MANCHESTER 1824

The University of Manchester

Understanding Network Level Changes in Multiple Sclerosis: Relationships with Cognitive Dysfunction

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy

in the Faculty of Biology, Medicine & Health

2022

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Contents

Word Count: 62,585

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List of Abbreviations

All abbreviations are defined at first mention in each chapter. Below are those most commonly used throughout the thesis.

ACM = Anatomical Connectivity Mapping

BOLD = Blood Oxygenation Level Dependent

BRB-N = Brief Repeatable Battery of Neuropsychological Tests

CBF = Cerebral Blood Flow

DMN = Default Mode Network

dMRI = Diffusion Magnetic Resonance Imaging

FA = Fractional Anisotropy

FC = Functional Connectivity

FPN = Frontoparietal Network

fMRI = Functional Magnetic Resonance Imaging

GM = Grey Matter

HC = Healthy Controls

ICA = Independent Component Analysis

MRI = Magnetic Resonance Imaging

MS = Multiple Sclerosis

NBV = Normalised Brain Volume

NGMV = Normalised Grey Matter Volume

NWMV = Normalised White Matter Volume

PASAT = Paced Auditory Serial Addition Test

RRMS = Relapsing-Remitting Multiple Sclerosis

ROI – Region Of Interest

Rs-fMRI = Resting State Functional Magnetic Resonance Imaging

RSN = Resting State Network

SPMS = Secondary Progressive Multiple sclerosis

SDMT = Symbol Digit Modalities Test

T = Tesla

WM = White Matter

Abstract

Cognitive impairment is a debilitating symptom of multiple sclerosis (MS). The pathological mechanisms are poorly understood, making it difficult to monitor decline clinically and develop interventions. Advanced MRI measures are increasingly used to identify the brain structural and functional substrates of cognitive dysfunction. This thesis aimed to build upon this work by investigating network changes in people with MS and assessing links with cognitive impairment.

A systematic review established that functional network changes are commonly associated with cognitive impairment across studies and MS phenotypes, but there was no consistent pattern of connectivity increases or decreases, likely due to the large heterogeneity of methods and population studied in the literature to date.

This review highlighted the need for more model-led studies which probe the mechanisms of functional connectivity changes. The second study of the thesis combined resting state functional MRI with diffusion MRI and cerebral blood flow to test the *nodal stress* hypothesis, which predicts that the high metabolic demands of network hub regions make them susceptible to degeneration. We found altered anatomical connectivity and cerebral blood flow measures around the networks with abnormal functional connectivity in cognitively impaired relative to non-impaired patients, thus providing preliminary support for the nodal stress hypothesis.

The third study of the thesis tested the *energy failure* hypothesis, which is based on observations of sodium accumulation in demyelinated axons, resulting from inadequate energy to pump sodium out of the cell. By combining resting state functional MRI with sodium MRI we were able to show evidence of sodium accumulation in functional network regions, which correlated with cognitive test performance, providing further support for altered metabolic function of brain networks supporting cognitive function.

The final study of the thesis explored whether damage to structural connectivity in MS is dominated by one or more patterns of pathology that resemble those seen using typical functional network assessments, to understand if damaged anatomical connections could be a driver of functional connectivity abnormalities. No evidence of a network structure within white matter was found, but results highlight the need to understand the relationship between anatomical and functional connectivity better.

Together, these studies confirm the importance of network changes as a correlate of cognitive impairment in MS and highlight the need for more model-led research into the mechanisms of network changes. A better understanding of what causes connectivity changes has the potential to provide an MRI marker of cognitive decline for clinical translation.

Declaration of Originality

I hereby confirm that no portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Authorship & Collaboration

Danka Jandric was responsible for the design and execution of all research studies presented within this thesis, with support from PhD supervisors Dr Nils Muhlert, Professor Geoffrey Parker and Dr Laura Parkes.

Several of the chapters in this thesis were scientific collaborations with researchers at other institutions. In Chapter 3 the study design, data collection and analysis were carried out jointly with Dr Anisha Doshi at University College London, the joint first author of the publication of this work. All writing and editing was done by Danka Jandric. Remaining study authors provided essential feedback on manuscript drafts. Chapters 4 and 6 were collaborations with Dr Ilona Lipp, the joint first author for the publication of the work presented in chapter 4, who collected the data used in this work at Cardiff University. All analyses and writing were done by Danka Jandric. Remaining study authors provided feedback on analysis steps and manuscript drafts. Chapter 5 was conducted fully by Danka Jandric, with support by Dr Nils Muhlert and Professor Geoff Parker during study design, and all co-authors during writing.

Acknowledgements

First and foremost, thank you to all research volunteers who contributed to this work. Your generosity with your time has made this research possible. Thank you from the bottom of my heart. This work is likely to only make a dent in understanding cognitive symptoms in MS, but I remain committed to the conviction that enough dents will make a break in how cognitive impairment is understood and managed.

Thank you to the Medical Research Council for funding and enabling this research.

Thank you to my amazing supervisors, Dr Nils Muhlert, Prof Geoff Parker, and Dr Laura Parkes, for guiding me through the many challenges of research with expertise and kindness. And to Dr David Rog and Dr David Paling for providing a clinical perspective and advice for not losing focus of the clinical relevance of this work. I am also deeply grateful to all wonderful researchers I have had the privilege to have collaborated or otherwise worked with, with special mentions of Dr Ilona Lipp and Dr Anisha Doshi, for making me think that little bit harder and do that little bit better. Thanks also to all R&D, clinical and administrative teams at the University of Manchester and the NHS Trusts where this research was conducted, without whom even the best idea for a project would not be possible to carry out.

To Josh, to my family and my friends - thank you for being there on both good days and bad. For providing endless support and making me feel like anything is possible, and for offering warm hugs, gentle words and wine when everything felt impossible. I am lucky beyond words.

Many people have been in my life during the nearly 4.5 years this thesis has taken to complete, and most have made an impact on me and my work. Thank you to all.

Chapter 1

Introduction

1.1 An introduction to multiple sclerosis

1.1.1 Prevalence and aetiology

Multiple sclerosis (MS) is a chronic neurodegenerative disorder of the central nervous system affecting 2.8 million people worldwide (MSIF, 2020), making it a leading cause of neurological disability in young adults (Compston and Coles, 2008). In the UK it is estimated that 131,720 people are living with MS, one of the highest prevalence rates in the world. MS is commonly diagnosed around the age of 30, is more prevalent in women than men with a ratio of about 3:1, and shows a latitude effect whereby it is more prevalent in people living in higher latitudes, such as Northern Europe and North America (MSIF, 2020). The causes of MS are unknown, but several risk factors have been established, including lifestyle factors such as smoking, environmental factors including vitamin D deficiency, viral infections, and to some extent genetic factors (Kamm et al., 2014; Baranzini and Oksenberg, 2017).

1.1.2 Diagnosis and clinical course

MS is highly heterogenous and the clinical course can vary substantially between individuals. Common symptoms include visual disturbances, incoordination, limb weakness, sensory abnormalities, fatigue, pain, bowel and bladder problems, and cognitive dysfunction. The diagnosis of MS can be made on the basis of either clinical symptoms or radiological evidence obtained with magnetic resonance imaging (MRI), and requires dissemination in space and time to rule out alternative diagnoses (Thompson et al., 2018).

