior temporal gyrus (V5/MT+, BA 37 and 39), **D)** superior parietal lobule (BA 5 and 7), **E)** cerebellum (I-IV, VI, Vermis VI, Crus I/II) and brainstem (anterior of 4th ventricle). Correlations were found with lower limb force error, lesion load and EDSS scores. **F)** During upper limb force tracking, pwMS displayed lower brain activity within the inferior occipital cortex, compared to controls. Maximum z-stat values are plotted on the x-axis and variables of interest (EDSS, lesion load, task performance) on the y-axis.

Description anatomical regions (peak and overlap)	Peak MNI coordinates		
	X	Y	Z
Lower limb movements (MS < HC)			
Rostral area 7 (Superior Parietal Lobule)	23.84	-60.04	61.01
Intraparietal area 7 (Superior Parietal Lobule)			
Lateral area 5 (Superior Parietal Lobule)			
Caudal area 7 (Superior Parietal Lobule)			
Ventrolateral area 37 (Inferior Temporal Gyrus)	52.87	-63.98	-1.56
V5/MT+ (Lateral Occipital Cortex)			
Dorsolateral area 37 (Middle Temporal Gyrus)			
Ventrolateral area 37 (Inferior Temporal Gyrus)			
Rostrodorsal area 39 (Inferior Parietal Lobule)			
Inferior occipital gyrus (Lateral Occipital Cortex)	40.30	-79.71	-12.83
V5/MT+ (Lateral Occipital Cortex)			
Lateroventral area 37 (Fusiform Gyrus)			
Medioventral area 37 (Fusiform Gyrus)			
Cerebellar Right I-IV	2.81	-44.55	-3.50
Left I-IV			
Cerebellar Right VI	7.66	-79.77	-19.06
Right Crus I / II			
VI			
Vermis VI			
Brainstem (Anterior of 4th ventricle, reticular formation et al.)	5.88	-37.43	-33.12
Cerebellar right I-IV			
Cerebellar right V			
Medioventral area 37 (Fusiform Gyrus)			
Upper limb movements (MS < HC)			
Inferior Occipital Gyrus (Lateral Occipital Cortex)	25.03	-93.81	-10.71
Occipital Polar Cortex (Lateral Occipital Cortex)			

Table 2. MNI coordinates and anatomical description. This table shows the location of the max z-stat of the significant clusters (group differences) in MNI coordinates. Cortical and subcortical regions are localised using the Brainnetome atlas,¹⁴ cerebellar regions using SUIT (included in FSL 6.0.1, FMRI 2012, Oxford, UK, <https://fsl.fmrib.ox.ac.uk/fsl/>) and brainstem regions using Swenson atlas of the (<https://www.dartmouth.edu/~rswenson/Atlas/BrainStem/index.html>). LL = Lower limb; UL = Upper limb; BA = Brodmann area.

Activation patterns related to upper and lower limb movements specifically

In both pwMS and HC activity specific to lower limb movements was observed in the contralateral medial primary somatosensory cortex and M1, whereas activation patterns related to upper limb movements were observed in lateral precentral gyrus, consistent with known somatotopy. Within the cerebellum, activity specific to upper limb movements was seen largely in the ipsilateral V-VI and lower limb activation was observed in the ipsilateral I-IV. Activation within the primary sensorimotor cortices and cerebellum largely overlapped and were not significantly different between group (Figure 4).

Discussion

In the present study, we aimed to investigate the complex upper and lower limb motor control in pwMS with minimal sensorimotor impairments using ultra-high field fMRI and a visually-guided force-matching task. We found that pwMS displayed altered task performance and hypoactivation of several regions within the cerebellum (I-IV, V, VI, Crus I/II), occipital and superior parietal cortices during lower limb movements, compared to HC, with the level of activation related to task performance, clinical disability and lesion load. During the upper limb task, motor performance was preserved in pwMS, yet activity within the inferior occipital cortex was lower compared to controls.

Lower limb functional activation changes

We detected several small loci with hypoactivation within the cerebrum and cerebellum during a visually guided force matching in pwMS, compared to healthy controls. Activation changes were associated with lower limb force error and EDSS scores, suggesting a role of functional disturbances in clinical disability early in the disease. We observed cortical changes during lower limb movements in visual-motor regions including BA 5 and 7, involved in somatosensory association processes including visuomotor attention and location of objects in space (integration proprioception and vision), as well as during foot and hand movements in the occipital cortex including BA 37, occipital pole (primary visual processes) and middle temporal visual area (processing of motion). In addition to cortical changes, we observed reduced cerebellar Crus I/II and cerebellar I-IV, V and VI activation during lower limb force tracking. Cerebellar Crus I/II are suggested to be primarily involved in higher-level cognitive processes. These regions are included in cerebral networks involving cognitive control¹⁵ and activation within Crus I/II was reported higher during a cognitive compared to a motor task¹⁶. Besides cognitive control, lesions in these regions have been associated with worse motor task performance in stroke patients.¹⁷ Cerebellar I-IV and VI are suggested to be primarily involved in sensorimotor processes. Lobule IV and VI, along with V VIIIB map to the somatosensory cerebral network encompassing hand and foot representations.¹⁵ Overall, the areas implicated by our study include both sensorimotor and visual-motor

regions, suggesting that motor control impairments in MS are related to dysfunctions in visuomotor integration.

This is one of the first studies to use a complex motor control task in MS.^{18,19} Previous functional task imaging studies have used simple motor tasks such as finger tapping^{4–7,20,21} and foot flexion and extension or increased complexity by performing a task with both hand and foot⁴. Using simple motor tasks resulted in increased activation in motor regions such as M1,^{4,5,20–22} often interpreted to be a compensatory mechanism in response to the pathological damage, and reduced cerebellar activation^{4,7,8,23}. To determine whether changes in activation are adaptive or maladaptive and are predictive of progressive decline would require longer term follow-up that was outside the scope of the current study. In contrast to more simple tasks, increasing complexity resulted in more widespread activation in supplementary motor area, thalamus, and frontal regions. Compared to controls, CIS patients displayed cortical and subcortical activity changes, but no cerebellar differences,⁴ potentially due to clinical field strength used. Imaging at higher field strengths and a task involving complex motor control revealed more subtle deficits in early stages, providing markers for early motor progression.

Pathological factors influencing lower limb functional impairments

The aim of this study was to investigate functional mechanisms underlying impairments in movements, so we did not undertake detailed structural investigations. However, we did identify gross atrophy of cortical and deep grey matter and white matter and the extend of cortical and cerebellar activation was negatively correlated with the lesion load, i.e., the higher the lesion volume the lower the brain activity. These results suggest that structural damage within the brain could explain activation and behaviour changes observed during lower limb movements specifically. Besides the thalamus and precentral gyrus (M1), areas important in motor control, the cerebellum is one of the first regions to become atrophic in relapse-onset disease,²⁴ possibly affecting brain function. The cortical regions on the other hand were not within the somatotopically mapped lower limb regions identified in the lower > upper contrast, but in visuomotor integration areas and therefore cannot be attributable to reduced sensory input or motor output to lumbar spinal cord motor neurons. In addition, we did not observe

differences in cervical spinal cord cross-sectional area between patients and controls. We were unable to fully characterise cervical cord lesions due to the limited field of view afforded by the use of a transmit/receive head coil necessary for 7T imaging. However, lesions are primarily observed in the cervical spinal cord (59%),²⁵ clearly affecting both upper and lower limb function and early MS spinal cord lesions are often asymptomatic²⁶ and do not predict disability progression²⁷.

Lower and upper limb performance in pwMS with minimal impairments: different processes?

In our study using a motor control task we were able to differentiate between upper and lower limb performance and brain activity. PwMS displayed delayed and more erroneous force tracking compared to HC for lower but not upper limbs. Whereas during upper limb force tracking only a single region differed between groups, multiple clusters were observed during lower limb movements in pwMS. These lower limb changes were also clinically meaningful as activation correlated significantly with markers of clinical disability including EDSS severity and lesion load, these associations were not found with upper limb activation. Together, this suggests that, at least in part, divergent pathological processes drive progression of upper and lower limb impairments. This interpretation is supported by a previous study showing only a moderate correlation between a hand-to-mouth task and walking revealed using advanced kinematic analyses.¹ Also, clinical observations of pwMS often report impairments in lower limb function early in the disease.^{28–30} Potentially, lower limb impairments precede upper limb disability, which would require a longitudinal design to investigate.

Force-matching for studying visuo-motor integration in pwMS

Here we used a force matching task to elicit submaximal contractions of the tibialis anterior (lower limb task) or hand flexion (upper limb task) performed in separate runs adapted from a study.¹⁰ With this task we aimed to model not only basic sensorimotor behaviour, but complex motor control behaviours required for daily life. Similar tasks have been used previously to study the basic neurophysiology involved in visuomotor control,^{11,31} showing that visual feedback resulted in stronger activation, that scaled with MVC. Stronger correlations were found between activation and behavioural

performance when participants performed low MVC contractions (10%), potentially due to greater neuronal recruitment required for finer motor control. We used an MVC of 5% that was selected to both increase the difficulty of the task, and to minimise head motion, resulting in the activation of a broad network of visual and oculomotor, premotor and motor areas. We conclude that force-matching tasks provide a relatively straightforward means to investigate complex visuomotor integration.

Methodological considerations

In this study we used ultra-high field MRI with the goal of detecting more specific activation loci with better accuracy due to high sensitivity. For low resolution fMRI data, large spatial smoothing kernels are commonly used to enhance signal-to-noise ratio and to minimise the effects of misregistration. However, this also reduces accuracy as it can lead to incorrect estimation of true spatial localisation and therefore type-I error.^{32,33} We therefore chose to create a study-specific template to improve the accuracy of spatial co-registrations and therefore we were able to use very limited smoothing in our data (2.5 mm). To conclude, using ultra high field and a study-specific template led to identification of detailed activation patterns specific to movement without losing sensitivity.

The comprehensive study design involving both MRI testing and laboratory gait analysis in two different locations resulted in long assessment times and a relatively small sample size. Future studies including a larger sample size would be recommended to confirm findings. Despite the sample size, we used focused inclusion criteria which resulted in a relatively homogeneous group of patients and the motor performance assessments at 7T were sensitive enough to detect subtle changes in brain activity in patients with minimal disabilities. While there are significant advantages for the use of ultra-high field for functional MRI such as higher raw signal to noise ratio,³⁴ stronger susceptibility effects,³⁵ and signal more closely attributable to venules than feeding veins³⁶), worse magnetic field inhomogeneities can lead to signal dropout and warping around the inferior temporal and orbitofrontal cortices. For the current study these areas were not of major interest and the study-specific template demonstrated the very high signal-to-

noise ratio across the brain with very little signal loss in areas of interest such as the sensorimotor cortices and cerebellum.

Conclusion

These results demonstrate that ultra-high field fMRI during complex hand and foot tracking can identify subtle impairments in movement and brain activity in otherwise minimally impaired pwMS, with differential effects for upper and lower limb impairments. Minimally disabled pwMS displayed altered lower limb movements and brain activation with preserved upper limb function but altered brain activation. Such techniques might find use in tracking and predicting the emergence of impairments in mobility and dexterity that reflect some of the most disabling symptoms of MS.

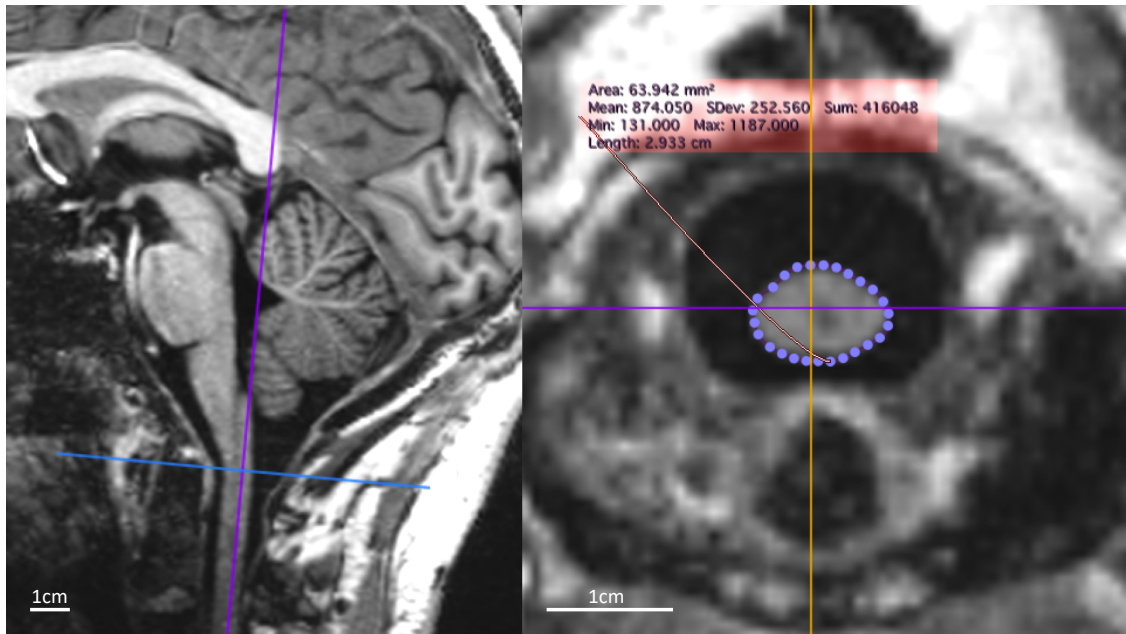
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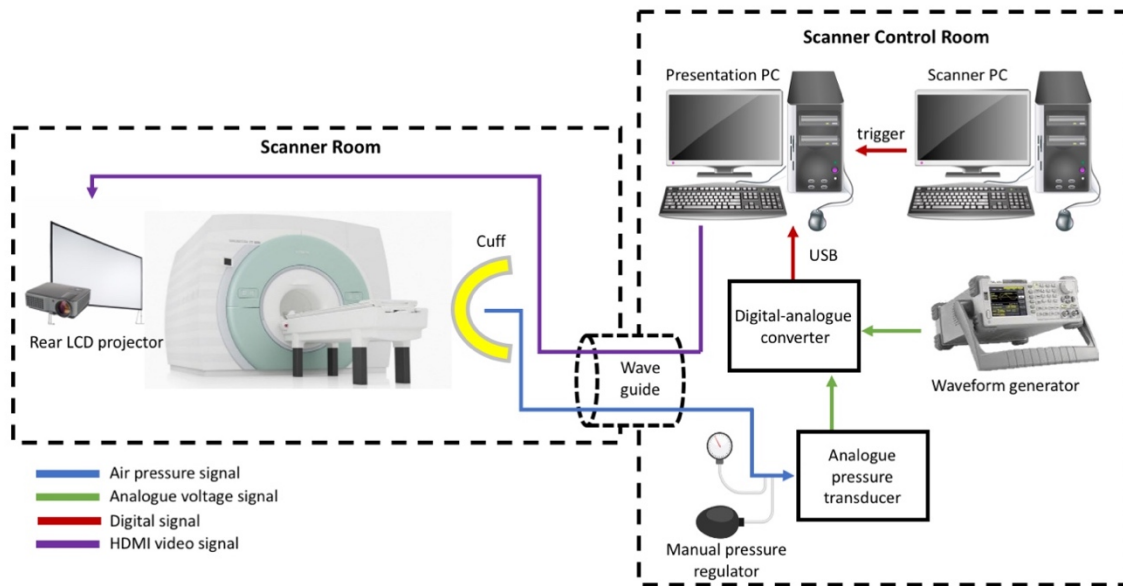
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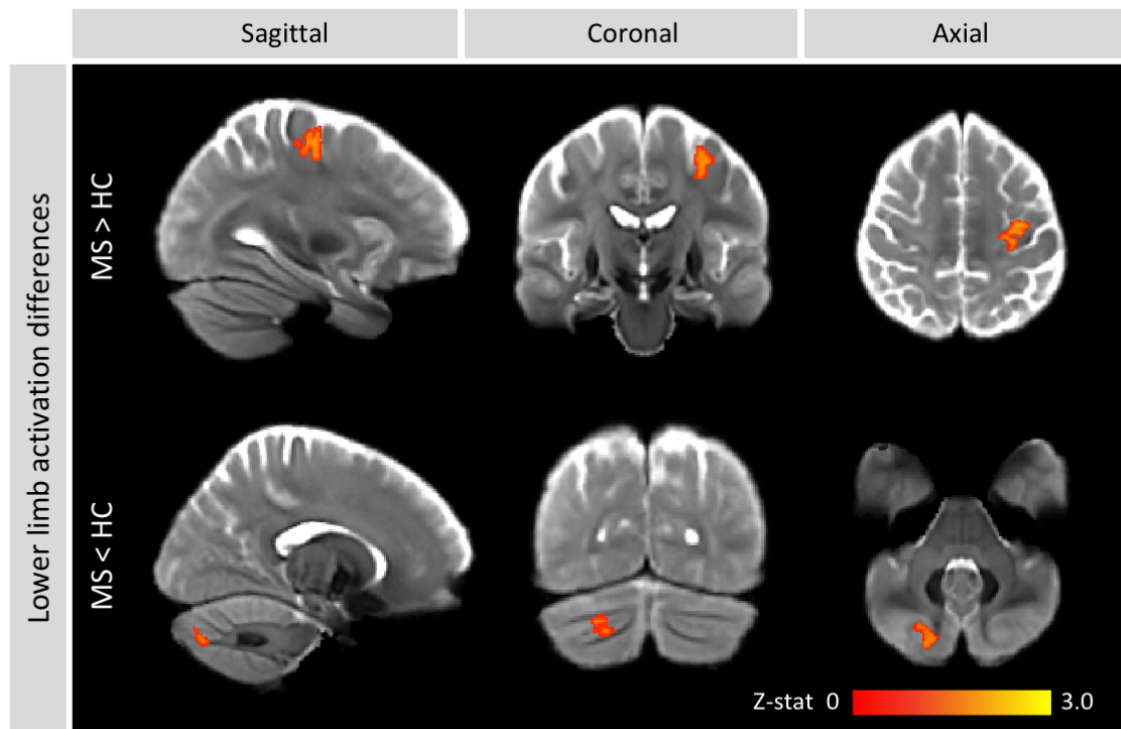
Supplementary material