Typically, patients will present with a single attack, known as a Clinically Isolated Syndrome (CIS). Most CIS cases will progress to develop a relapsing-remitting form (RRMS), affecting about 80% of people with MS, in which episodes of the presence of neurological symptoms (relapses) over the course of days or weeks are followed by a period of remission in which symptoms recede. Over time recovery from relapses is increasingly incomplete, and disability accumulates (Compston and Coles, 2008). This

rate of disease progression varies between patients, from those with relatively little disease activity and progression, termed benign MS (BMS) (Reynders et al., 2017), to those with rapidly progressive MS, termed rapidly evolving severe (RES) MS (Huisman et al., 2017). About 65% of those diagnosed with RRMS will develop secondary progressive MS (SPMS) about 10-15 years after diagnosis, a stage of the disease characterised by a lack of remission and as a result, progressive accumulation of disability. A small number of those diagnosed with MS, about 20%, experience this progressive form of the disease, known as primary progressive MS (PPMS), from the onset (Compston and Coles, 2008). Occasionally, findings on MRI are made which are consistent with MS, but lack a history of neurological symptoms. This radiologically isolated syndrome (RIS) may convert to MS, but its diagnosis and management is a topic of debate among clinicians (Hosseiny et al., 2020).

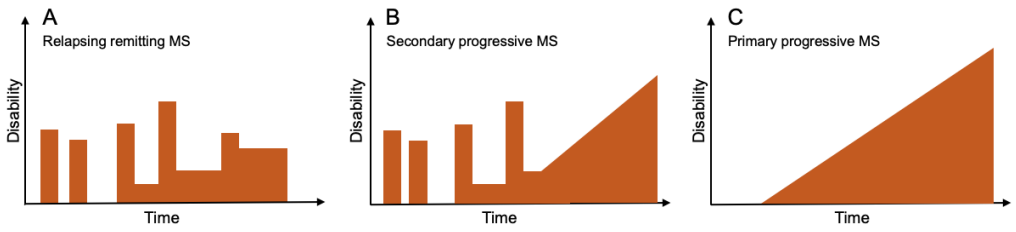

Figure 1.1: Subtypes of multiple sclerosis
Disability progression over time occurs in different patterns across the three main subtypes of MS. In relapsing-remitting MS (A) episodes of neurological symptoms, relapses, are followed by periods of remission during which there is complete or partial recovery of symptoms. Most patients with relapsing-remitting MS will develop secondary progressive MS (B), in which disability gradually accumulates in the absence of relapses. A small proportion of patients experience gradual accrual of disability without relapses from disease onset, in the primary progressive MS subtype (C).

1.1.3 MS pathology

MS is characterised by focal inflammatory demyelination of neuronal axons, leading to what is commonly called ‘lesions’ or ‘plaques’ (Charcot, 1877; Lassmann, 2018). While traditionally viewed as a disease affecting only the white matter, it is now known that lesions also occur in the grey matter, and atrophy develops across the brain parenchyma (Lassmann, 2018) at a rate of 0.5-1% greater than in normal aging (Klawiter, 2013). The trigger of inflammatory activity is not established, but it involves autoreactive immune cells, including T and B lymphocytes, crossing the blood-brain barrier into the central nervous system and attacking axonal myelin sheaths and myelin-producing oligodendrocytes. In the early stages of the disease some remyelination occurs, but with increasing oligodendrocyte loss this becomes less common. Lesions

can be either active with ongoing inflammatory activity, or inactive. Inactive lesions are a result of immune attacks and subsequent repair processes and reflect the scarring following inflammation, demyelination, remyelination, astrocytosis and gliosis, and finally axonal and neuronal loss. Due to the appearance of lesions of scarred tissue, sclerosis, and abundance in the MS brain, they have given the disease its name (Compston and Coles, 2008; Lassmann, 2018).

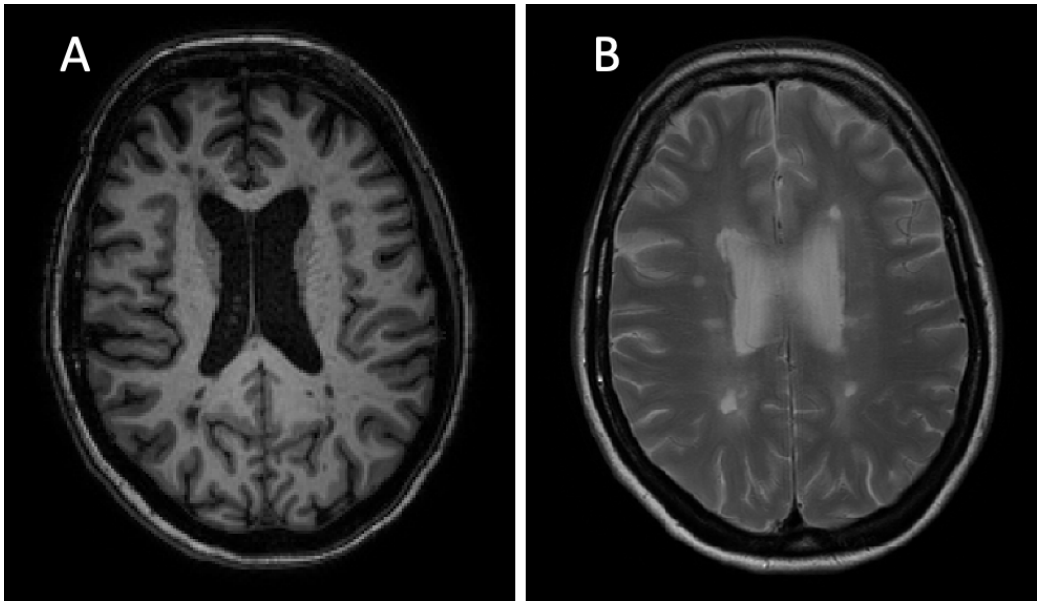

Figure 1.2: Lesions in multiple sclerosis
Axial slices from T1-weighted (A) and T2-weighted MRI scans of a 53-year-old female with diagnosed RRMS, showing inactive and active lesions, respectively. This data is from an individual example MS patient from Chapter 5.

1.1.4 Treatment

At present there is no cure for MS, but over the last 20 years a number of disease modifying treatments (DMTs) have been developed. Though they differ in their mechanism of action, all DMTs aim to reduce inflammation and thereby reduce clinical and radiological aspects of the disease, commonly the number of relapses, new lesions and atrophy. Based on their mechanism of action DMTs vary in their effectiveness and safety profile, with higher efficacy treatments commonly being associated with more severe side effects (Torkildsen et al., 2016; Doshi and Chataway, 2017). The prescribing decision is typically guided by the patient's disease activity and the licensed indication of the DMT, which can vary between countries. In the UK the National Institute for Health and Care Excellence (NICE) sets out criteria for the use of DMTs based on their efficacy and safety (Perry et al., 2014). Within this framework, the choice of suitable DMT remains the decision of the treating neurologist. Two common treatment approaches

include prescribing higher efficacy DMTs earlier in the disease, so called induction treatment, or escalation treatment involving the initial use of lower efficacy treatments, followed by gradual escalation to higher efficacy DMTs (Doshi and Chataway, 2017).

Currently most DMTs in the UK are licensed for the treatment of RRMS only, and it is not yet established whether any DMT can prevent or delay conversion to SPMS. In recent years only two treatments have been licensed for progressive forms of MS in the UK (EMC, 2021a, b), and so treatment for this degenerative stage of the disease largely remains an unmet need.