Supplementary Figure 1. Spinal cord cross-sectional area measurement. The spinal cord region of interest for was identified on the MP2RAGE scan (left image). A transverse slice was selected (blue line) at the rostral margin of the odontoid peg orthogonal to the caudally extending spinal cord (purple line). This slice was used to manually outline the spinal cord and to record the cross-sectional area.



Supplementary Figure 2. The apparatus setup. This flowchart demonstrates the connectivity of the various apparatus used in the experiment. The sphygmomanometer cuff was connected to an air-tight plastic tube and linked through the wave guide to the pressure transducer unit. The pressure transducer was fitted with a manual pressure regulator that was used to fill the apparatus with a reasonable air pressure to allow sensitive pressure change measurements (~40 psi). The transducer was connected to the analogue to digital converter (ADC) via a BNC connected cable. An analogue voltage waveform generator was also connected to the ADC to generate the stimulus waveforms to be tracked. The ADC was connected to the presentation PC via a USB connection. Both target force and the force produced by the participant were displayed (Figure 1) at the rear of the scanner bore using an LCD projector and viewed through a head-coil mounted mirror.



Supplementary Figure 3. Group comparisons without hand dominance as covariate. To contextualise the effect of adding hand dominance to the analysis, we performed the group analysis without hand dominance. During the lower limb force-matching task, greater activation was observed in the contralateral primary motor cortex and premotor cortex and lower activation within cerebellum Crus I/II in people with multiple sclerosis (MS) compared to healthy controls (HC). No differences were observed between groups during upper limb force tracking.

		Upper lag	Upper force error	Lower lag	Lower force error	EDSS
WMV^a	<i>r</i>	-0.143	-0.421	0.254	0.190	-0.532
	<i>p</i>	0.504	0.040	0.231	0.374	0.007
DGMV^a	<i>r</i>	-0.037	-0.284	0.160	0.052	-0.487
	<i>p</i>	0.864	0.179	0.455	0.809	0.016
CGMV^a	<i>r</i>	-0.205	-0.666	0.028	0.124	-0.454
	<i>p</i>	0.336	0.000	0.898	0.565	0.026
Ventricles^a	<i>r</i>	-0.213	0.361	-0.134	0.079	0.032
	<i>p</i>	0.317	0.083	0.534	0.714	0.881
Spinal cord, mm²	<i>r</i>	0.158	0.046	0.112	0.108	0.033
	<i>p</i>	0.461	0.830	0.603	0.616	0.876
Lesion volume, log mm³	<i>r</i>	0.004	0.324	-0.008	-0.031	0.248
	<i>p</i>	0.986	0.122	0.969	0.887	0.232

Supplementary Table 1. Correlations between brain volumetrics, behavioural performance and clinical disability in people with multiple sclerosis. At an uncorrected level, several moderate correlations were observed in people with multiple sclerosis. The correlation between cortical grey matter volume (CGMV) and upper limb force error survived False Discovery Rate correction (in bold, $p < 0.05$). Abbreviations: EDSS = Expanded Disability Status Scale; WMV = white matter volume; DGMV = deep grey matter volume. ^aBrain volumes were normalised for intracranial volume.

Chapter 3.2

Axonal loss in major sensorimotor tracts is associated with impaired motor performance in minimally disabled multiple sclerosis patients

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Abstract

Multiple sclerosis is a neuroinflammatory disease of the CNS that is associated with significant irreversible neuro-axonal loss, leading to permanent disability. There is thus an urgent need for *in-vivo* markers of axonal loss for use in patient monitoring or as endpoints for trials of neuroprotective agents. Advanced diffusion MRI can provide markers of diffuse loss of axonal fibre density or atrophy within specific white matter pathways. These markers can be interrogated in specific white matter tracts that underpin important functional domains such as sensorimotor function. This study aimed to evaluate advanced diffusion MRI markers of axonal loss within the major sensorimotor tracts of the brain, and to correlate the degree of axonal loss in these tracts to precise kinematic measures of hand and foot motor control and gait in minimally disabled people with multiple sclerosis. Twenty-eight patients (Expanded Disability Status Scale < 4, and Kurtzke Functional System Scores for pyramidal and cerebellar function ≤ 2) and 18 healthy subjects underwent ultra-high field 7 Tesla diffusion MRI for calculation of fibre-specific measures of axonal loss (fibre density, reflecting diffuse axonal loss, and fibre cross-section reflecting tract atrophy) within three tracts: cortico-spinal tract, interhemispheric sensorimotor tract, and cerebello-thalamic tracts. A visually-guided force-matching task involving either the hand or foot was used to assess visuo-motor control, and three-dimensional marker-based video tracking was used to assess gait. Fibre-specific axonal markers for each tract were compared between groups and correlated with visuomotor task performance (force error and lag) and gait parameters (stance, stride length, step width, single and double support) in patients. Patients displayed significant regional loss of fibre cross-section with minimal loss of fibre density in all tracts of interest compared to healthy subjects (family-wise error corrected p -value < 0.05), despite relatively few focal lesions within these tracts. In patients, reduced axonal fibre density and cross-section within the cortico-spinal tracts and interhemispheric sensorimotor tracts was associated with larger force tracking error and gait impairments (shorter stance, smaller step width and longer double support) (family-wise error corrected p -value < 0.05). In conclusion, significant gait and motor control impairments can be detected in minimally disabled people with MS that correlated with axonal loss in major sensorimotor pathways of the

brain. Given that axonal loss is irreversible, the combined use of advanced imaging and kinematic markers could be used to identify patients at risk of more severe motor impairments as they emerge for more aggressive therapeutic interventions.

Introduction

Multiple sclerosis (MS) is an autoimmune mediated disorder of the central nervous system, common in young adults. Over the longer term, many patients develop progressively worsening walking and dexterity impairments that ultimately affect performance of daily-life activities substantially.¹ Progression of motor disabilities is associated axonal loss in clinically eloquent white matter pathways such as the cortico-spinal tract (CST)², with significant axonal loss occurring prior to clinical progression. Thus, identifying patients at greatest risk of progressive sensorimotor decline requires both *in-vivo* markers for axonal loss and sensitive functional markers that can detect subtle impairments and walking and hand function that predate overt clinical impairment.

Axonal loss can be detected *in vivo* using diffusion MRI that is sensitive to the microscale motion of water molecules in tissue that are restricted in healthy white matter due to the multitudinous collinear membranes. In white matter structures containing a single fibre population, diffusion can be adequately modelled using a tensor ellipsoid (diffusion tensor imaging, DTI). Previous studies have reported loss of diffusion anisotropy in important sensorimotor pathways such as the CST²⁻⁴, corpus callosum^{5,6} and thalamus,^{7,8} and DTI metrics have been shown to more strongly correlate with neurological disabilities than conventional MRI measures such as lesion load⁹. Recently, it has become recognised that DTI is insufficient to model axonal loss due to: (a) sensitivity to crossing or merging fibres that include >90% of brain voxels,¹⁰ and (b) sensitivity to non-axonal cellular pathologies such as increased extra-cellular fluid and non-neuronal cells such as inflammatory cells¹¹. These limitations have led to the development of more advanced diffusion MRI acquisition and signal modelling techniques to allow estimation of tract-specific axonal fibre density and fibre cross-section using fixel-based analysis (FBA).¹²⁻¹⁵ In multiple sclerosis, fixel-based analysis has been shown to more sensitively and specifically identify microstructural changes in damaged white matter (WM) compared to DTI.^{16,17} Moreover, using the high signal-to-noise ratio (SNR) afforded by ultra-high field (7 Tesla) MRI and simultaneous multi-slice imaging techniques, diffusion MRI can be acquired with spatial resolution approaching anatomical imaging and high angular resolution within reasonable scan times (~10mins).

Kinematic analyses of video or force tracking can reveal subtle changes in upper and lower limb motor control before clinical impairments become overt.^{18,19} Previous studies have shown that DTI changes within the corticospinal tracts are associated with impairments in simple gait assessments such as timed walks, strength or up and go tests.^{3,20,21} Reduced strength²⁰ and slower walking speed were associated with reduced CST fractional anisotropy²⁰ and increased radial and axial diffusivity^{3,21}. These walking metrics explained more variance in CST diffusivity than basic clinical measures such as EDSS, age and gender.²⁰ Another study did not find a relation between timed up and go and walking with DTI metrics, but reported a link with grey matter volume loss.²² However, there has been limited work comparing modern and more sensitive kinematic assessments such as 3D motion or force tracking with sensorimotor tract damage,³ particularly in minimally disabled patients. So it is not clear whether the additional sensitivity to change of these new techniques relates to physiological (potentially modifiable through physical or symptomatic treatment) or structural (irreversible but can be delayed through more aggressive disease modifying treatment) brain changes. Sensitive tracking of both functional impairments and associated brain changes is therefore important for assisting treating clinicians to better manage the progression of the disease.

This study therefore aimed to examine relationships between advanced diffusion MRI markers for axonal loss in major sensorimotor pathways of the brain and kinematic analyses of gait and upper/lower limb motor control in people with multiple sclerosis and minimal clinical disability. We hypothesized that patients would display subtle impairments in gait and motor control that would be associated with axonal loss in major sensorimotor tracts.

2. Methods

2.1. Participants

This study was conducted in accordance with the Declaration of Helsinki with the protocol and informed consent forms approved by the Human Research Ethics Committees of the Royal Melbourne Hospital. All participants provided voluntary, written consent to one of the investigators. We recruited 28 multiple sclerosis patients (age = 41.9 ± 10.0 years; 23 females) from the Royal Melbourne Hospital, Melbourne, Australia, and 18 healthy participants (age = 39.2 ± 7.1 years; 10 females) through word-of-mouth advertising. At the time of assessment, all patients were diagnosed with clinically definite multiple sclerosis according to the revised criteria of McDonald.²³ All patients presented with minimal disability (EDSS < 4, and Kurtzke Functional Scale for pyramidal and cerebellar function ≤ 2). No patients performed physical rehabilitation and or specific physical activities before the study enrolment. Patients were excluded with a disease duration of more than 20 years, evidence of a clinical relapse in the 6 months prior, neurological conditions other than multiple sclerosis, orthopaedic conditions causing disability of the lower limbs, or cardiovascular diseases.

2.2. Upper and lower limb tracking task

Two behavioural task parameters were computed from data acquired during a functional MRI (fMRI) acquisition performed during the same MRI acquisition session (Supplementary Figure 1) with methods related to these tasks reported elsewhere.²⁴ To briefly reiterate, participants performed two runs of a visually guided force-matching task that involved a controlled, low force contraction of either (i) the ankle (the tibialis anterior) or (ii) hand muscles. The lag and force error of the upper and lower limb tasks was calculated from the force data collected during testing. Lag was the delay between the force target stimulus and the actual force response, calculated using the maximum cross-correlation between stimulus and response time series (milliseconds). The force error, a measure of accuracy and sensorimotor integration error, was the root mean square difference between the stimulus and the lag-corrected response time series (Newtons).

2.3. Spatial-temporal gait assessments

Gait assessments were performed on the same day as MRI (n=9), or within a median time of 14.5 days, (IQR=0.75-151.50) from the MRI scan (n=19). For gait assessments, reflective markers were placed on participant's specific body landmarks following the full-body plug-in-gait model (VICON, Oxford, UK) (Supplementary Figure 1). We asked participants to walk barefoot on a 12 m long walkway at 1.4 ms^{-1} with a tolerance of $\pm 5\%$ of the target speed, which was monitored using two timing gates separated by 3 m in the middle of the walkway. Participants were requested to modify walking speed, if necessary, by providing verbal feedback on the speed recorded. All walking trials were recorded until five left and five right gait cycles at each speed were collected. Marker kinematics was collected using an 8-MX VICON camera system and Nexus 2 software (VICON, Oxford, UK). Raw data was exported and analysed using a customized Matlab R2020a (Mathworks, Natick, MA, USA) script to calculate basic spatiotemporal measures of gait based on feet markers (heel, ankle and toe) including: stride length; step width; and time percentage spent in single and double support and stance gait phases. Markers at the sacrum level were used to calculate overall gait speed. For each participant, spatiotemporal measures obtained from five left and five right gait cycles at each speed were averaged for further statistical analyses.