1.2 Cognitive impairment in MS

1.2.1 Prevalence

Cognitive impairment in MS was first described by Charcot in 1877 as “marked enfeeblement of the memory” and “conceptions [that] are formed slowly” (Charcot, 1877) and is today still one of the common symptoms of MS. Cognitive dysfunction is estimated to affect 40-70% of MS patients, depending on the clinical and demographic characteristics of the sample and the tests and criteria used (Chiaravalloti and DeLuca, 2008; Sumowski et al., 2018). It is present across subtypes, and affects several cognitive domains, including memory, attention, information processing, executive function, verbal fluency and visuospatial perception (Chiaravalloti and DeLuca, 2008). Cognitive impairment is hugely debilitating and associated with greater unemployment (Morrow et al., 2010; Campbell et al., 2017; Srpova et al., 2021), increased concordant depression (Feinstein, 2011), and reduced quality of life (Campbell et al., 2017). Moreover, it correlates with progression of physical disability throughout the disease (Lynch et al., 2005), and predicts disability progression longitudinally (Deloire et al., 2010), demonstrating that patients with cognitive impairment accumulate greater disability.

Although rarely a presenting symptom, cognitive deficits have been documented early in the disease, both in CIS (Feuillet et al., 2007; Glanz et al., 2007; Khalil et al., 2011; Brochet and Ruet, 2019) and in newly diagnosed RRMS patients (Jønsson et al., 2006; Glanz et al., 2007; Brochet and Ruet, 2019), and it has been suggested that the presence of cognitive impairment can predict which CIS patients will develop RRMS (Zipoli et al., 2010). Rates of impairment in CIS vary across the literature, from 12.3% to 57%, depending on the characteristics of the sample and tests used (Feuillet et al., 2007; Uher et al., 2014), however, rates around 20-30% are more commonly reported (Brochet and Ruet, 2019). Similarly, reported rates of cognitive impairment in early

RRMS vary, but are often around 30-40% in cross-sectional studies (reviewed in Brochet and Ruet, 2019). However, there is an effect of disease duration, by which cognitive impairment becomes more common and more severe with increasing disease duration. In a large cross-sectional study of 1500 MS patients cognitive impairment was found to increase over 25 years, from 16.6% at year 1 after diagnosis, 20.9% at year 5, through to 44.6% at year 25, when defined as scores of 2 standard deviations below normalised mean scores on a global cognitive score (Achiron et al., 2013). Similarly, a recent retrospective study of patients diagnosed with RRMS and followed up prospectively for up to 15 years showed a rate of cognitive impairment of 12.8% at baseline, increasing to 24.4% after 10 years (Carotenuto et al., 2021). Compared to baseline, 51.4% of patients maintained a stable test performance while 48.6% showed a worsening (Carotenuto et al., 2021). In addition to disease duration, aging causes accelerated brain atrophy which is likely to influence cognitive function (Azevedo et al., 2019). Evidence shows that those diagnosed with MS later in life are more likely to experience cognitive impairment (Butler Pagnotti et al., 2021).

1.2.2 Measurement and diagnostic criteria

A wide range of neuropsychological tests are used to assess cognitive function in MS (das Nair, 2021). These are typically used either in isolation, in combination with other stand-alone tests, or as part of neuropsychological testing batteries. Two commonly used stand-alone tests are the Paced Auditory Serial Addition Test (PASAT) and the Symbol Digit Modalities Test (SDMT). The PASAT is considered a test of attention, information processing speed and working memory. It requires the test-taker to listen to single digits presented every two or three seconds and add each new digit to the digit presented immediately prior to it. The total score is based on the accuracy of the responses. However, it has been shown that this test is not always well tolerated, causing the test-taker anxiety which may affect the test results (Tombaugh, 2006). The SDMT requires the test-taker to match geometric symbols to corresponding numbers using a reference key provided to them. It taps into several cognitive functions, including attention and information processing and has been found to be most sensitive for cognitive monitoring in MS (Benedict et al., 2017; Sumowski et al., 2018). Batteries have been developed which include several tests to measure different aspects of cognition, including attention, information processing speed, working memory, executive function, verbal episodic memory and visuospatial episodic memory. The three perhaps most commonly used in MS are the Brief Repeatable Battery (BRB, Rao, 1990), the Minimal Assessment of

Cognitive Function in MS (MACFIMS, Benedict et al., 2002) and the Brief International Cognitive Assessment for MS (BICAMS, Langdon et al., 2012).

While all of these batteries have proven sensitivity to cognitive deficits in MS (reviewed in Sumowski et al., 2018), an ongoing challenge is how to define cognitive impairment. Common definitions are based on a comparison to healthy controls or normative values and set at a threshold of a number of standard deviations (SD) below the control or normative mean on a certain number of tests, for example 1.5 or 2 SD on two or more tests (reviewed in Fischer et al., 2014). One challenge with this approach is that different study participants might fail different tests, e.g. memory or processing speed, yet meet the same definition for cognitive impairment, raising concerns about heterogenous samples which makes interpretation of results challenging. Nevertheless, performance across tests tends to correlate, and impaired patients tend to be impaired on more than one domain, so this approach, while complicated, is still commonly used (Sumowski et al., 2018).

Another challenge with defining cognitive impairment is establishing the threshold for impairment. There is increasing evidence to show that the threshold set influences the prevalence rate of cognitive impairment, with more lenient definitions typically resulting in higher rates (Fischer et al., 2014; Doshi et al., 2019). This effect could result in an overestimation of the prevalence of cognitive impairment, or it could be argued to detect more subtle impairment. While more stringent definitions seem to be more reliable over time (Zipoli et al., 2010), evidence is lacking of how definitions of impairment reflect the patient's functioning in their daily life (Fischer et al., 2014). This issue of ecological validity of neuropsychological tests is further complicated by the discrepancy between the perceived cognitive impairment by patients and formalised definitions of cognitive impairment on tests. Many patients will experience a worsening of their cognition, but fall within the normal range on formal tests, possible due to an above average baseline cognitive function (Sumowski et al., 2018). Finally, several other aspects of MS may influence cognitive function, including fatigue, depressive symptoms, pain and sleeping problems (Damasceno, 2020), and may not always be accounted for in formal assessments of cognitive function.

1.2.3 Management

Despite cognitive symptoms being common in MS, cognitive function is not routinely monitored in the clinical setting (Sumowski et al., 2018; DeLuca et al., 2020), and even when it is assessed deficits are not diagnosed with great accuracy (Romero et al.,

2015). The development of improved neuropsychological tests, including an electronic SDMT for self-administration by patients (Lam et al., 2021), is expected to help. A major challenge is management of cognitive impairment even when it is accurately diagnosed. At present there are no licensed treatments for cognitive symptoms in MS, and cognitive endpoints are still uncommon in phase 3 clinical trials of DMTs (DeLuca et al., 2020). Nevertheless, some data suggest beneficial effects of DMTs. A recent study reported lower rates of poor cognitive performance in a large sample of DMT-treated patients compared to previous estimates, suggesting a possible protective treatment effect on cognition (Harel et al., 2019). Similarly, a meta-analysis reviewed available evidence on the effect of DMTs on cognitive function and found positive, if small, effects. However, the findings reflected mainly well-established DMTs including interferon-beta and natalizumab, with data missing for newer treatments (Landmeyer et al., 2020).

Non-pharmacological approaches to improving cognitive function in MS focus on cognitive rehabilitation programmes, which involve training on some cognitive task or function. This is a recent and growing research field, and to date the evidence is mixed, with some studies obtaining positive results of sustained improvements over time (reviewed in DeLuca et al., 2020), but systematic reviews finding only preliminary and/or inconclusive evidence across the body of literature (Rosti-Otajärvi and Hämäläinen, 2014; Mitolo et al., 2015; das Nair et al., 2016). The field faces several methodological challenges to produce stronger evidence, including the use of larger sample sizes and stronger control conditions and blinding procedures.