2.4. Imaging acquisition

All participants were imaged using a whole-body Siemens MAGNETOM 7 Tesla MRI system (Siemens Healthcare, Erlangen, Germany) with a combined single-channel transmit and 32-channel receive head coil (Nova Medical, Wilmington MA, USA). High resolution diffusion weighted imaging (DWI) was acquired using a simultaneous multi-slice 2D spin-echo EPI sequence based on a published sequence²⁵ with the following parameters: repetition time (TR)=7000 ms, echo time (TE)=72.4 ms, flip angle (FA)=90 /180 degrees, multiband factor (MB)=2, GRAPPA phase acceleration factor=3, 128 slices, phase encoding direction (PE) anterior-to-posterior (AP), 1.24 mm isotropic resolution, whole brain coverage and 103 directions. DWI imaging was acquired using multiple b-shells including 5 b=0 s/mm², 17 b=1000 s/mm², 31 b=2000 s/mm² and 50 b=3000 s/mm² images. In addition, 6 non-diffusion weighted images (b=0 s/mm²) with opposite phase

encoding (posterior-to-anterior) were acquired to correct for susceptibility-induced off-resonance field distortion.

Two runs of functional MRI were acquired (upper and lower limb motor tasks performed in separate runs) with the following parameters: TR=1700 ms, TE=34.4 ms, FA=65 degrees, MB=6, GRAPPA phase acceleration factor=2, 120 slices, 1.2 mm isotropic resolution, 165 volumes, image matrix = 168x168).

A 3D T1-weighted structural scan (MP2RAGE: TR=4900 ms, TE=2.89 ms, inversion time (TI)=700/2700 ms, flip angle (FA)=5/6 degrees, 192 slices, AF=4, Phase Encoding direction=AP, voxel size=0.9 mm isotropic, image matrix 256x256) and 2D fluid-attenuated inversion recovery scan (FLAIR) (TR = 10000 ms, TE =96 ms, TI = 2600 ms, flip angle = 145 degrees, 45 axial slices, GRAPPA = 3, PE = AP, voxel size = 1.2x1.2x3.0 mm) were acquired for volumetric measurements and lesion identification.

All patients were also imaged using a Siemens Prisma 3 Tesla MRI system (Siemens Healthcare, Erlangen, Germany) using a 64 channel combined head neck receive coil with body transmit for collection of a sagittally acquired 3D fluid-attenuated inversion recovery (FLAIR) scan for spinal cord lesion identification (TR=5000 ms, TE=408 ms, inversion time (TI)=1800 ms, flip angle (FA)=120 degrees, 160 slices, AF=4, phase encoding direction=AP, voxel size=0.98 mm isotropic, image matrix 256x256).

2.5. Anatomical MRI processing

White matter lesions were segmented on the MP2RAGE using a semi-automated thresholding technique in MRICron (<https://www.nitrc.org/projects/mricron>). To avoid inclusion of cerebrospinal fluid, the 7T FLAIR image was used as visual reference. To create a lesion probability map, individual lesion maps were warped to the diffusion group template using Advanced Neuroimaging Tools (ANTs, v.2.3.1, <http://stnava.github.io/ANTs/>) antsRegistrationSyNQuick and antsApplyTransforms scripts, binarized and merged. In addition, the percentage intersection between the probability map and tracts of interest was calculated. MP2RAGE images were lesion filled with lesion maps using SLF (<http://atc.udg.edu/nic/slfToolbox/index.html>) in

Statistical Parametric Mapping (SPM8), which were subsequently used for brain segmentation analyses using FreeSurfer version 6.0-patch (<https://surfer.nmr.mgh.harvard.edu/>). FreeSurfer volumetrics used in statistical analysis included the WM, cortical grey matter (GM), deep GM, ventricular volumes and right and left thalamus, cerebellum, precentral gyrus and postcentral gyrus. All volumetrics were normalized by total intracranial volume. One subject's data could not be reliably segmented, even after lesion filling, and was excluded from volumetric analyses.

Spinal cord cross-sectional area was measured on MP2RAGE scans at the level of the superior margin of the odontoid peg (Supplementary Figure 2). Sagittal images were used to reconstruct transverse images perpendicular to the cord and the cord was manually marked using Horos (v4.0, www.horosproject.org). While no dedicated spinal cord imaging was performed in this study, cervical spinal cord lesions were manually segmented up till cervical cord area 4 using 3T FLAIR scans.

2.6. Diffusion weighted MRI analyses

Diffusion imaging analyses was performed with MRtrix3, using the multi-shell multi-tissue constrained spherical deconvolution method (Figure 1). Having multiple b-shells allowed us to differentiate between macroscopic tissue types including the WM, GM and cerebrospinal fluid (CSF) resulting in a more complete modelling of the diffusion weighted signal and more precise estimation of the fibre orientation distribution and accurate measures of axonal damage.²⁶

2.6.1. Pre-processing diffusion-weighted imaging data

Diffusion-weighted MRI pre-processing involved denoising, motion and distortion correction including eddy current-induced distortions (EDDY, FSL, v.6.0.3) and susceptibility-induced EPI distortions using the anterior-posterior phase encoded non-diffusion weighted images (TOPUP, FSL, v.6.0.3). One subject did not have reverse phase encoded data correctly acquired and therefore was not processed for EPI distortion correction. Next, images were corrected for bias field signal (ANTs, N4 algorithm, <http://stnava.github.io/ANTs/>) and global intensity normalisation was performed across

all participants using the median $b=0$ s/mm² intensity within a WM mask for robust voxel-wise comparisons across participants and to correct for residual intensity inhomogeneities.¹³

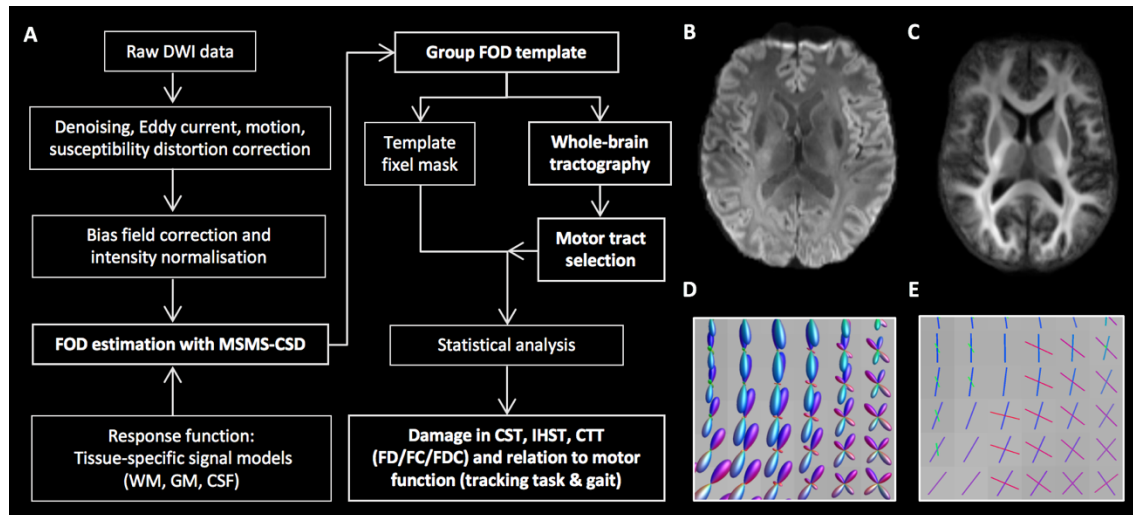


Figure 1. Overview processing pipeline. **A-B)** The diffusion weighted imaging (DWI) processing pipeline. **B)** DWI after pre-processing steps. **C)** The white matter (WM) fibre orientation distribution (FOD) group template. **D)** WM FODs colour coded by direction (green: dorsal-ventral, red: left-right, blue: cranial-caudal) and **E)** corresponding fixels. Abbreviations: CST = corticospinal tracts; CTT = cerebello-thalamic tracts; FC = fibre cross-section; FD = fibre density; FDC = fibre density and cross-section; GM = grey matter; IHST = interhemispheric sensorimotor tracts; MSMT-CSD = multi-shell multi-tissue constrained spherical deconvolution.

2.6.2. Fibre orientation distribution estimation

To accurately and specifically estimate the fibre orientation distributions (FOD) of the WM, multi-shell multi-tissue constrained spherical deconvolution was applied using tissue-specific response functions for WM, GM and CSF by exploiting the unique b-value dependencies of each tissue type.²⁶ The response function represents the diffusion signal of in a pure voxel of WM (single fibre), GM or CSF, and is then used to estimate the fibre orientation distribution for each tissue class using constrained spherical deconvolution of the diffusion weighted signal. The estimation of the response function and distribution of fibres was fully automated and driven by the DWI data itself. While we focussed solely on the WM FODs as measure WM tissue damage, the GM and CSF tissue classes in the model accounts for the remainder of the complete diffusion-

weighted signal making the WM FOD estimation more accurate. In addition, modelling 3-tissue FODs results in more precise FOD estimates at tissue boundaries what results in more accurate measures of axonal damage and fibre tracking.²⁶

2.6.3. Group template construction

A study-specific group WM FOD template was created using a randomly chosen subset of participants (10 patients and 10 healthy controls) by repeatedly registering (non-linear) the WM FOD map of each subject to a continually updated average template image (Figure 1). The WM group template was used in all subsequent steps including calculations of fibre specific measures of axonal damage and statistical analysis. For each subject, the FOD images were registered to the group WM FOD template generating a deformation field that encodes nonlinear transformations. To ensure subsequent analysis steps are performed in voxels with data in all participants, an intersection mask is created and used to segment fixels, individual fibre populations, from the FOD template, i.e. the fixel template analysis mask. Each subject's WM FOD image was nonlinearly aligned to the WM FOD group template and correspondence between each subject's fixel map and the template fixel mask was identified (Figure 1). For each fixel, three measures were calculated: fibre density (FD), fibre cross-section (FC) and the fibre density modulated by cross-section (FDC which equals $FD \times FC$).

2.6.4. Identification of tracts of interest

Firstly, whole brain probabilistic fibre tractography was performed on the group WM FOD template using the following stopping criteria: minimum angle = 22.5, maximum length = 250 mm, minimum length = 10 mm, power = 1.0, 20 million seeds randomly assigned within the WM template mask (Video 1). To improve on the reconstruction of the structurally connected fixels and to reduce biases in tractogram densities, the tractogram was filtered to 2 million tracts using the SIFT2 algorithm.²⁷ Subsequently, the following tracts were selected using inclusion and exclusion regions of interest:

i) Corticospinal tracts (CST)

The regions used to select the corticospinal tracts from the whole brain tractogram included pre-central (M1) and post-central (S1) gyrus grey matter masks included in the

Harvard Oxford Atlas (provided by FSL, FMRIB, Oxford, UK), which were registered to the diffusion group template using ANTs (ANTs, v.2.3.1, <http://stnava.github.io/ANTs/>), thresholded at 30% (voxels that were in 30% of subjects for those ROIs) and combined to form one inclusion region (Figure 2, Video 2). In addition, two midbrain masks were manually drawn and used as inclusion regions. Regions of interest were manually drawn to exclude tracts entering the corpus callosum, cerebellum and thalamus, and tracts terminating anterior or posterior to the CST.

The CST tract was further subdivided into M1 and S1 tracts (Figure 2) and tracts related to upper and lower limb movements (Figure 2) using the follow method:

- a) M1 and S1 tracts were selected using the M1 as inclusion region for M1 fibres and as exclusion regions for S1 tracts and vice versa.
- b) The upper limb and lower limb tracts within the CST were selected using the functional MRI task data.²⁴ Patients performed two visually guided force-matching tasks involving a low force contraction of the lower limb (tibialis anterior) or upper limb (hand muscles). First, functional activation patterns specific to upper or lower limb movement were estimated using a two-level analysis (run and higher-level) using FEAT (FSL, FMRIB, Oxford, UK, v.6.0.3). Significant voxels were identified using family-wise error correction (threshold of z-stat > 2.3 and cluster size significance $p < 0.05$). Run-level activation maps from all participants were used as input to higher level analyses to calculate activation maps specific to either upper or lower limb movements (upper > lower and lower > upper). Significant upper and lower activation maps were registered to the diffusion group template using ANTS and subsequently used as inclusion regions to select tracts specific to either upper or lower limb movements within the CST.

ii) *Interhemispheric sensorimotor tracts (IHST)*

The interhemispheric tracts were selected using the left and right M1 and S1 as inclusion regions (Figure 2). To exclude tracts running anterior or posterior, two coronal planes were used and an axial plane to prevent tracts running inferior. The IHST was further subdivided into M1 and S1 interhemispheric tracts (Figure 2, Video 3). These tracts were

selected using the right and left M1 as inclusion regions for M1 interhemispheric connections and as exclusion regions for the S1 interhemispheric tracts and vice versa.

iii) *Cerebello-thalamic tracts (CTT)*

To select the cerebello-thalamic tracts, we used the 20 million tract tractogram instead of the SIFT filtered tractogram to increase the number of tracts available. We manually created regions of interest for the left superior cerebellar peduncle, right red nucleus and right thalamus as well as right superior cerebellar peduncle, left red nucleus and left thalamus. Two sagittal planes were used to prevent tract running across hemispheric, just inferior and superior of where the CTT tracts decussate (Figure 2, Video 4). We combined both tracts to form one tract of interest and tracts running from the thalamus to the cortex were not included in the statistical analyses.