Some lifestyle factors seem to reduce somebody’s risk of developing cognitive impairment. Higher education and greater intellectual enrichment have been shown to contribute to a so called cognitive reserve which makes people less susceptible to cognitive impairment, even in the presence of lesions and atrophy (Sumowski and Leavitt, 2013; Sandroff et al., 2016; Estrada-López et al., 2021; Randolph et al., 2021). Similarly, physical exercise is thought to have a positive effect on cognition in MS, possibly mediated through improved neuroplasticity (Sandroff et al., 2018; DeLuca et al., 2020).

Still, despite several promising avenues for managing cognitive impairment, the limited evidence for any given strategy highlights monitoring cognition, identifying those at risk of cognitive decline and effective interventions to reduce cognitive decline as major unmet needs in the management of MS. An improved understanding of the pathological changes underlying cognitive impairment will be key to addressing this.

1.3 MR imaging of cognition in MS

1.3.1 Lesions and atrophy

The pathological mechanisms of cognitive impairment are not established and are the topic of active research. MRI is an important clinical and research tool for investigating brain changes in MS. Conventional MRI measurements include lesion count and volumetric measurements of lesions and normal appearing brain tissue (Filippi and Rocca, 2011). A large body of research has focused on the relationship between white matter lesion count or volume and cognitive function. While such associations are commonly found, overall the evidence suggests low to moderate correlations, leading to the so called clinic-radiological paradox (Barkhof, 2002; Rocca et al., 2015; Paul, 2016).

Although traditionally thought of as a white matter disorder, lesions also occur in the grey matter. However, it is difficult to measure GM lesions and it is estimated that only a small number of cortical lesions are detected with current MR methods, including double inversion recovery (DIR), which supresses the signal from both cerebrospinal fluid (CSF) and WM to detect cortical lesions with more accuracy (Geurts et al., 2005; Faizy et al., 2017) and phase-sensitive inversion recovery, which has higher signal to noise for detecting cortical lesions (Sethi et al., 2012). Nevertheless, studies have shown correlations between cortical lesions and cognitive test performance in MS (Roosendaal et al., 2009; Calabrese et al., 2012; Geurts et al., 2012), highlighting an important role in the disease pathology for cortical tissue which appears normal on conventional MRI.

In addition to lesions, neurodegeneration occurs in MS and a research focus has been the link between brain atrophy and cognitive decline. While several longitudinal studies show correlations between increases in atrophy measures and decline in cognitive function over time (Zivadinov et al., 2001; Deloire et al., 2011), whole-brain atrophy is a non-specific measure of brain damage and does not distinguish between the contribution of different tissue classes to cognitive decline or provide information about the potential pathological mechanisms. The study of patterns of grey matter (GM) atrophy shows more promise as it shows some regional specificity. Several studies have found different patterns of GM atrophy between cognitively impaired and unimpaired MS patients, including a greater degree of cortical thinning in frontal and temporal areas and more atrophy of deep GM structures including the thalamus and hippocampus, in cognitively impaired patients (Morgen et al., 2006; Sicotte et al., 2008; Calabrese et al., 2010; Sbardella et al., 2013; Preziosa et al., 2016; Tillema et al., 2016). Importantly, some regions seem more susceptible to cortical thinning than others, as shown in a study

that found anatomical patterns of co-varying cortical thickness in a large sample of 208 MS patients with long standing disease (Steenwijk et al., 2016). Two of the ten patterns identified, including the posterior cingulate cortex and temporal pole, were strong predictors of cognitive test performance. Similarly, a recent study found covarying patterns of regional GM volumes in 988 SPMS patients. Fifteen patterns were identified, of which one consisting mainly of basal ganglia atrophy was most strongly associated with cognitive function (Colato et al., 2021).

These results raise important questions about the disease pathology, including what causes shared susceptibility of cortical tissue to atrophy. The finding of cognitively relevant co-varying patterns of GM atrophy is consistent with the brain's organisation into cortical networks, which may atrophy as a result of Wallerian degeneration following damage to shared white matter tracts (Bodini et al., 2009; Mühlau et al., 2013).

1.3.2 Normal appearing white matter

More advanced MR techniques are able to provide information about brain tissue which appears unaffected by lesions and atrophy on conventional MRI. White matter (WM) microstructure outside of lesions is known to be affected by both demyelination and axonal loss in MS. The extent to which these occur as a secondary effect of, or independently from, tissue damage in lesions, is not known, although evidence shows that axonal loss can occur both as a consequence of lesional inflammatory activity and extra-lesional demyelination (Trapp et al., 1998; Bitsch et al., 2000; Allen et al., 2001; Trapp and Stys, 2009). Diffusion MRI (dMRI) enables the study of microstructural white matter and provides information about the anatomical connectivity of the brain. dMRI relies on the random motion of water molecules throughout the brain. The motion is restricted by microscopic structures, such as axonal membranes, where diffusion tends to follow the direction of the axon. In contrast, in regions of the brain with few structures, such as the ventricles, random diffusion is greater. Diffusion weighted imaging provides information about the directionality and magnitude of the diffusion of water, and this information can be used to infer the structural integrity of tissue at a microscopic level (Jenkinson and Chappell, 2017). The most commonly used diffusion metric is fractional anisotropy (FA), a measure of directionality of diffusion.

Many studies have found associations between abnormal dMRI metrics in normal appearing white matter (NAWM) and cognitive test scores, across disease subtypes and disease durations, indicating subtle widespread damage (Rovaris et al., 2002; Benedict et al., 2007; Dineen et al., 2009; Hulst et al., 2013; Sbardella et al., 2013;

Llufriu et al., 2014, Meijer et al., 2016b; Preziosa et al., 2016; Zhao et al., 2020). Most studies have used whole-brain voxelwise analyses of the white matter, such as tract-based spatial statistics (TBSS) (Smith et al., 2006) and found abnormalities in most major WM tracts associated with cognitive impairment, including the arcuate fasciculus, cingulum, corona radiata, corpus callosum, corticospinal tract, forceps major, fornix, inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, superior longitudinal fasciculus, thalamic radiations. Interestingly, in some studies such tract localisations of cognitive impairment seem to overlap only partially with lesion volume and GM damage (Dineen et al., 2009; Hulst et al., 2013), demonstrating an important unique role for microstructural WM damage in cognitive impairment. Recently, it has also been demonstrated that WM damage occurs in consistent patterns based on covarying FA, suggesting that some WM tracts are similarly affected by neurodegeneration (Meijer et al., 2016a), and demonstrating the role of network changes in the MS brain.

However, despite reports of diffusion metric abnormalities in specific WM tracts from these studies, the TBSS method provides only limited information about WM tracts. This method was designed to overcome the difficulty of achieving accurate registration of the WM, by reconstructing a skeleton of the WM based on high FA values in the tract centre (Smith et al., 2006). As a result, a large amount of data is lost from each tract (see Chapter 2.3.2 for a discussion). An alternative approach is tractography, which tracks axon bundles through the white matter to reconstruct tracts. However, due to the presence of WM lesions it has been viewed as a challenging method in MS and only a few studies have applied it to the study of cognitive symptoms (Lin et al., 2005; Ozturk et al., 2010; Mesaros et al., 2012; Bozzali et al., 2013; Valdés Cabrera et al., 2020). While correlations are reported between diffusion metrics and cognitive test scores in these studies, more research is needed to understand the relationship between the brain's anatomical connectivity and cognitive symptoms in MS.