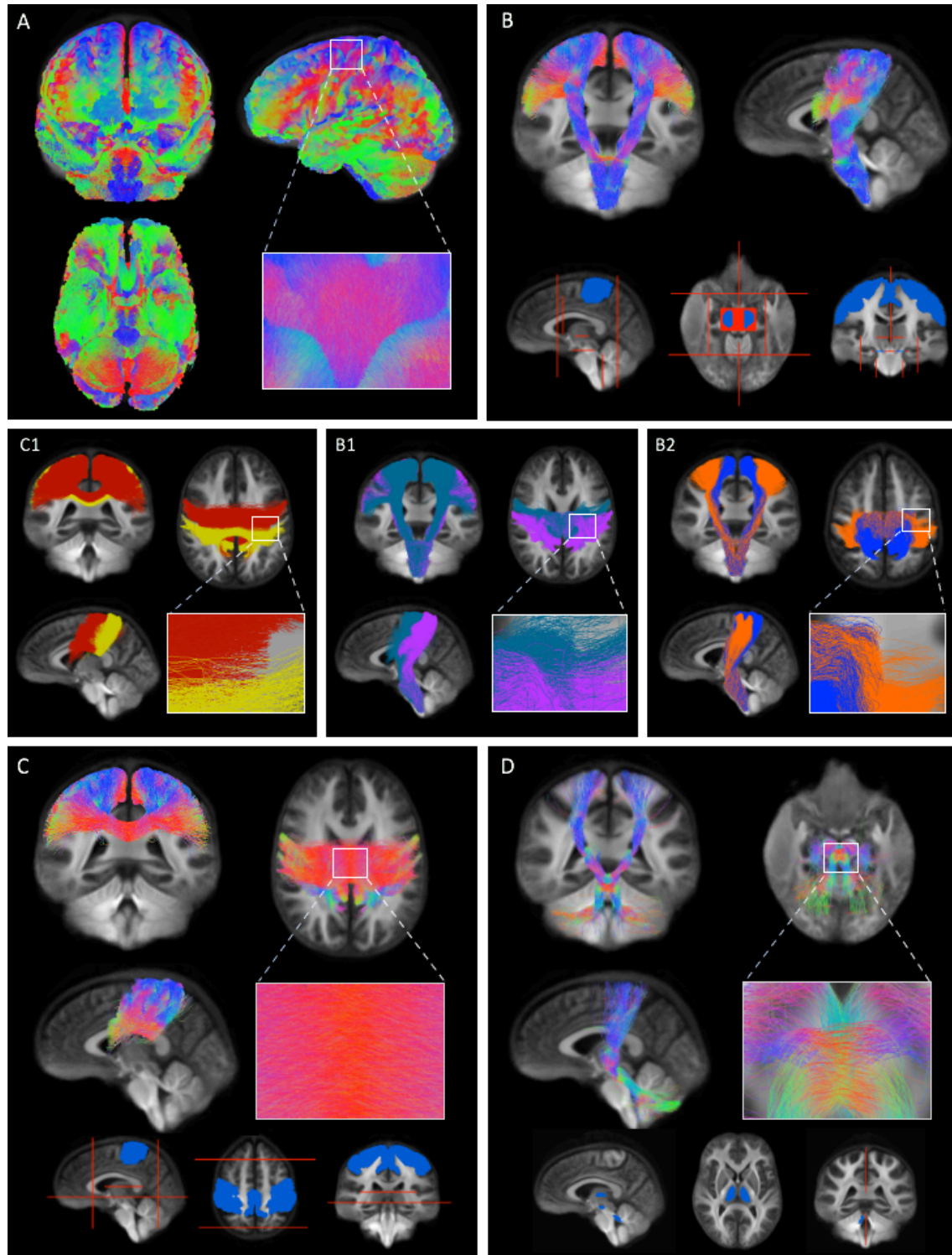


Figure 2. The tracts of interest. **A)** Whole brain tractogram was created on the group white matter fibre orientation template using probabilistic tractography. **B)** The corticospinal tract (CST) was identified from the whole-brain tractogram using seed regions of interest located in the primary motor cortex (M1), primary somatosensory cortex (S1) and midbrain (blue). Exclusion regions were drawn manually to exclude tracts entering the cerebellum, thalamus and corpus callosum, and tracts terminating posterior or anterior to the CST (red). Within the CST,

*we investigated the proportion of damage observed within **B1** M1 (blue) and S1 (pink) tracts using either the precentral or postcentral gyrus as inclusion region and **B2** tracts related to upper (blue) or lower limb (orange) movements specifically using fMRI activation maps. **C**) The interhemispheric sensorimotor tracts were selected using the right and left pre- and post-central gyrus (blue) and to prevent tracts from running anterior, posterior or inferior several exclusion planes were used (red). **C1**) The interhemispheric tracts between left and right S1 (yellow) and left and right M1 (red). **D**) The cerebello-thalamic tracts were selected using seed regions within the superior cerebellar peduncle, red nucleus and thalamus (blue) and 2 sagittal exclusion planes were used superior and inferior of the decussation (red). **A-D**) The colour of the tracts indicates the local fibre orientation (green: dorsal-ventral, red: left-right, blue: cranial-caudal).*

2.7. Statistical analysis

Groups were compared on demographics, brain volumetrics, spatiotemporal gait and functional behavioural parameters using independent samples t-tests, or when data was non-normally distributed, Mann-Whitney U tests and Chi-square test for nominal variables. Five additional healthy control participants with gait analysis but no MRI data available were included in group comparisons involving gait parameters to improve statistical power.

Fixel-wise general linear models with connectivity-based fixel enhancement ($p_{fwe} < 0.05$) and non-parametric permutation testing ($n=5000$ samples)¹² were used to compare FD, FC, and FDC within the CST, IHST and CTT tracts between multiple sclerosis patients and healthy controls, and for correlations between fixel metrics and spatial-temporal gait measures (stance, stride length, step width, single and double support) and behavioural parameters (lag and force error).

Post-hoc analyses were performed to calculate the proportional volume of M1, S1, upper limb and lower limb tracts within the CST and M1 and S1 interhemispheric tracts within IHST that displayed significant group differences. In addition, linear regression analyses were performed between the mean significant fixel-metrics within damaged tracts and EDSS, and volumetrics in multiple sclerosis patients. Residuals were tested for normality and partial rank correlations were performed when non-normally distributed.

For all regression analyses, age and sex were included as covariates, significance was set at $p < 0.05$. Corrections for multiple comparisons were performed using false discovery rate (FDR).²⁸

2.8 Data Availability

Deidentified data for this study are available upon reasonable request to the corresponding author.

3. Results

3.1. Demographics, volumetric measures and clinical disability

Demographics, volumetric measures and clinical disability scores (EDSS) are summarized in Table 1. Multiple sclerosis patients did not significantly differ in sex, age or hand dominance from healthy controls.

Multiple sclerosis patients displayed significant reduction in whole brain volumetrics including cortical GM ($p_{\text{FDR}}=0.020$), deep GM ($p_{\text{FDR}}=0.009$) and WM volume ($p_{\text{FDR}}=0.006$), compared to controls. In addition, multiple sclerosis patients showed significant right ($p_{\text{FDR}}<0.001$) and left ($p_{\text{FDR}}<0.001$) thalamic atrophy and reduced volume of the left post-central gyrus (S1) ($p_{\text{FDR}}=0.044$), compared to controls.

Multiple sclerosis participants displayed greater force error ($p_{\text{FDR}}=0.010$) and a longer lag ($p_{\text{FDR}}=0.010$) during lower limb force matching but not during upper limb movements (Table 2). Multiple sclerosis patients walked with a shorter stride length compared to healthy controls, but the p-value did not survive FDR correction ($p=0.021$, $p_{\text{FDR}}=0.070$) (Table 2)

	Healthy controls (n=18)	MS patients (n=28)	p-value	p-value FDR
Demographics				
Sex, F/M	10/8	23/5	0.051●	0.108
Age	39.22 (7.13)	41.75 (10.01)	0.358	0.380
Disease duration		6.50 (3.94)		
Dominant hand (R/L), n	15/3	27/1	0.124●	0.211
Disability scores				
EDSS ^a		1.50 (1.0, 1.5)		
Pyramidal FSSa		1.00 (0.0, 1.0)		
Cerebellar FSSa		0.00 (0.0, 1.0)		
Brain volumetrics				
BP, mm3	1143921 (100661)	1061714 (90033)	0.002*	0.007*
WM, mm3	423592 (47671)	380951 (48597)	0.001*	0.006*
CGM, mm3	399123 (36891)	377639 (32317)	0.007*	0.020*
DGM, mm3	75246 (8571)	67692 (7090)	0.002*	0.009*
Ventricles, mm3	26334 (18082)	34350 (17808)	0.106	0.200
M1 L, mm3	13515 (1760)	12725 (2636)	0.196	0.278
M1 R, mm3	13034 (1852)	12444 (2814)	0.336	0.381
S1 L, mm3	9843 (1202)	9013 (1954)	0.018*	0.044*
S1 R, mm3	9339 (1046)	8870 (2024)	0.195	0.301
Cerebellum L, mm3	49466 (4570)	46611 (9783)	0.231	0.280
Cerebellum R, mm3	49639 (4496)	46745 (10128)	0.218	0.285
Thalamus L, mm3	6962 (707)	5832 (1190)	<0.001*	<0.001*
Thalamus R, mm3	6673 (625)	5695 (1113)	<0.001*	<0.001*
Lesion volume, mm3		3303 (5248)		
Spinal cord lesion volume, mm3		176 (248)		
Spinal cord, mm2	69.55 (6.64)	71 (10)	0.703	0.703

Table 1. Demographics, clinical, and MRI characteristics. Compared to healthy controls, multiple sclerosis patients displayed significantly reduced brain parenchymal (BP), white matter (WM), cortical grey matter (CGM), deep grey matter (DGM), right and left thalamus and left primary somatosensory cortex (S1) volume. Besides lesion load and spinal cord area, all volumetrics were normalized for intracranial volume. One subject was excluded from volumetric analyses as we were unable to reliably calculate volumetrics even after lesion filling. Abbreviations: F = female; M = male; R = right; L = left; EDSS = Expanded Disability Status Scale; M1 = primary motor cortex. All variables were tested using independent samples t-test and values represent means and standard deviations unless denotes otherwise. ^aMedian and interquartile range. ●Chi-Square test. *Significant difference between multiple sclerosis patients and healthy controls.

	Healthy controls	Multiple sclerosis patients	<i>p</i> -value	FDR <i>p</i> -value
Force matching performance				
Number of participants	17	28		
Upper limb lag, ms	184.71 (113.75)	216.07 (90.08)	0.201	0.503
Upper limb force error, N	0.31 (0.07)	0.34 (0.09)	0.226	0.452
Lower limb lag, ms	142.35 (116.49)	266.43 (120.68)	0.002*	0.010*
Lower limb force error, N	0.30 (0.05)	0.45 (0.16)	0.001*	0.010*
Spatiotemporal gait measures				
Number of participants	15	28		
Speed, ms	1.39 (0.04)	1.38 (0.03)	0.308	0.513
Single support, % gait cycle	40.19 (3.26)	40.45 (2.92)	0.760	1.000
Terminal double support, % gait cycle	7.79 (1.30)	7.78 (1.54)	0.985	0.985
Stance, % gait cycle	58.52 (1.26)	58.44 (2.35)	0.899	1.000
Stride length, mm	1426.26 (88.09)	1358.05 (89.16)	0.021*	0.070
Step width, mm	75.51 (22.68)	77.26 (24.50)	0.919	1.000

Table 2. Functional motor performance and spatiotemporal gait measures. Multiple sclerosis patients showed worse behavioural performance during the lower limb visually-guided motor task during fMRI testing, but no differences were observed during upper limb movement. Multiple sclerosis patients displayed longer lag ($p=0.002$) and more force error ($p=0.001$) during lower limb movements, compared to control participants. In addition, during walking patients showed a shorter stride length compared to controls ($p=0.021$). *Significant difference between patients and healthy controls.

3.3. Sensorimotor network damage and conventional MRI

For the CST, FC and FDC were significantly reduced in multiple sclerosis patients in large regions of the tracts with no difference in FD compared to healthy controls (Figure 3, Video 5). Within the CST, we assessed the proportional volume of M1, S1, upper limb and lower limb connected tracts that overlapped with significant group differences. The largest volume of significant difference was observed for FC. This region overlapped with 20.2% of M1 tracts, 27.3% of S1 tracts, 35.7% of lower limb tracts and 29.7% upper limb tracts. Areas with reduced FDC overlapped with 6.8% of M1 tracts, 11.6% of S1 tracts, 14.2% of lower limb tracts and 12.4% upper limb tracts.

For the IHST, multiple sclerosis patients showed significantly reduced values for all fixel-specific metrics compared to healthy controls, mostly within the corpus callosum (Figure 3). The strongest significant differences were observed for FC (Video 6) and this region

intersected with 4.6% of M1 interhemispheric tracts and 10.9% of S1 interhemispheric tracts. More significant fixels were observed for FDC, which overlapped with 2.6% of M1 and 13.2% of S1 interhemispheric tracts. Reduction in FD was observed to a much smaller extent and overlapped with only 0.02% of M1 and 0.38% of S1 interhemispheric tracts.

For the CTT, patients showed significantly reduced FC (Video 7) and to a lesser extent FDC, but not for FD, compared to controls (Figure 3).

We calculated the intersection between the motor tracts of interest and the lesion probability map to explore the relation between lesion load and fibre-specific damage (Figure 4). While the probability map overlapped with 19.9% of the CST, 19.2% of the IHST and 12.6% of the CTT, the maximum proportions of patients with lesions intersecting with these tracts were only 28.6% for the CST and IHST and 14.3% for the CTT.

We observed a range of moderate correlations between conventional markers and fibre metrics (Supplementary Table 1), however none survived FDR correction.

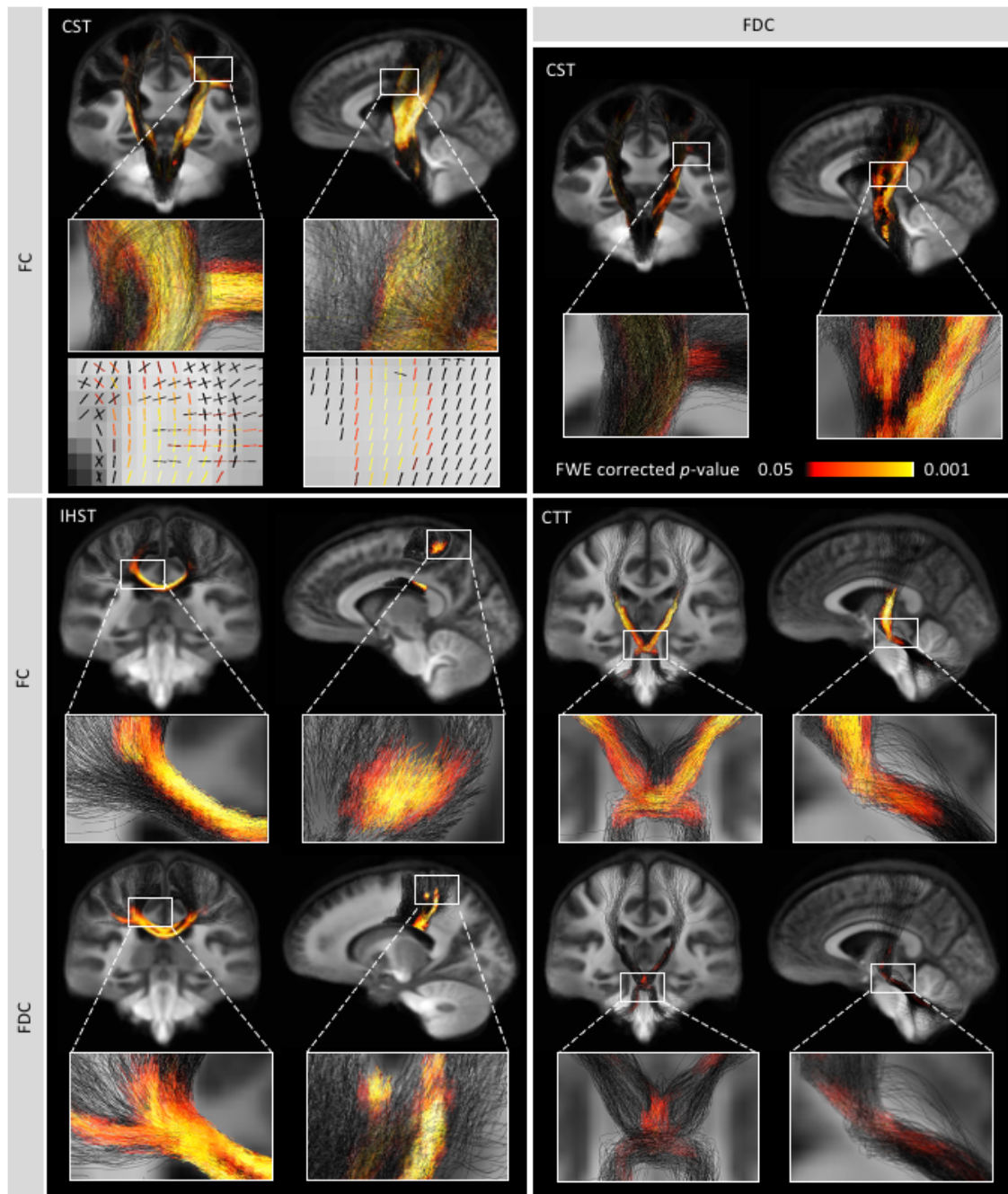


Figure 3. Damage within the corticospinal tracts. Multiple sclerosis patients showed substantial loss of fibre cross-section (FC) and to a lesser extent loss of fibre density and cross-section (FDC) in the corticospinal tracts (CST), interhemispheric tracts (IHST) and cerebellar-thalamic tracts (CTT), compared to healthy controls. Significant fixels (family-wise error (FWE) corrected p -value < 0.05) are represented on the tractogram and coloured with the p -value and a close-up of the significant fixels are displayed for FC in the upper panel.

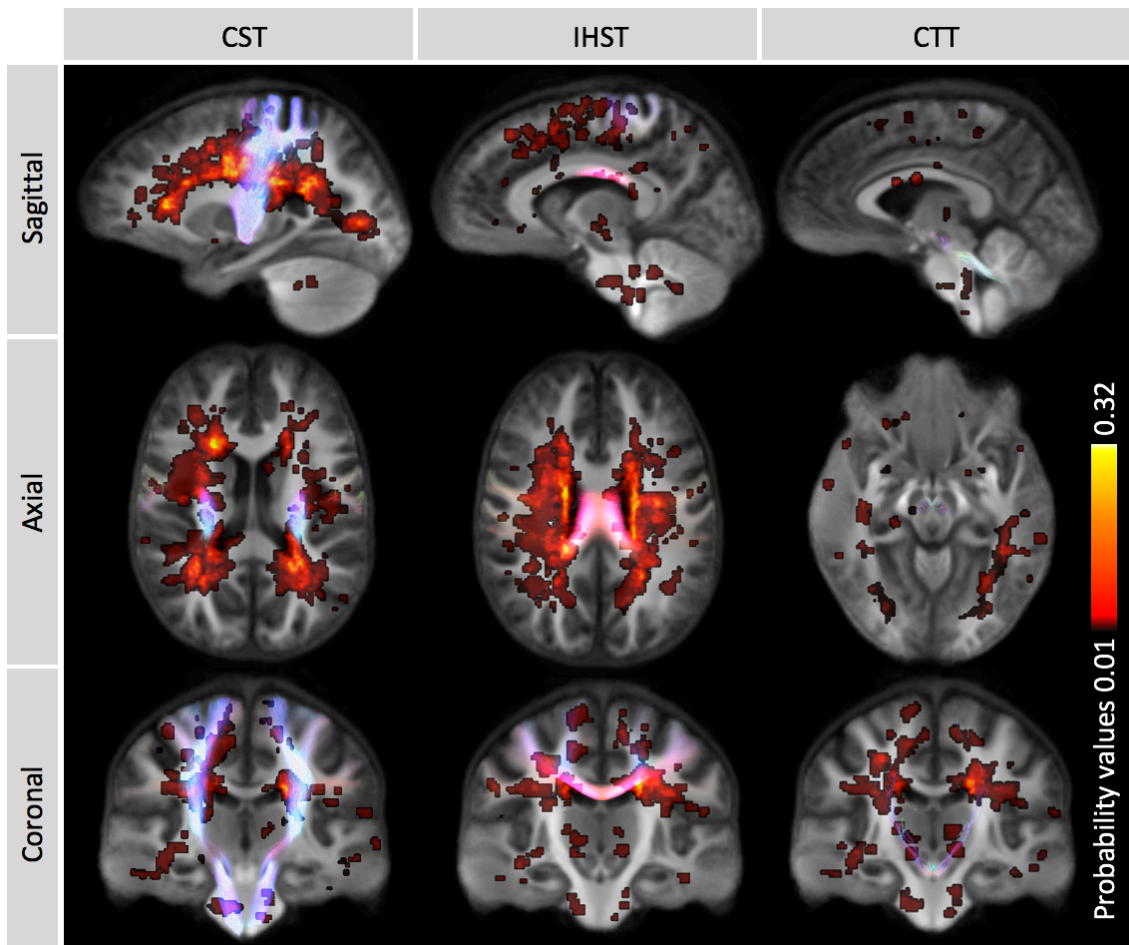


Figure 4. Lesion probability map and tracts of interest. This figure demonstrates the probabilities of lesions occurring across the brain across multiple sclerosis patients with the motor tracts visualized in an overlying manner. Low probability values are shown in red and higher values are shown in yellow. The maximum probability was 32%, indicating that lesion pathology occurred in the same location in 32% of the patients. The probability map intersected with 19.9% of the corticospinal tracts (CST), 19.2% of the interhemispheric sensorimotor tracts (IHST) and 12.6% of the cerebello-thalamic tracts (CTT). The maximum proportions of patients with lesions intersecting with these tracts were only 28.6% for the CST and IHST and 14.3% for the CTT.

3.4. Correlations with disability, motor performance and gait

Greater force error during upper limb force matching was associated with lower FDC in CST and IHST, lower FD within the CST, and lower FC within the IHST (Figure 5). Shorter stance was associated with lower FD within the CST. Smaller step width was associated with reduced FD in the CST and CTT. Increased double support time was associated with reduced FC within the IHST (Figure 5). While the associations between axonal loss and

functional measures appeared to be quite localised, uncorrected p-value maps (Supplementary Figure 3) showed that the effect was widely distributed throughout the tracts, suggesting that family-wise error correction could lead to an underestimation of the anatomical extent of damage.

Fixel metrics, averaged across regions showing significant group differences, did not correlate with the EDSS in any tract.

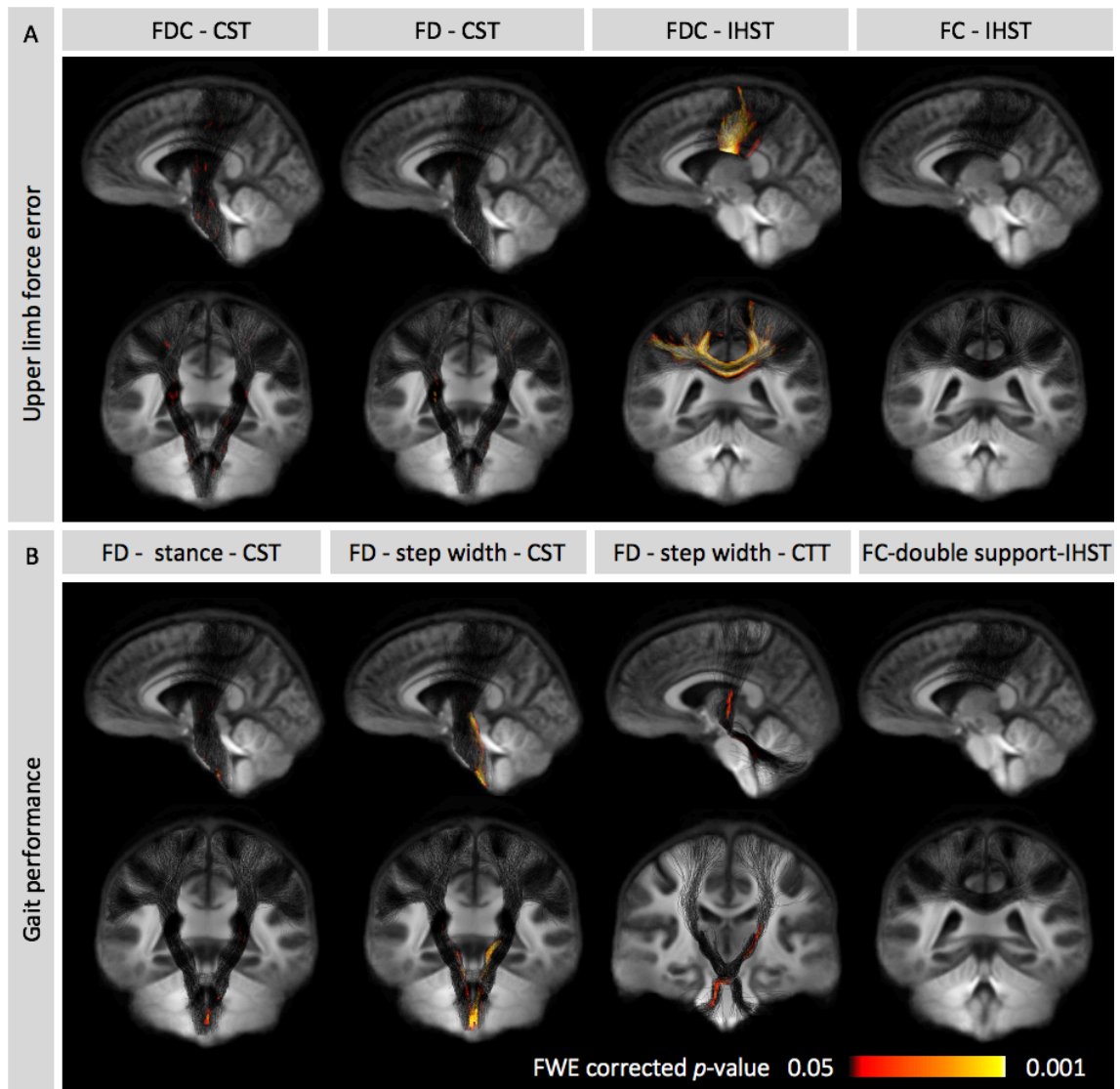


Figure 5. Motor dysfunction and axonal damage. A) The associations between upper limb force error and fixel-metrics within the motor tracts. Greater force error during the upper limb visually-guided force-matching task was associated with loss of fibre bundle density (FD) and fibre density and cross-section (FDC) in corticospinal tracts (CST) and reduced FDC and fibre bundle cross-section (FC) within the interhemispheric sensorimotor tracts (IHST). **B)** Shorter stance was associated with reduced FD within the CST. Smaller step width was associated with reduced FD within the CST and cerebello-thalamic tracts (CTT). Increased double support time was associated with reduced FC within the IHST. Areas of significance are coloured with the p -values (family-wise error (FWE) correction) and visualized on tracts using p -value.

Discussion

Here we aimed to investigate and compare impairments in gait and motor control using advanced kinematic technologies, and axonal loss within key sensorimotor tracts of the brain in minimally disabled people with MS. The main findings of this study were: minimally-disabled multiple sclerosis patients i) showed substantial loss of FC and FDC but minimal to no loss of FD along all motor tracts compared to healthy controls, and ii) pathway-specific disruptions were associated with variation in upper limb force tracking performance and altered spatiotemporal patterns of gait.

Extensive axonal loss along motor pathway in multiple sclerosis

As hypothesized, we observed axonal damage within all tracts of interest. The greatest effects were observed as loss of cross-section of fibre bundles, an indication of tract-specific atrophy. When we subdivided the CST into upper/lower limb and sensory/motor fibres, a larger proportion of S1 tracts compared to M1, and a larger proportion of lower compared to upper limb tracts were affected. We observed moderate correlations (not significant at an FDR corrected level) between CST FC and whole brain lesion load, S1 atrophy and thalamic atrophy, but no correlation with cervical cord lesion load or cross-sectional area where most CST tracts terminate²⁹ and lesions are commonly located³⁰. Consistent with recent reports,³¹ we observed relatively little primary lesion activity was present within CST, so axonal loss within these tracts could be attributable to spinal cord lesions or trans-synaptic degeneration due to lesions in second order tracts. While we did not see a correlation with cervical cord lesion volume, these results must be interpreted with caution as we could only identify lesions within the rostral segments (C1-C4) due to a lack of dedicated spinal cord imaging consistently acquired during routine monitoring scans.

Significant axonal loss was observed within the IHST within medial subcortical areas as well as within the corpus collosum. Similar to the CST, when subdividing the IHST into motor and sensory tracts, a larger portion of the tracts connecting the two sensory cortices were damaged compared to M1 fibres. The corpus callosum is involved in control of unimanual movements by inhibiting interference of the other hemisphere and

asynchronous bimanual movements by transcallosal passage of motor signals and sensory feedback.³² Structural damage within the corpus callosum observed here, is congruent with findings of white matter abnormalities reported in DTI literature.^{5,33,34}

Less is known about the clinical effects of injury to the cerebello-thalamic tracts in multiple sclerosis,³⁵ compared to CST and IHST. Atrophy of regions at either end of the CTT tracts, the cerebellum and thalamus, is observed even at early stages of multiple sclerosis,^{36,37} and has been associated with clinical disability^{38,39} and tremor³⁵.

While FC and to a lesser degree FDC changes were widespread in the tracts studied, FD reduction was only observed within a small part of the IHST. Further work is required to map the spatial and temporal patterns of loss of axonal density compared to tract atrophy across the disease course. At face value, one might expect that initially density is reduced due to axonal transection in lesions and diffuse axonal degeneration, and that subsequently the tract will atrophy depending on the rigidity of the extracellular matrix and the presence of glial proliferation and scarring. Consistent with this hypothesis, a previous study of patients with early stage multiple sclerosis shortly following acute optic neuritis showed preferential loss of FD and very little change in FC in the optic radiations.¹⁷ A more recent study examining FC in a sample of MS patients representing a broad spectrum of disability, showed substantial FC changes that were more pronounced in progressive than relapsing phenotype patients.¹⁶

Axonal damage, visuomotor control and kinematics of gait

In multiple sclerosis patients, worse visuomotor hand control was associated with greater axonal damage within the CST and IHST. Microstructural damage to the corpus callosum has been observed from early on in the disease, even prior to the presence of macroscopic lesions³³ and has been related to clinical disability worsening,⁵ dexterity impairment⁶ and disease progression.⁴⁰ In this study we found that the location of the WM damage in the IHST was mainly observed posteriorly, a region that is thought to be involved in complex somatosensory interhemispheric information transfer,⁴¹ with S1 tracts damaged in a greater extent than M1 interhemispheric tracts. Together with previous findings, our results suggest that the structural disruption within the corpus

callosum has an effect on upper limb motor functioning, possibly driven by disruptions of somatosensory interhemispheric integration.

Changes in gait were associated with axonal damage within the CST and CTT. Reduced FD was associated with shorter stance and smaller step width. This contrasts with previous studies that reported prolonged stance time⁴² and wider step width in multiple sclerosis.^{42,43} These studies either included various multiple sclerosis phenotypes⁴² or patients using walking aids and or pyramidal and cerebellar FSS scores equal or above 2⁴³. In contrast, patients in this study were selected based on having minimal pyramidal and cerebellar dysfunction. Potentially, patients employ different strategies to maintain stability during walking at different disease stages. We also observed that loss of FC within IHST was associated with longer double support times. This finding is congruent with previous research showing prolonged double limb support^{18,42,44} related to clinical disability scores⁴⁵. Structural damage within the sensory and motor interhemispheric tracts potentially interferes with the transcallosal passage of motor signals and sensory feedback needed for bilateral coordination.³² Prolonged double support may be therefore be a compensatory mechanism to increase stability as balance control deterioration has been previously reported in multiple sclerosis.⁴⁶

Methodological considerations

In this study we used ultra-high field diffusion MRI. While ultra-high field imaging has advantages in terms of higher signal to noise ratio and therefore the ability to image at higher spatial resolution, for diffusion imaging which utilises spin-echo echoplanar imaging sequences, ultra-high field is associated with worse B0 and B1 field inhomogeneities leading to signal attenuation and distortions, particularly observed in the inferior temporal and orbitofrontal cortices. These limitations on the regions available for study with diffusion imaging at ultra-high field were not of great relevance to this study as we selected tracts of interest distant to B0 and B1 inhomogeneity effects, allowing us to take full advantage of the SNR and resolution gains.