Due to the methodological difficulties of investigating white matter tracts in MS using dMRI, an increasing number of studies are using graph theory metrics to assess structural connectivity. In graph theory, GM regions are considered nodes and the connection between regions edges in a connectome representing the brain. Edges can be either WM tracts between GM regions, or some other, indirect, connection between regions. A range of metrics, such as small world networks, can be obtained from connectomes and give information about the integrity and efficiency of information transfer within a network (Sporns and Honey, 2006). This approach generated informative results about the network organisation changes associated with MS (Rocca et al., 2016; Lin et al., 2018; Koubiyr et al., 2019), including changes in structural

connectivity and cognitive impairment (Llufriu et al., 2017, 2019; Solana et al., 2018). While graph theory is a useful approach for understanding network efficiency in MS, it must be remembered that graph theory measures are abstract mathematical constructs of network organisation and provide limited anatomical information, particularly in the WM, as connectivity in a connectome (i.e. edges) can exist in the absence of known WM tracts connecting those network regions. In addition, graph measures are not pathologically specific, so it is not clear what might cause change in disease.

While limited by methodological constraints, the studies on the NAWM to date have shown evidence of abnormalities in anatomical connective tissue of the brain, emphasising the need to investigate network changes in cognitive impairment in MS.

1.3.3 Functional connectivity

Most of the evidence on network changes associated with cognitive impairment in MS comes from studies using resting state functional MRI (rs-fMRI). This method uses correlations between blood oxygenation level dependent (BOLD) fluctuations on a functional MRI scan in the absence of a task to infer connectivity between regions (Bijsterbosch et al., 2017). This principle is based on observations that spatially separate regions which work together to perform a function tend to have correlated BOLD patterns over time (Biswal et al., 1995).

Functional connectivity (FC) is typically assessed between one region of interest and the regions it is functionally connected to, or between several connected regions making up resting state networks (RSNs). A range of RSNs have been investigated in relation to cognitive symptoms in MS, most commonly the default mode network and frontoparietal network (reviewed in Jandric et al., 2021a). The default mode network was first described by Raichle et al., (2001) with positron emission tomography (PET) and confirmed by Greicius et al., (2003) with rs-fMRI. It typically includes the medial prefrontal cortex, posterior cingulate cortex and/or precuneus, and angular gyrus and was first identified as regions that seemed to ‘de-activate’ in the presence of a task. It is thought to be important for a range of complex functions, including cognition, and has been found to show abnormal FC in a range of neurological disorders that affect cognition function, including Alzheimer’s disease and MS (Badhwar et al., 2017, Jandric et al., 2021b). Similarly, the frontoparietal network (FPN), sometimes split into the left and right FPN, respectively, and consisting of the lateral prefrontal cortex and posterior parietal cortex, has been implicated in a number of neurological conditions, including MS (Marek and Dosenbach, 2018, Jandric et al., 2021b). Often, the salience network

or one of its key regions, the anterior cingulate cortex (the other being the anterior insula), are also studied in relation to cognitive function in MS (Jandric et al., 2021b). These networks consist of brain regions which are heavily interconnected structurally and functionally, so called network 'hubs' of the brain. Due to their prominent role in the networks organisation of the brain they are considered susceptible to pathological mechanisms in disease (van den Heuvel and Sporns, 2013).

A large and growing body of literature has investigated the relationship between FC and cognitive function in MS (see Chapter 3 and Jandric et al., 2021a) and generally found an association between abnormal FC and worse cognition. However, results are difficult to interpret as both increased and reduced FC seems to be related to worse cognition. The field suffers from substantial heterogeneity in study methods and clinical samples, which likely influences findings. As part of this thesis the consistency of findings between FC changes and cognitive dysfunction in people with MS has been assessed (Chapter 3).

1.3.3.1 Models of functional connectivity abnormalities

A number of models have been developed over the last decade which aim to explain the mechanisms of FC abnormalities. The testing of such models will be key for understanding what pathological events FC changes reflect and in turn to understand what causes cognitive impairment in MS.

An early model is based on observations in Alzheimer's disease that heavily interconnected network 'hubs' or 'nodes' tend to be more susceptible to metabolic damage than other brain regions (Buckner et al., 2009). A *nodal stress* hypothesis was thus developed, which proposes that the high activity of network hubs results in activity-related 'wear and tear.' Specifically, it is thought that the high activity of network nodes involves higher energy demands which place metabolic stress on these regions and make them susceptible to neurodegeneration when these demands are unmet (Zhou et al., 2012). Using graph theory models of rs-fMRI data the authors who outlined this hypothesis also found support for it by showing a correlation between nodal connectivity and disease vulnerability. However, although network hubs have been shown to be affected in MS (Jandric et al., 2021b), and there are hypotheses of metabolic changes in the MS brain (Paling et al., 2013), the *nodal stress* hypothesis has not been formally investigated in MS.

The *network degeneration hypothesis* similarly proposes that network nodes are susceptible to degeneration, specifically atrophy. A meta-analysis of 33 studies in MS,

including 2935 participants and 562 brain coordinates, showed that the most atrophied GM regions of the brain have the strongest functional connections to each other, and weaker connections to non-atrophied regions. Based on this it was proposed that atrophy affects functionally connected regions and an atrophy-based functional network (AFN) model was developed (Chiang et al., 2019). This work was validated in an independent sample of 22 RRMS patients in which the AFN model was a significant predictor of functional connectivity abnormalities (Chiang et al., 2021). The hypothesis has potential to explain atrophy in MS and provide early markers of degeneration before irreversible tissue loss. As such it will be an important aim for future research to test, particularly in relation to cognitive symptoms.

Finally, a model which was developed specifically to explain the relationship between FC and cognition in MS is the *network collapse* model. It considers the effect on cognition by the relationship of structural damage in the brain and network efficiency over time. It is proposed that early in the disease structural damage is low, allowing network efficiency to remain high, possibly through compensatory mechanisms, and cognitive dysfunction low. As the structural damage accumulates the compensatory mechanisms preserving network efficiency become less effective and network changes start to occur. However, it is only when structural damage accumulates up to a critical threshold that functional networks will no longer be able to compensate for the damage and a 'network collapse' occurs, resulting in low network efficiency and increased cognitive impairment (Schoonheim et al., 2015). While the body of rs-fMRI research on cognition as a whole does not support this model (reviewed in Jandric et al., 2021a), most studies have not explicitly tested it and are therefore hard to interpret in the context of the model. Emerging evidence from individual studies does however support this model, including computational modelling work showing initial FC increases as structural damage increases over time (Patel et al., 2018; Tewarie et al., 2018). Similarly, a longitudinal study of CIS patients found increasing structural reorganisation and increases in functional graph theory metrics in the absence of cognitive impairment, suggesting compensatory mechanisms in early MS (Koubiyr et al., 2019), as predicted by the *network collapse* model.

Given that FC abnormalities are a key, but poorly understood, finding in cognitive impairment in MS, an important priority for future research must be the testing of such models to explain the mechanisms of FC changes.

1.3.4 Sodium imaging

An additional avenue for understanding disease mechanisms underlying cognitive impairment in MS is the use of methods which tap into biochemical processes required for healthy neural functioning. One such method is sodium, or 23Na, MRI, which can be used to quantify concentrations of sodium, an essential chemical for healthy axonal functioning.

It is well established that a key driver of pathology in MS is axonal damage, but the mechanisms of cellular changes leading to axonal dysfunction following demyelination are not well understood. It has been suggested that abnormalities of neuronal energy metabolism may be a driver of neuroaxonal damage and ultimately death (Waxman, 2006; Paling et al., 2011; Inglese et al., 2013). The signal conduction of healthy, myelinated axons depends on a chemical balance of ions between the intra- and extracellular space. The sodium-potassium ion channel, or Na+/K+ pump, has a key role in maintaining this balance by pumping sodium out of the cell, and potassium into the cell, both against the concentration gradient, thus ensuring a higher concentration of sodium in the extracellular space (140-150 mM vs 10-15 mM in intracellular space) and potassium in the intracellular space (Madelin et al., 2015). The Na+/K+ pump is dependent on energy from adenosine triphosphatase (ATP) to maintain the resting potential of the axon (Hille, 2001).

Histological studies have shown that following demyelination, an upregulation of sodium channels occurs along the axon (Foster et al., 1980; Craner et al., 2004). The role of this upregulation is likely to restore electric conduction, but it places increased pressure on the Na+/K+ pump, increasing the energy requirement of the cell. Additionally, dysfunction of mitochondria, which produce ATP, has been documented in demyelinated axons (Mahad et al., 2008), and it has been proposed that nitric oxide impairs the functioning of mitochondria following demyelination by inhibiting mitochondrial respiration (Kapoor et al., 2003). Thus, mitochondria are not able to meet the increased demand for ATP to power the Na+/K+ pump, leading to axonal sodium accumulation. A reduction in N-acetyl-aspartate (NAA) measured using MR spectroscopy, which is synthesised in the mitochondria and can be used as a marker of mitochondrial function, has been shown to correlate with brain atrophy and disability in MS (Paling et al., 2011). A recent study exploring the metabolic counterparts of neural sodium accumulation using MR spectroscopy found a negative correlation between sodium concentration and NAA levels, demonstrating in vivo a relationship between increases in sodium concentration and mitochondrial dysfunction (Donadieu et al.,

2019). Accumulation of intracellular sodium reverses the direction of the sodium-calcium exchanger, resulting in calcium influx into the cell and in turn calcium mediated axonal dysfunction and ultimately death (Kapoor et al., 2003).

Because of its ability to measure sodium concentrations in vivo, sodium MRI can be used to show ongoing metabolic dysfunction and thus may provide a surrogate marker of axonal injury before more widespread tissue damage has occurred (i.e. MR visible lesions). Sodium MRI is acquired using a head coil sensitive to the signal from sodium nuclei, the second most abundant source of an MR signal in biological tissue. While only a small number of studies have used sodium MRI in MS, all have found evidence of sodium accumulation related to MS (Petracca et al., 2016). Increases in sodium concentration have been reported in acute and chronic lesions, as well as in NAWM and NAGM, of brains of people recently diagnosed with RRMS (Inglese et al., 2010). Moreover, sodium accumulation in the GM has been shown to differentiate people with advanced RRMS from those with early RRMS in the absence of differences in GM atrophy (Zaaraoui et al., 2012).

Several correlational studies have found evidence of a link between sodium accumulation and cognitive dysfunction in MS (Paling et al., 2013; Maarouf et al., 2017; Brownlee et al., 2019). Such associations have been reported in both RRMS, SPMS and PPMS and in all tissue types, including lesions, NAWM, deep GM and cortical GM (Paling et al., 2013). Moreover, higher sodium concentrations in the GM correlate with cognitive test performance in patients progressing from CIS to RRMS or SPMS over the course of 15 years, independent of GM atrophy (Brownlee et al., 2019). The same study found no increase relative to healthy controls in patients who remained CIS after 15 years. Both Paling and Brownlee studies found a higher degree of sodium accumulation in SPMS than RRMS, demonstrating the relationship with disease progression. These studies suggest an important role for sodium accumulation in the MS pathology, however, the evidence from early MS is more mixed. A recent study assessed patients newly diagnosed with CIS or MS, within three months of a first demyelinating event, and found sodium accumulation in NAWM and GM compared to healthy controls, but no associations with cognitive test scores. However, the cognitive test performance in this sample did not differ substantially from normative values (Collorone et al., 2021). In a separate study, when RRMS with a short disease duration (mean 3.1 years) patients were split into subgroups based on the cognitive test performance, increases in sodium concentration were found in both NAWM and GM in cognitively impaired relative to preserved patients (Maarouf et al., 2017). In regression analyses sodium concentration predicted cognitive test scores better than atrophy measures.

These results point to an early pathological role of sodium accumulation in cognitive impairment and should be investigated further, particularly in multimodal studies. As sodium accumulation has been shown to occur in normal appearing tissue and is a better predictor of cognitive functioning than atrophy (Maarouf et al., 2017; Brownlee et al., 2019), it should be considered whether sodium accumulation may be a key mechanism of network breakdown in the MS brain. An important priority for future sodium MRI studies is therefore to combine sodium MRI with rs-fMRI to investigate sodium accumulation in network hubs of the brain networks supporting cognitive function in MS.

1.4 Overview of the thesis

Management of cognitive impairment in MS is a large unmet need in the clinical setting. Currently, there is a limited understanding of why some people develop cognitive impairment and others don't and the brain mechanisms responsible. Shedding light on the brain correlates of cognitive dysfunction is an essential step towards improving monitoring and management of this debilitating symptom. Current evidence suggests a key role for central 'hub' regions in the brain networks that support cognition, but there are several outstanding questions. The overarching aim of this thesis was therefore to build on this body of evidence by assessing network changes in people with multiple sclerosis and exploring links between these and cognitive symptoms.

1.4.1 Review of evidence on the links between functional connectivity and cognitive impairment in MS

Studies using rs-fMRI to assess the links between functional network connectivity abnormalities and cognitive impairment have produced promising results (Chard et al., 2021), but interpretations of findings are challenging. Both increases and decreases in functional connectivity have been associated with worse cognition, and while models exist which aim to provide interpretations, such as the *network collapse* model (Schoonheim et al., 2015), they are often not formally tested.

Therefore, we conducted the first systematic review of the literature on functional connectivity abnormalities and cognitive impairment. This review summarised research findings to identify the factors which might influence functional connectivity abnormalities. Moreover, we assessed how well results fit the predictions of the *network*

collapse model. This was done with the aim to understand the state of the field to date and identify outstanding research questions.

1.4.2 Mechanisms of network changes associated with cognitive impairment in MS

Our systematic review showed a strong link between functional connectivity abnormalities and cognitive dysfunction in MS, but also revealed a great amount of heterogeneity in the study literature which limits the conclusions that can be drawn about this relationship. One key point identified is the need to understand the mechanisms of network changes in MS, in order to elucidate their pathophysiology.

We conducted a multimodal MRI study to test another model of functional connectivity abnormalities in neurodegenerative disease. The *nodal stress* hypothesis suggests that the high metabolic demands on network 'hub' regions makes them more susceptible to degeneration (Zhou et al., 2012). If such 'wear-and-tear' changes influence functional connectivity abnormalities, we expected them to also show as abnormalities on other MRI metrics. Therefore, we investigated whether anatomical connectivity and cerebral blood flow are abnormal in and around functional network hub regions which show functional connectivity changes in cognitively impaired, relative to non-impaired, MS patients.

1.4.3 Sodium accumulation in functional network hub regions

Sodium MRI has been used to point to evidence of unmet metabolic demands of demyelinated axons. It is thought that upregulation of sodium-potassium channels along an axon occurs after demyelination, in order to restore signal conduction. However, these additional channels put a greater energy demand on the cell, which cannot be met, leading them to dysfunction and for sodium to accumulate in the intracellular space (Paling et al., 2011).

This method can be informative about metabolic changes in functional networks, and so we conducted the first study that combines rs-fMRI and sodium MRI to test whether functional network hubs show sodium accumulation suggestive of energy failure. Further, we tested whether sodium concentrations in network hubs correlate with cognitive test performance.

1.4.4 White matter tract structure in MS

The relationship between functional and structural connectivity in the brain is not fully understood. It has been suggested, in the *network collapse* model, that functional connectivity is a result of increased structural damage over time (Schoonheim et al., 2015), and it is possible that this occurs through degradation of anatomical connections between network regions (Catani and ffytche, 2005; Dineen et al., 2009). If white matter tracts are connected to each other to support functional networks, it can reasonably be expected that pathological damage to one tract will affect connected tracts too. Thus, it is important to understand the white matter network in the brain to assess whether pathological change occurs in patterns which make some tracts more susceptible than others.