There are several additional methodological issues affecting the interpretation of our data. Firstly, the number of participants included was relatively small in comparison to

large clinical studies. This reflects barriers to participation related to the amount of testing required (~2 hour visit for MRI and ~2 hour visit for comprehensive gait laboratory testing). However, more comprehensive phenotypic assessments of smaller yet more homogeneous sample of patients ($EDSS < 4$ and pyramidal $FSS \leq 2$) can provide useful convergent evidence. Indeed, despite the relatively small sample size, our assessments were sensitive enough to identify axonal damage and motor impairments in minimally disabled patients. Such sensitivity is important for developing markers that could be deployed in smaller and short duration phase II clinical trials required to justify larger trials with clinical endpoints. However, associations should be interpreted with caution and larger study sizes would be recommended in future studies to confirm findings. Whilst the gait markers used here would be difficult to implement in large scale studies due to the requirement for 3D video tracking in a laboratory setting, these techniques have a high reproducibility. However, other techniques such as wearable sensors are easy to deploy and have reasonable reproducibility.⁴⁷ Secondly, longitudinal studies will be required to evaluate the within-patient sensitivity of fixel-based markers of axonal loss, and to determine whether longitudinal change in axonal density and/or atrophy is associated with motor progression. Thirdly, we interpret loss of fibre density and cross-section as markers of axonal loss based on a theoretical understanding of the molecular diffusion processes in brain tissue and histopathological data regarding the specific pathologies evident in the multiple sclerosis brain. Other advanced diffusion models have been proposed as markers of axonal density (e.g. NODDI⁴⁸), however few of these can be analysed within a fixel-based analysis framework as they do not model individual fibre elements. Lastly, although we focussed on three major motor tracts that are highly relevant to motor behaviours, there is potential for damage to other tracts to play a role in motor dysfunctions.

Conclusions

In conclusion, significant gait and lower limb motor control impairments were detected in minimally disabled people with MS that correlated with axonal loss in major sensorimotor pathways of the brain. Given that axonal loss is irreversible, the combined use of advanced imaging and kinematic markers could be used to identify patients at

risk of more severe motor impairments as they emerge for more aggressive therapeutic interventions.

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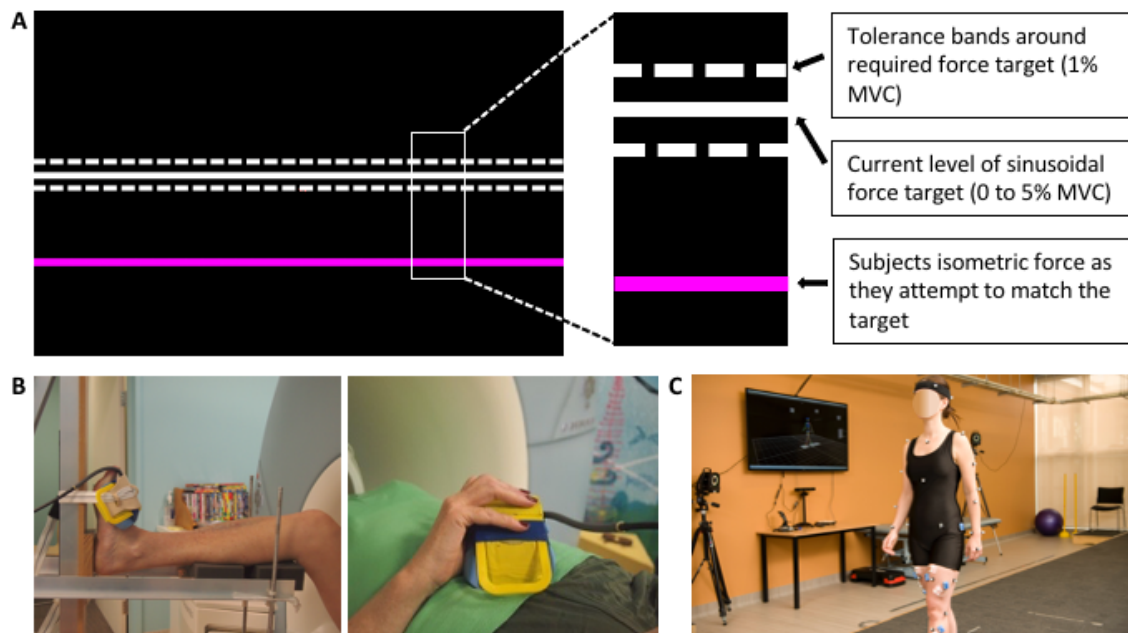
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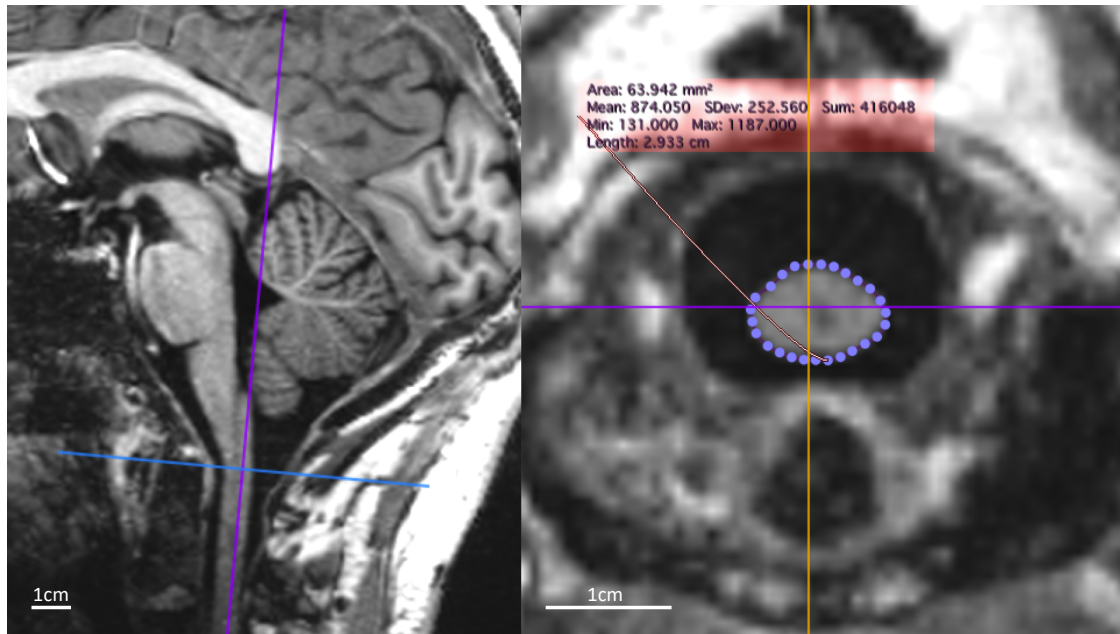
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Supplementary material

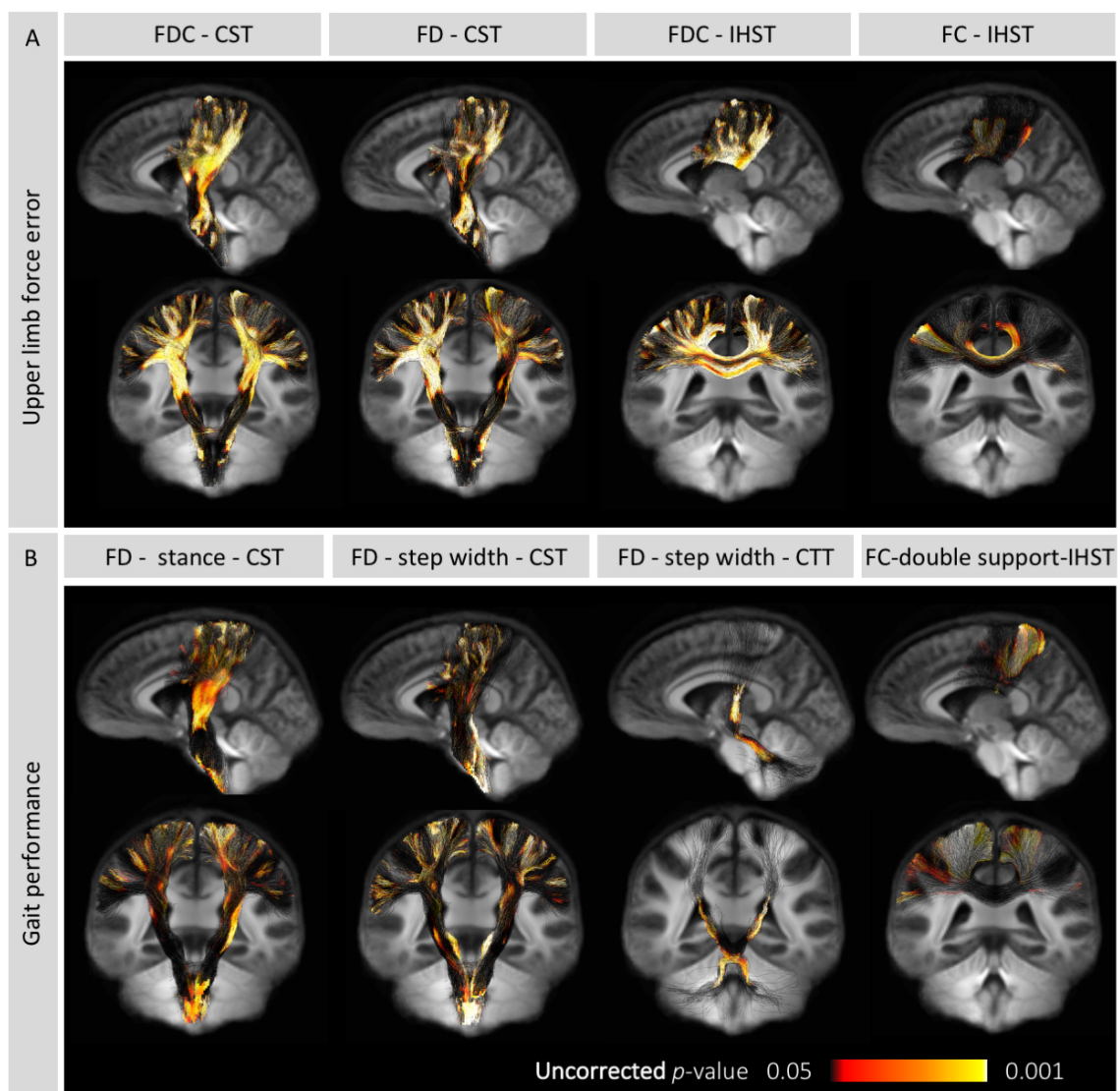


Supplementary Figure 1. Experimental set-ups for gait assessment and force tracking fMRI task. **A)** The visually-guided force-matching task presented to participants. The white line is the target force and in pink the participants' isometric force as they attempt to match the target force, which would slowly move up and down. Participants were asked to follow the target force as accurately as possibly by pulling their foot up and down or squeezing or releasing their hand. **B)** MR compatible rig and sphygmomanometer cuff positioned over the dorsum of foot, used to measure force production. **C)** Reflective markers were placed on specific body landmarks and a motion capture system was used to assess spatiotemporal patterns of gait. Patients were instructed to walk on a walkway at 1.4 ms^{-1} .



Supplementary Figure 2. Region of interest for spinal cord cross-sectional area measurement.

The mid sagittal image was identified on MP2RAGE brain scans (left) and a transverse slice was identified at the rostral margin of the odontoid peg orthogonal to the caudally extending spinal cord (blue line). This slice was used to manually outline the spinal cord for all participants and the cross-sectional area was recorded.



Supplementary Figure 3. Associations between motor dysfunction and axonal damage using uncorrected p -value. This image shows the same associations visualized in Figure 6, but with the uncorrected p -value plotted on the tractograms. As results of the relatively strict family wise error correction used in the analyses the location of the association between motor dysfunction and axonal damage seems to be quite moderate and localized. However, without correction associations were observed throughout the tracts, suggesting that loss of motor function is presumably not associated with localized damage but rather the entire tract might be involved.

	FC			FD			FDC		
	<i>p</i> -value	Beta _{st} _d	<i>p</i> _{FDR}	<i>p</i> -value	Beta _{st} _d	<i>p</i> _{FDR}	<i>p</i> -value	Beta _{st} _d	<i>p</i> _{FDR}
CST									
Cerebellum, % ICV	0.333	0.228	0.642	0.125	0.293	0.422	0.074	0.361	0.375
Thalamus, % ICV	0.006	0.629	0.243	0.729	-0.071	0.937	0.192	0.280	0.536
S1, % ICV	0.030	0.520	0.347	0.169	-0.279	0.527	0.762	0.067	0.935
M1, % ICV	0.815	0.059	0.917	0.566	-0.119	0.917	0.816	-0.052	0.905
NCGM, % ICV	0.321	0.231	0.667	0.319	0.191	0.912	0.150	0.291	0.486
NWM, % ICV	0.011	0.600	0.223	0.328	-0.201	0.680	0.449	0.167	0.791
Brain lesions	0.009	-0.585	0.243	0.889	-0.028	0.664	0.100	-0.343	0.386
Spinal cord lesions*	0.757	-0.064	0.943	0.493	-0.141	0.940	0.337	-0.196	0.635
Spinal cord CSA	0.813	0.058	0.928	0.888	0.028	0.922	0.720	0.077	0.941
IHST									
Cerebellum, % ICV	0.205	-0.300	0.536	0.050	0.372	0.312	0.210	0.261	0.532
Thalamus, % ICV	0.792 *	0.056	0.930	0.081	0.349	0.365	0.006 *	0.533	0.486
S1, % ICV	0.578	0.141	0.918	0.710	0.078	0.943	0.330	0.216	0.652
M1, % ICV	0.364	-0.232	0.670	0.630	0.102	0.963	0.890	0.031	0.901
NCGM, % ICV	0.062	-0.429	0.359	0.038	0.388	0.308	0.285	0.221	0.624
NWM, % ICV	0.624	0.125	0.972	0.259	0.234	0.617	0.088	0.372	0.375
Brain lesions	0.729	0.086	0.923	0.014	-0.467	0.227	0.045	-0.418	0.304
Spinal cord lesions*	0.873	-0.033	0.930	0.765	0.062	0.925	0.932	0.020	0.932
Spinal cord CSA	0.122	-0.373	0.430	0.276	0.223	0.621	0.671	0.092	0.937
CTT									
Cerebellum, % ICV	0.491	0.171	0.829	0.886	-0.039	0.932	0.704	0.121	0.950
Thalamus, % ICV	0.074	0.449	0.400	0.036	-0.564	0.324	0.368	-0.297	0.662
S1, % ICV	0.635	0.125	0.953	0.035	-0.576	0.354	0.170	-0.454	0.510
M1, % ICV	0.489	-0.183	0.843	0.830	-0.062	0.909	0.832	-0.073	0.899
NCGM, % ICV	0.651	-0.111	0.942	0.670	-0.114	0.952	0.691	-0.125	0.949
NWM, % ICV	0.092	0.432	0.373	0.017	-0.645	0.230	0.267	-0.371	0.618
Brain lesions	0.120	-0.379	0.442	0.190	0.356	0.550	0.767	0.097	0.914
Spinal cord lesions*	0.511	-0.135	0.845	0.204	-0.257	0.551	0.217	-0.250	0.533
Spinal cord CSA	0.651	-0.116	0.959	0.043	-0.537	0.317	0.076	-0.562	0.362

Supplementary Table 1. Relationships fibre-specific measures and conventional MRI metrics.