The study of normal appearing white matter has been constrained by methodological limitations in MS, but recent advances have led to increased ease and accuracy. We used one such new methodological development to conduct individual, automated tractography in MS. We extracted microstructure metrics from non-lesioned parts of tracts and used a tractometry approach to assess covariance among all major white matter tracts in the brain. The aim was to investigate whether tracts show a network structure which could inform us of pathological patterns in the normal appearing white matter in MS. Given the limited prior evidence on this topic, this was an exploratory study conducted during the COVID-19 pandemic, while data collection for other parts of the thesis was on hold.

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Chapter 2

Methods

The purpose of this chapter is to provide an overview of some of the magnetic resonance (MR) methods which are currently used in the diagnosis, treatment and research of multiple sclerosis (MS), and in particular those which have been used in this thesis to research cognitive impairment in MS. MR methods are the focus of the thesis because MR imaging (MRI) is used routinely in the diagnosis and disease monitoring of MS (Thompson et al., 2018). In addition, markers of cognitive decline which are detectable on MRI could have clinical utility and be incorporated into existing clinical protocols. This chapter also aims to outline considerations and limitations of the different MR methods which have influenced the methodology in this thesis and which need to be considered when interpreting the findings.

2.1 A brief introduction to MRI

MRI is a powerful tool in the diagnosis and treatment of MS (Bakshi et al., 2008; Thompson et al., 2018). To understand the measures derived from MRI it is important to briefly outline how MRI works.

The human body, including the brain, is mostly made up of water. Each water molecule is made up of one oxygen and two hydrogen atoms. Because hydrogen atoms are so abundant in the human body the magnetic field of their nuclei is used for MRI. These positively charged nuclei, called protons, have a positive electrical charge and an intrinsic property of ‘spin’ which gives the nucleus angular momentum and allows it to develop magnetism. Through these spins the protons are acting like tiny bar magnets with their own magnetic fields and can interact with and be manipulated by other magnetic fields. This forms the basis for MRI.

In the absence of an external magnetic field the protons are oriented in different directions (see Figure 1), thus there is no overall orientation of their magnetic fields and no signal. In an MR scanner a strong magnetic field is always present. This field is created by a large electronically conducting wire, called a superconducting coil, which creates magnetic fields through the passing of electrical currents through the coil. The strong magnetic field created by this coil is called the B0 field and determines the strength of the scanner, in units of tesla (T).

In the presence of this strong magnetic field the protons rotate around the axis of the B0 field, in a conical shape, a process called precession. The frequency of precession is proportional to the strength of the external magnetic field and referred to as the resonant frequency. By transmitting electromagnetic fields at the resonant frequency, called radiofrequency (RF) pulses, the protons can be excited to change their alignment away from the B0 field. The protons absorb the energy from RF pulses, causing them to go from a low energy state parallel to the B0 field to a high energy state not parallel to the B0 field. The RF pulse leads to a subset of the protons precessing in phase and producing a net magnetisation that is detected by the head coil. This forms the MRI signal that is used to form an image. When the RF pulse is stopped the protons will start releasing the energy they absorbed and change their spin direction back again, see Figure 1. The rate of this return is called relaxation. The rate of relaxation is different in different types of tissue, and exploiting this difference forms the basis of visualisation of different tissue classes in an MR image (Jenkinson and Chappell, 2017).

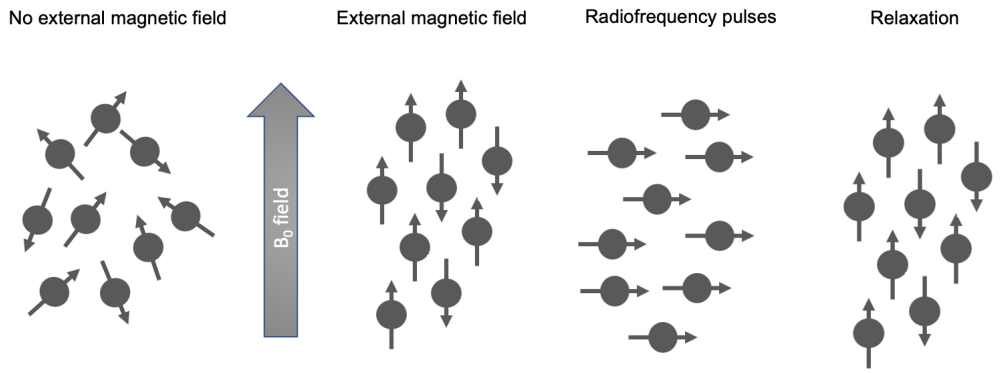

Figure 2.1: Illustration of proton spin
In the absence of an external magnetic field protons are oriented in different directions. When an external magnetic field is applied their orientation changes to parallel to the B0 field. Protons absorb the energy from radiofrequency pulses transmitted during an MRI scan and change their direction away from the B0 field. When the pulse is stopped and energy is released from the protons they will change their orientation back to the superposition of two states parallel to the external field.

There are several MR modalities that are all based on this simple principle, but with important differences in how MR parameters such as excitation and relaxation are manipulated. These different types of MR modalities capture different information about the brain that can be complementary in achieving an understanding of pathology. The aim of this PhD is to investigate the pathological changes underlying cognitive symptoms, and we have made use of several different MR modalities to try to understand the brain abnormalities associated with this aspect of the disease. Each modality used is

described below with a brief discussion of methodological considerations of importance to this PhD.

It is also important to note that while most MR modalities described in this thesis use hydrogen nuclei, the nuclei of other atoms, such as sodium, can be used to provide complementary information to that derived from hydrogen. Sodium MRI has been used in chapter 5 and is discussed below.

2.2 Use of MRI in MS diagnosis and treatment

The pathological hallmark of MS that can be detected by MRI is the demyelinating lesion in the white matter (Lassmann, 2018). Detection of lesions on MRI is a sensitive marker of MS and has become part of the diagnostic criteria, which requires dissemination of clinical episodes or radiological measures in space and/or time (Thompson et al., 2018). In other words, lesions should be present in more than one anatomical location and show development across two or more time points. In addition, lesion measures are used clinically to track disease progression and evaluate the efficacy of disease modifying treatments (DMTs), and in clinical trials of DMTs as an objective endpoint for disease progression.

While lesions can occur anywhere in the brain or spinal cord, they are commonly seen in the periventricular and juxtacortical white matter. New lesions can appear to resolve over time, either through remyelination or resolution of oedema (Trip and Miller, 2005) and different varieties of MR acquisition are used to determine the stage lesions are at.

In the clinical setting T2-weighted images, on which lesions appear as bright hyperintensities, are usually acquired (Figure 2B). However, because the cerebrospinal fluid (CSF) also appears bright on T2-weighed images, periventricular lesions can be difficult to identify. Proton density (PD) images, on which CSF signal is low, are therefore often acquired together with T2-weighted images (Figure 2C). Alternatively, a fluid attenuated inversion recovery (FLAIR) sequence can be used to supress the CSF signal on a T2-weighted image. One limitation of measuring lesions on T2-weighted images is the lack of specificity of the underlying pathological processes. Such lesions can reflect demyelination, inflammation, axonal damage, gliosis, remyelination or oedema (Pirko, 2010). Additional information can therefore be gained by acquiring T1-weighted images on which the fat in myelin makes white matter appear bright. Permanent damage to myelin or axons (i.e. axonal loss or severe demyelination) thus appears as dark hypointensities, commonly called ‘black holes’ (Figure 2A). T1-weighted images can

also be acquired after intravenous administration of gadolinium and used to identify new active inflammatory lesions. Focal inflammatory activity increases blood brain barrier permeability and gadolinium-enhanced lesions thus indicate areas of active inflammation (Pirko, 2010; Thompson et al., 2018).