Linear regression were performed to assess the relationship between fibre-specific measures within areas of significant damage and volumetrics. Significant relations are highlighted in bold, but these associations do not survive FDR correction. All brain volumetrics were normalized for intracranial volume. Partial rank correlations were performed for ∇ non-normally distributed data or when *residuals were non-normally disturbed. Abbreviations: S1 = primary somatosensory cortex; M1 = primary motor cortex; CGM = cortical grey matter; WM = white matter; CSA = cross-sectional area.

CHAPTER 4

Summary and general discussion

The aim of this thesis was to investigate the mechanism underlying different severities of motor disability in multiple sclerosis (MS). We approached this aim using advanced functional and structural MRI analysis methods in two separate cohorts of people with MS (pwMS).

In the first part of this thesis, we studied the functional sensorimotor network disturbances in a large cohort of pwMS with mild to severe disabilities (Chapter 2). We investigated the functional disturbances within the sensorimotor network in relation to severe disabilities cross-sectionally, and longitudinally in pwMS that progressed or remained stable in terms of upper or lower limbs disabilities. The second cohort included patients with no to minimal clinical impairments and we investigated more subtle motor disabilities (Chapter 3). Here, we used ultra-high field MRI and sensitive measures of motor behaviour to investigate the neuroplasticity of the brain while performing a task and the white matter integrity in relation to subtle changes in motor performance. Together, these four projects aimed to investigate the structural and functional mechanisms as comprehensive as possible across the span of disability in MS. The key questions of this thesis were the following:

- 4. Which functional network changes underlie sensorimotor impairments in patients with more severe disabilities, and are these predictive of subsequent progression?**
- 5. In early disability stages, prior to more severe impairments, which functional activation pattern underlie subtle deficits in the upper and lower limbs?**
- 6. In people with MS with minimal physical disabilities, to what extent is axonal loss prevalent in of the major motor pathways of the brain, and does it relate to subtle changes in hand function and walking?**

In this general discussion, the key findings are summarised and discussed below, as well as my perspective on the directions of future research in MS.

1. Which functional network changes underlie sensorimotor impairments in patients with more severe disabilities, and are these predictive of subsequent progression?

A network perspective to better understand disability in MS?

The sensorimotor network does not only comprise of ‘typical’ motor regions and tracts such as the primary motor cortex (M1) and corticospinal tracts, but it is a highly intricate system comprised of multiple regions and several pathways that exhibits a high degree of interconnectivity. As MS is characterized by widespread pathological processes, it is highly likely that inflammatory and neurodegenerative processes affect the sensorimotor system. Whereas damage can be focal and sensorimotor functions reside in specialized regions to some degree, the disease is highly heterogeneous, and regions do not act in isolations. The integration and segregation of information flow within and between brain networks lead to complex motor behaviours. As such, the heterogeneity of the disease and the complexity of the system stresses the need to understand the communication within the sensorimotor network. To investigate the functional disturbances within brain networks, resting-state functional MRI has been extensively used. Functional connectivity variations specific to the motor system are present from earliest stages of the disease, but have not clearly been related to clinical symptoms.^{1,2} In fact, previous studies have mostly shown complex patterns of increases and decreases in connectivity that have been difficult to interpret. For instance, studies have reported higher connectivity in early stages of MS^{1,2}, but also reduced connectivity³⁻⁷, both with conflicting and limited relations to clinical impairments. In addition, methodological issues were frequent, often driven by the incomplete representation of the sensorimotor system. For example, either just the ‘typical’ motor regions were investigated, such as the primary sensorimotor cortices, overlooking relevant areas, or a whole brain approach was used, potentially including irrelevant regions.

As such, we wanted to take a different approach and study the sensorimotor system in its entirety. We included regions beyond the ‘typical’ motor regions such as frontal, parietal and subcortical regions, based on previous literature.⁸ In addition to investigating the raw connectivity between regions, we studied the system as a whole and investigated how sensorimotor regions worked together as a network. We used the

graph theoretic concept of efficiency to study functional disturbances within the sensorimotor network. Network “efficiency” is well validated metric that can be calculated for the entire sensorimotor system (i.e. global efficiency) or for each region separately (i.e. local efficiency). Whereas the global efficiency is based on the average number of steps along the shortest path and reflects efficiency of information transfer at a global level, local efficiency is based on how well-connected neighbour regions, i.e. the ability to integrate information at a local level.

Sensorimotor network abnormalities explain physical disability in MS

Using aforementioned network approach, we were able to demonstrate different patterns of connectivity and efficiency within the sensorimotor system in pwMS with overt disability, while the network of pwMS with no to mild disability remained normal. In pwMS with more serious disabilities (walking difficulties and in need of aid or assistance) three out sensorimotor regions displayed increased local efficiency including the thalamus, premotor cortex and primary somatosensory (S1) cortex. This binary measure of disability was further deepened by also looking at patients with no to very minimal disability (EDSS<2) compared to high disability, showing similar results. It should be noted, however, that we did not assess the specific type of disability nor effects of laterality, which might be of interest. The functional changes within the sensorimotor system were especially around the S1 as this region correlated the strongest of disability severity, independent of structural damage. Increased S1 efficiency reflects higher clustering, which means stronger connectivity with neighbour regions. Potentially, increased efficiency of S1 could be caused by pathological processes in the ascending tracts that disrupt sensorimotor information from the periphery to S1, which results in reduced or disturbed signals arriving in S1. Alternatively, damage in descending pathways or in second order pathways connecting parietal or prefrontal cortices to S1 cortices could lead to increased connectivity and efficiency. Changes were observed locally, but not globally. This could be explained by the fact that global and local efficiency metrics are based on different principles. Global efficiency is based on the shortest path length of all individual network links, whereas local efficiency is based on the clustering coefficient which reflects how neighbours of individual nodes are interconnected.

Further, loss of inhibitory inputs might play a role, or the primary sensory cortices might be just more susceptible to damage than other regions in the brain. Also, signals within the system can become more similar due to widespread pathological processes that could lead to higher connectivity and higher efficiency. Unfortunately, no causal claims can be made as a cross-sectional design was used. Nonetheless, given that no network changes were seen in patients without more severe disability, the results from this study suggest that functional network disturbances are clinically disadvantageous in MS.

Sensorimotor network dynamics predict upper and lower limb disability progression

Next, we were interested in studying functional alterations to the sensorimotor network that were related to upper or lower limb impairments specifically and their value in explaining progression of disability. We used a longitudinal design to assess disability progression over 5 years and 24 pwMS were classified as upper limb progressing and 67 as lower limb progressing. Interestingly, only 21 patients converted to SPMS, which indicates that the current definition of SPMS does not overlap our current definition of progression on lower or upper limb motor function. This could indicate that our clinical definition of conversion to SPMS was more conservative than our research definition of progression, although it should be noted that we did not take relapses into account. Further, in addition to measurements of network efficiency across the entire functional scan (i.e. static approach), we also studied fluctuations over time (i.e. network dynamics). As static measurements are not sensitive to the fundamental dynamic nature of both brain and behaviour,^{9,10} studying temporal fluctuations of brain connectivity and networks is imperative, but limited research. Whereas a static approach is commonly used in MS research, studying the temporal fluctuations has only been recently validated, mostly focused on cognition.^{11,12} In relation to physical disability, we observed increased network efficiency dynamics and connectivity at baseline in pwMS who subsequently progress. The dynamics of the thalamus and supplementary motor area were independent predictors of upper and lower limb disability progression respectively. The higher dynamics could reflect a network that is unstable and therefore unable to perform as needed. Alternatively, higher network efficiency dynamics could be an adaptive response to ongoing pathology, what might reach a threshold after which dynamics reduces and subsequent progression of disability. In cognitively

impaired patients reduced centrality dynamics was observed in the same group of pwMS.¹¹ Lower centrality dynamics reflects a less fluctuating network that might be interpreted as a network that is 'stuck', what could lead to cognitive dysfunctions. However, from these results it is not clear how these different network metrics and disability and cognition potentially relate to each other. Nonetheless, our results suggest that high fluctuations in sensorimotor network efficiency is a sign of imminent progression of upper and lower limb impairments.

Summary Chapter 2

To conclude, from the studies in Chapters 2.1 and 2.2 we have learned that functional disturbances within the sensorimotor network are more prominent in pwMS who develop more serious motor disabilities. Our results show that while static efficiency increases relate to more severe disability cross-sectionally, dynamic efficiency increases were able to predict imminent clinical progression. These findings highlight the added value of using a network approach and studying the temporal features of a system in understanding the mechanisms underlying disability in MS.

2. In early disability stages, prior to more severe impairments, which functional activation pattern underlie subtle deficits in the upper and lower limbs?

Whereas in aforementioned studies we assessed functional sensorimotor network disturbances in pwMS with severe motor impairments, next we were interested in investigating the functional mechanisms underlying more subtle impairments in early stages of disability. We were able to detect changes in brain activity and subtle changes in motor performance in minimally disabled pwMS using a complex visually guided force-matching task and ultra-high field imaging. Compared to healthy controls, during lower limb force tracking, pwMS displayed a delayed response to the task cue and more erroneous movements, as well as lower activity in regions involved in visuomotor integration including cerebellar, occipital and superior parietal areas. Lower activity within these visuomotor regions correlated with worse disability and more structural damage. During upper limb movements, pwMS displayed lower inferior occipital cortical activation, but interestingly we did not observe any correlation with sensorimotor

disability or structural damage and any between-group differences in task performance. Together these results suggest that upper limb function is preserved in pwMS with no to minimal impairments and that partially divergent functional mechanisms might underlie upper and lower disabilities.

The neuronal mechanisms and pathways of upper and lower limb movements in brain and spinal cord are highly complicated and are not fully understood. Compared to walking, upper limb movements are more complex, have a higher degree of freedom and are less repetitive and thus less spinal cord processing dependent. Possibly it is easier to adapt upper limb motor functioning due to higher dependence on brain function. The disassociation between upper and lower limb motor performance in MS is in line with previous studies reporting moderate relation between walking and upper limb function¹³ and lower limb impairments early in the disease.^{14–16} As for brain activity, studies investigating the lower limbs in limited, either in clinically isolated syndrome¹⁷ or primary progressive MS¹⁸. Studies investigating hand function often used simple motor task at clinical field strength in a different population of pwMS.^{19–22} These approaches could have impaired the detection of subtle changes and more nuanced motor mechanisms underlying impairments in both upper and lower limbs, particularly in early disability. From our study we have learned that motor control impairments in MS are related to dysfunctions in visuomotor integration. Furthermore, ultra-high field imaging during complex upper and lower limb force tracking can reveal subtle impairments in movement and brain activation and differentiate upper and lower limb impairments in minimally disabled MS.

3. In people with MS with minimal physical disabilities, to what extent is axonal loss prevalent in of the major motor pathways of the brain, and does it relate to subtle changes in hand function and walking?

In pwMS with no to minimal clinical impairments substantial axonal loss was observed within major sensorimotor tracts, despite minimal focal inflammatory demyelination lesions within the tract, the classical pathological hallmark of MS. Unlike conventional diffusion tensor imaging measures, we used novel axonal markers associated with

specific fibre bundles, even in voxels that contain multiple fibre populations. The combination of recent advances in diffusion MRI and signal modelling enabled us to derive markers of axonal loss including fibre bundle density, indicative of diffuse axonal loss, and fibre cross-section, a marker of tract atrophy. The greatest effects were observed at a macrostructural level in the cross-section of the fibre bundle in all three tracts: corticospinal tract, interhemispheric sensorimotor tract, and cerebello-thalamic tracts. The degree of axonal fibre loss was associated with more erroneous hand movements and altered spatiotemporal gait patterns (shorter stance, smaller step width and prolonged double support).

To examine the added value of using a fixel-based approach in understanding disease pathology and the relation to structural damage elsewhere in the system, we examined the correlations between conventional brain volumetrics and fibre specific damage. The sensorimotor tracts were relatively devoid of focal inflammatory demyelination lesions, with less than 21% overlap with the tracts of interest in only up to 29% of pwMS. A moderate correlation was observed between whole brain lesion load and tract damage. While we cannot infer causality, this might suggest that the field of injury driving axonal loss is widespread rather than focal. Potentially, focal damage in other pathways or regions connecting to primary sensorimotor cortices could lead to trans-synaptic degeneration of the fibres in the sensorimotor tracts. In addition, Wallerian or retrograde axonal degeneration resulting from spinal cord injury could be a contributing factor to the WM damage within the corticospinal tracts. However, within the cervical cord, an area where most corticospinal tracts terminate²³ and lesions are commonly located (59%)²⁴, we observed no atrophy and no correlation between cervical lesion load and axonal damage in the motor tracts. This might argue against spinal cord lesions contributing to motor tract damage in the brain. In addition, early in the disease spinal cord lesions are often asymptomatic²⁵ and do not seem to predict disability progression²⁶. Further, we observed grey matter atrophy of S1 and thalamus, structures containing neurons whose axons comprise significant proportions of the motor tracts, which correlated with axonal loss in the corticospinal tracts.

Further, from our results it appears that axonal loss in minimally disabled patients is rather non-specific as substantial axonal damage was observed throughout all three motor pathways. However, within the corticospinal tracts we observed that the lower limb tracts were affected to a slightly greater extent than tracts related to upper limb movements. Also, a larger proportion of tracts originating from S1 compared to M1 were damaged. Therefore, it is reasonable to believe that some parts of the sensorimotor network might be more vulnerable to MS pathology and potentially sensory processes might be affected to a greater extent, leading to changes in hand function and walking patterns.

Summary Chapter 3

To conclude, we have gained insight into the changes in functional activation patterns and microstructural damage in relation to subtle changes in upper and lower limb function in pwMS with no to minimal motor impairments. We found subtle changes in brain activity and subtle impairments in movements during lower limb force tracking and preserved upper limb function. In addition, substantial axonal loss was observed within major sensorimotor tracts in the brain, despite relatively few white matter lesions, associated with subtle impairments in hand function and walking.

Observations brought forward in this thesis: bringing it all together

A key role for the primary sensorimotor cortices in disability progression in MS?

Considering all the results of this thesis, of the primary sensorimotor regions S1 was frequently implicated to play a role in disability in MS. In pwMS with minimal disabilities we observed significant S1 atrophy, but M1 was not atrophic, and S1 tracts were affected to a greater extent than axons descending from M1 (Chapter 3.2). As S1 is highly connected to other cortical and subcortical regions, S1 atrophy and tract damage early in disability potentially leads to functional activation changes in other regions such as cerebellar and superior parietal areas as observed during upper and lower limb movements (Chapter 3.1). While in early stages of disability sensory regions and tracts could be more vulnerable to structural damage, subsequently in more severe disabilities, functional alterations within S1 were observed, i.e. S1 efficiency and

dynamics predicted disability severity (Chapters 2.1 and 2.2). In more severe disability stages, axonal loss within the ascending pathways might have exceeded a threshold leading to functional disturbances in S1 as well, that consequently leads to progression of motor impairments. However, from our results we cannot infer any causality in disturbances within the primary sensorimotor regions and ascending and descending tracts. To understand the order of events and to unravel the complex relation between structure and function, multimodal approaches and a longitudinal design are needed. In addition, focussing solely on S1 might be too simplistic as the motor system is an intricate network comprised of multiple pathways and a high degree of interconnectivity, a network approach could be useful.

What is the role of the thalamus in MS?

Besides the primary sensorimotor cortices, our results indicate a significant role for the thalamus in disability. The thalamus is an important hub in the brain and is widely connected to other (sub)cortical regions. In minimally disabled pwMS we observed thalamic atrophy which is in line with previous studies showing that thalamic atrophy precedes clinical symptoms in MS²⁷ and progresses rapidly throughout the disease with strong correlation to physical disability²⁸ and cognitive dysfunction.²⁹ In addition, we observed functional disturbances of the thalamus, i.e. the functional efficiency of the thalamus fluctuated more (i.e. higher dynamics) in more severe disability stages. Increased network dynamics is in line with previous research which shown higher thalamic functional connectivity²⁹ and increased centrality³⁰ related to clinical symptoms in MS. Whereas most previous studies focused on either cognition or physical disabilities, motor and cognitive dysfunctions are related to each other³¹ possibly due to the integrative role of the thalamus in the entire system. In this thesis, the assessment tools used, and the sensorimotor network studied are highly likely to not only involve pure motor function but also comprise cognitive aspects. For example, we used the 9-HPT to assess hand dexterity, but cognitive aspects are likely to be involved in this test as 9-PHT performance was found to relate to cognition functioning.³² In addition, the sensorimotor network studied did not comprise of pure motor regions, but also included regions involved in cognition function such as the frontal cortex, deep grey matter structures and the cerebellum. As the brain is a complex system, using a more holistic

approach and studying cognition and physical disability together might lead to better understanding of the mechanisms underlying symptoms in MS. Nonetheless, regional information may also be useful, as regions such as the thalamus are a central hub that is highly responsible for the network to perform as needed.

Upper and lower limb impairments in MS

In pwMS with minimal clinical disability, we observed worse motor performance during lower limb movements but preserved upper limb function (Chapter 3.1). In more severe disease stages both upper and lower limb disabilities progressed over 5 years, but more patients displayed a decline in lower limb than upper limb function (65% vs 11%) (Chapter 2.2). These findings suggest that differential functional mechanisms might underlie upper and lower limb disabilities in MS, possibly with the lower limbs being affected earlier in the disease and more severely. However, even though we did not observe significant between-group differences in pwMS with mild impairments, pwMS did perform slightly worse during upper limb force tracking including more erroneous and delayed responses that correlated with more severe damage within major sensorimotor tracts (Chapter 3.2). These findings suggest that upper limb dysfunction is already present early on, in line with findings of previous studies,^{16,33} but were possibly not detected properly and more sensitive tools are needed. Similarly, in later stages, the assessment tools might not have been sensitive enough used to detect upper limb progression accurately.

Alternatively, upper limb disabilities might emerge slightly later and/or more mildly than lower limb impairments and are not clinically present until more severe stages of the disease. We studied either pwMS with minimal motor impairments or pwMS with more serious disabilities, however we did not focus on the group in between. Taking into account that the clinical course of MS is highly heterogenous, studying pwMS with moderate impairments potentially gives more insights into the emerge of upper and lower limb impairments in MS.

Future directions

From clinical rating scales to more sensitive measures of upper and lower functioning

In minimally disabled pwMS we were able to detect subtle lower limb impairments including more erroneous movement and a delayed response during force tracking and altered patterns of gait. We did not observe any changes in upper limb function using the visually guided force-matching fMRI task. Possibly, hand dysfunction emerges in later stages or we were unable to detect subtle dysfunction with the assessment tool used. Even though the task involved complex visuomotor integration and reflects daily motor performance more closely than simple fMRI tasks such as finger tapping, it is limited due to restricted movements possible in the scanner. However, detecting subtle changes in upper limb function early on disease is not only useful in research to understand evolution of disease progression, but is potentially important in clinic as well in order to optimize treatment strategies as early as possible to delay or prevent progression of disabilities.

The gait patterns and behavioural performance measures acquired during visuomotor fMRI task were assessed in minimally disabled pwMS only but might be informative in more severe disability stages as well. In pwMS with more serious motor disabilities, we used the EDSS, 9-HPT and the T25FW to assess disability severity and the progression of upper and lower limb impairments. Whereas these tests are relatively easy to perform and quick to implement, evaluation is not very detailed. The EDSS has limited reliability,³⁴ is predominantly sensitive to lower limb impairments particularly at the higher ends of the scale. In MS, the 9-HPT is recommended as a golden standard for measuring fine dextrous manual movements.³⁵ However impaired dexterity can be accompanied by reductions in upper limb strength, altered hand sensation,^{33,36} tremors,^{37,38} changes in vibration,³⁹ abnormal tactile sensibility,³⁶ and impaired coordination³⁹. Unfortunately currently there is no single outcome measure that covers all upper limb related disabilities in MS.⁴⁰ Similarly, the T25FW essentially measures walking speed in a straight line, but does not capture other aspects of gait including balance, turning, endurance and subtle changes in walking that require advanced technologies.^{14,41}

To detect subtle changes in walking and assess the spatiotemporal aspect of the gait cycle 3-dimensional video tracking gait analyses can be used. While laboratory gait analysis has many advantages over simple physical assessments, this method requires a complex experimental set up with a dedicated laboratory and advanced motion capture systems, sensor embedded walkways and force platforms. To obtain more simple but accurate measures of walking performance, wearable wireless sensors can be used.^{41,42} Wearable sensors can be placed on different parts of the body to measure acceleration, orientation, velocity and gravitational forces or under the foot to detect changes in pressure and force, both used to assess walking and balance.⁴¹ Sensors give less detailed information but are easy to use, lower in costs and can potentially improve monitoring of lower limb impairment in clinic as well as in daily life between visits to detect changes as early as possible for optimal treatment.⁴¹

Whereas wearable sensors could potentially be very useful, current assessment tools such as the 9-HPT and T25FW might be used more optimal. In Chapter 2.2 we defined progression based on > 20% decline in performance, but we lacked healthy control data and were therefore unable to define who was disabled at baseline. This would be useful not only in research but also in clinic to define whether upper or lower limb function is actually impaired. Currently, treatment strategies are optimized when a relapse occurs, or when progression is clinically measured, but when deviations from normal are detected earlier, potentially treatment strategies can be adapted or formulated to prevent functional decline.

Clinical and ultra-high field imaging

Our ultra-high field imaging findings have highlighted the added value of 7T MRI in understanding the underlying neuronal mechanisms of motor impairments in MS. We studied the sensorimotor system using 3T imaging in a large heterogenous group of pwMS and performed a more focussed study in minimally disabled pwMS using ultra-high field MRI. Whereas 3T MRI scanners are widely available, ultra-high field MRI allows to image the brain in more detail and with higher accuracy. The high signal-to-noise ratio that can be achieved with ultra-high field allows the visualisation of finer structures and

has shown to improve detection of cortical grey matter pathology^{43,44} and diagnosis⁴⁵ in MS. Besides better structural imaging, functional imaging at ultra-high field has major advantages over 3T imaging. The higher signal-to-noise can be invested in higher spatiotemporal resolutions and better contrasts improving the statistical power and modelling of the signal, which allows to detect subtle functional activation changes with higher specificity.^{46,47} At lower field strengths the SNR is enhanced commonly by using large spatial smoothing kernels to improve inter-subject alignments, but also leads to incorrect estimation of true localisation and consequently to type-I errors.^{48,49} Ultra-high field imaging comes with the challenge of worse magnetic field inhomogeneities. Signal dropout and warping is particularly observed around the inferior temporal and orbitofrontal cortices. B0 and B1 field inhomogeneities leading to signal distortions are particularly seen with diffusion weighted imaging.

In this thesis, 7T imaging allowed us to detect subtle changes in brain activation and microstructural damage in absence of lesion pathology in relation to subtle changes in upper and lower limb function pwMS with no to minimal impairments. The clinical application of ultra-high field imaging is still limited, but these findings highlight the added value of 7T to detect subtle changes early in the disease. This could be useful in monitoring and predicting the emergence of motor impairments and could lead to more effective treatment strategies to delay or prevent significant neurological decline.

Multi-model approaches

Given that the brain is an incredibly complex and highly connected system, it is very likely that the sensorimotor network overlaps and is connected with other brain networks involved in for example cognitive function. A more holistic approach could be useful in understanding the mechanisms underlying disabilities in MS. In addition, brain function and structure are often studied separately, but likely the interplay between different modalities underlies disabilities in MS. Spatially structural and functional resting-state network largely resemble each other.⁵⁰ However whereas the structure of the brain can be considered relatively fixed, brain function is more dynamic. Damage to one pathway could result in several variations in functional activation patterns. Potentially the change in coupling between structural and functional networks lead to

motor deficits. A multi-model approach and longitudinal studies are needed to decode the complex coupling between structure and function and the evolution of sensorimotor impairments. In addition, current developments in artificial intelligence now allow for a reliable modelling of brain function. Such models have been used to simulate progression in MS, by simulating structural network disconnection due to lesions, and evaluating functional consequences to the network.⁵¹ These explorations have shown that such structural damage initially induces increased functional connectivity, which transitions to reduced functional connectivity in later stages. In addition, a sudden loss of network efficiency (a “network collapse”) was identified during this transition towards reduced connectivity. These approaches could be useful to further our understanding of the network changes we have observed in this thesis, as well as their relevance for motor dysfunction as well as cognitive impairment.

From advanced MRI methods to imaging biomarkers in clinic

Whereas we have learned a great deal about motor disability in MS using advanced imaging methods, the translation of these methods to clinic is challenging. Whereas network measures and/or markers of axonal loss might be useful in monitoring and predicting the emergence of mobility and dexterity impairments in MS, the studies were largely exploratory involving complex analysis and were based on a group rather than the individual. While these techniques might be quite far away from clinical monitoring, phase II trials could be a good way to translate these techniques because secondary endpoints often involve advanced imaging.^{52,53} In addition, as MS is highly heterogeneous, there is an urgent need to predict the individual trajectories, but current knowledge is limited on how to do this. To capture the clinical heterogeneity and understand the individual brain mechanisms underlying each disability course, ultra-high field imaging could be useful because of the high sensitivity which allows to image the brain at high spatiotemporal resolutions. In addition, machine-learning could be helpful in building better predictive models of individual disease courses, however the application in neuroscience is still relatively new and these techniques require large and variable datasets to train.

Histopathology of axonal loss and network disturbances

We have learned a great deal about motor disability in MS using advanced imaging methods, however the histopathology underlying the MRI changes were not studied. We have inferred lower fibre cross-section and fibre density in major motor tracts as axonal loss. Even though this was based on solid theory and high-quality imaging, histopathological validation is needed. Histopathological measures of axonal density and cross-section would be valuable for accurately interpreting fibre bundle changes in pwMS. For instance, recent work has investigated the underlying substrate of diffusion changes in cortical lesions,⁵⁴ while another study focused on diffusion changes within white matter tract connected to damaged cortical areas.⁵⁵ The use of our techniques based on fixel analyses and 7T imaging could provide even more information. Modelling the fibre orientation distributions and deriving fibre specific metrics of axonal density and cross-section, as well as the high spatial resolutions acquired with 7T imaging,^{56,57} might allow for more accurate estimation of pathology and diffusivity. In addition, our network effects could also be investigated in more detail using histopathological studies, as was done recently.⁵⁸ This study focussed on the relation between neuronal size and axonal density and structural network topology measures in pwMS. Smaller neurons and lower density at a micro-scale related to a higher macro-scale clustering coefficient, a measure of network segregation. Fibre length, a measure of integration, related to neuronal size, i.e. longer fibers were associated with larger neurons.⁵⁸ These relations not only show the potential underlying pathological mechanisms of network changes, but also suggests that a network approach might be informative in understanding the neuroaxonal pathological processes in MS. Even though these initial findings are promising, the histopathological basis of particularly functional network changes remains poorly understood. Improving our understanding of the pathology processes underlying these network disturbances might lead to novel treatment targets.

Key Findings

The key findings of this thesis can be summarised as follows:

1. *Functional network efficiency disturbances and severe disabilities*

- Out of 222 MS patients, 185 patients displayed minimal impairments and 37 patients presented with more serious disabilities.
- In pwMS with no to mild disability the sensorimotor network remained normal
- PwMS with severe disabilities displayed increased sensorimotor network efficiency
- Functional changes in highly disabled patients were centred around the somatosensory cortex (S1)
- S1 connectivity was increased with prefrontal and secondary sensory areas
- Out of 214 pwMS, 24 showed upper and 67 showed lower limb progression
- Progression was predicted by increased network efficiency dynamics
- No change in static network measures observed in progressing compared non-progressing patients
- Thalamic and supplementary motor area dynamics predicted upper and lower limb disability progression respectively
- Functional network effects were predictors of progression independent of structural damage

2. *Functional task activation patterns and motor behaviour in minimally disabled pwMS*

- Differential functional activity patterns and motor performance were observed for hand and foot impairments in patients with no to minimal disabilities
- PwMS displayed delayed and more erroneous lower limb force tracking compared to healthy controls
- Lower cerebellar, occipital and superior parietal cortical activation was observed during lower limb movements in pwMS compared to controls
- Despite no differences in upper limb task performance, pwMS displayed lower activation in the inferior occipital cortex

- Ultra-high field during a complex visuomotor fMRI task is useful to detect subtle impairments in movement and subtle changes in brain activity

3. *Axonal loss and subtle changes in hand function and walking*

- Minimally disabled pwMS displayed substantial axonal loss within major sensorimotor tracts, despite relatively few focal lesions
- The degree of axonal loss associated with worse hand function and altered patterns of walking

Future studies are needed to further investigate

Network disturbances and progression of sensorimotor impairments

- Can we unravel whether network metrics initially increase till a certain threshold is reached, after which connectivity and network efficiency decreases, leading to progression of motor disability in MS?
- What is the interplay between structural damage and functional changes in ascending (S1) and descending (M1) pathways in relation to motor disability progression?
- Can we more precisely unravel the drivers of disability using a multi-modal approach?
- What are the histopathological substrates of the disturbed network patterns observed in this thesis?
- Will the integration of a network perspective and more sensitive measures (i.e. high field imaging + gait analysis) help to further unravel disability progression?

The use and development of sensitive tests and ultra-high field imaging

- Could axonal fibre metrics provide useful markers for emerging sensorimotor network dysfunction in early disease stages?
- Could decline in motor function be detected earlier in clinic with more sensitive measures, and would this be useful to optimize treatment strategies to prevent neurological decline?
- Could ultra-high field MRI markers be useful in the motoring and predicting decline in hand and foot function?

Progression of upper and lower limb disabilities

- Are more sensitive measures needed to detect upper limb disabilities early on?
- We studied either minimally or severely impaired pwMS, but can we further elucidate the neural mechanism underlying upper and lower limb disability progression by studying the sensorimotor network of mildly impaired pwMS?
- What are the network disturbances of upper and lower limb impairments over a longer time frame, and would this help to unravel the order of impairments?

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