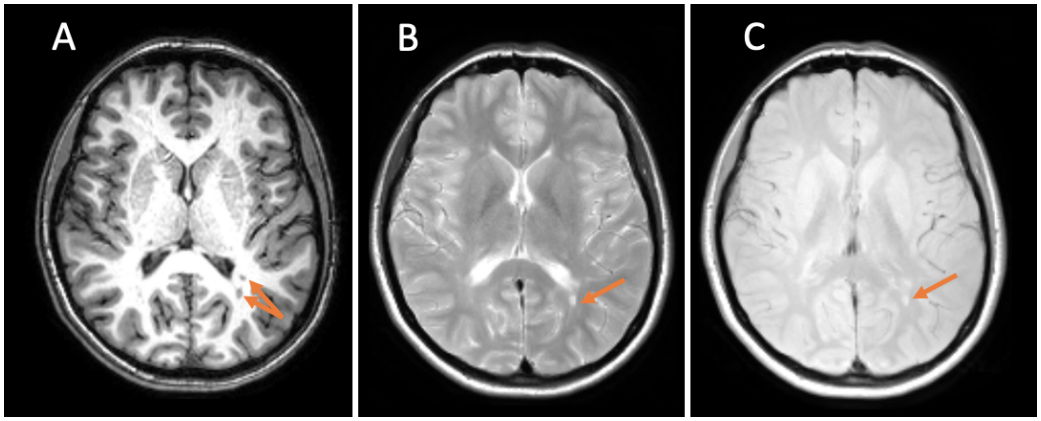

Figure 2.2: Axial MRI scans with T1-, T2- and PD-weighting
MRI scans of a 25-year-old female with diagnosed RRMS showing periventricular lesions on T1-weighed (A), T2-weighted (B) and proton density weighted (C) scans. Orange arrows indicate lesion location. This data is from an individual example MS patient from Chapter 5.

In clinical trials of DMTs, atrophy measures, usually normalised brain volume (NBV), are also collected and reported as a complementary radiological marker. However, atrophy is pathologically non-specific and brain volume measures are influenced by confounds such as patient's age, hydration status, and lifestyle factors like smoking (De Stefano et al., 2014).

While both lesion and atrophy measures are useful for establishing a diagnosis of MS and tracking disease progression, they correlate poorly to clinical symptoms, a common research finding termed the 'clinico-radiological paradox' (Barkhof, 2002). This is likely to be partly due to the low specificity of these measures and other ongoing pathological processes which are not captured by conventional MRI. MR measures which are more sensitive to clinical symptoms could improve MS care and provide clinical trial efficacy end points with higher specificity. Thus there has been a move to more advanced MR measures in the research of cognitive impairment in MS. Those techniques and measures which have been used in this thesis are described in the sections that follow.

2.3 Advanced MRI techniques

2.3.1 Resting state functional MRI

Much of this thesis is concerned with network changes in the MS brain and the relationship with cognitive function. An increasingly common approach to the investigation of brain networks in both healthy brains and neurological disorders is resting state functional MRI (rs-fMRI), described below.

2.3.1.1 Blood oxygenation level dependent signal

Unlike the MR measurements used in the clinical setting, which rely on anatomical changes, functional MRI, including rs-fMRI, is influenced by neuronal and vascular activity. These can be used to try to establish which regions of the brain are involved in tasks, such as cognitive processes. fMRI is based on the concept of increased oxygen and glucose delivery through the blood to regions with high neuronal activity. Increased blood delivery leads to a higher local concentration of oxygenated haemoglobin compared to deoxygenated haemoglobin. Deoxyhaemoglobin is paramagnetic and distorts the local magnetic field. This leads to a loss of phase coherence of the magnetisation of the surrounding water protons and so a reduced MRI signal. With an increase in the proportion of oxyhaemoglobin (which is not paramagnetic and so does not distort the local field) the signal increases. This MR detectable signal is called the blood oxygenation level dependent (BOLD) signal (Jenkinson and Chappell, 2017) and is the basis of functional MRI (fMRI).

2.3.1.2 Resting state fMRI and functional connectivity

A well-established form of functional MRI is task-based fMRI, which involves a research participant performing a task while scanned. The task is designed to test a specific brain function, and the aim of the scan is to identify the brain region or regions which ‘are activated,’ i.e. show an increase in BOLD signal, when the task is performed. Such localisation of function has many advantages when studying impairment or loss of function in disease. However, it is well-established that while different regions of the brain have their subspecialisations, they are connected to allow more complex function (Damoiseaux et al., 2006). In addition, many neurological diseases rarely impact isolated regions, but affect widespread areas. Even quite focal lesions have been shown to disrupt the networks that the affected region is part of (Boes et al.,

2015). Therefore, studying networks of functionally connected brain regions can be very informative about diffuse pathological changes. Rs-fMRI can be used to measure the connectivity of several networks in one scan, in the absence of a task, i.e. at rest.

It is possible to study brain networks with task-based fMRI, through the use of several paradigms. However, rs-fMRI has a number of pragmatic advantages that make it more attractive for clinical translation. Task-based fMRI is cost-, time- and labour-intensive. Scanner-compatible equipment is needed to deliver the paradigms to the research participant or patient, time and expertise setting up the paradigms and analysing the data are required from the researchers or radiographers, and the person being scanned needs to be able to perform the task they've been set. In contrast, no additional equipment or set up and little additional expertise are needed to run a rs-fMRI scan. Importantly, the person being scanned is not required to do anything, which is a great advantage when working with a population that might have impairments in cognitive or physical functions. In addition, unlike many MR techniques which require group-comparisons, functionally connected networks can be detected in individuals (Gordon et al., 2017), marking it further attractive as a potential biomarker.

Resting state functional connectivity is based on the observation of correlations between low frequency BOLD oscillations in spatially separate, but functionally related, regions by Biswal et al (1995). Such correlations between fluctuations of the BOLD signal over time exist across the brain and are thought to reflect fluctuations in neuronal activity of regions which work together to perform a function. It is believed that neuronal populations working together to enable a function, i.e. 'firing' together, have 'wired' together though synaptic plasticity (Lewis et al., 2009). Regions which show high correlations in BOLD time course are thus considered functionally connected (see Chapter 3, Figure 1). A set of functionally connected brain regions detected by rs-fMRI is called a resting state network (RSN). RSNs are localised in the grey matter (Damoiseaux et al., 2006) and thought to reflect the intrinsic functional organisation of the brain (Bijsterbosch et al., 2017). Studying how their connectivity is altered in clinical populations has the potential to provide biomarkers of disease.

A common criticism of rs-fMRI is that even at rest the brain is not doing nothing and the person being scanned can be engaged in a variety of mental processes, from day dreaming to complex problem solving. Despite this lack of experimenter control over cognitive processes during the scan, RSNs have been shown to be reliably and reproducibly identified across studies, different groups of participants, and even individual participants (Damoiseaux et al., 2006; Bijsterbosch et al., 2017; Teeuw et al.,

2021) and the networks identified in rs-fMRI scans correspond well to those induced by task-fMRI paradigms (Smith et al., 2009).

2.3.1.3 Acquisition

Rs-fMRI data is noisy. Because there is no model of expected activation and because low frequencies of the BOLD signal are measured, it is difficult to identify and remove noise in the data. Motion in particular can cause changes in the patterns and location of BOLD timeseries (Power et al., 2014), which can appear as group differences in the strength or location of functional connectivity between two groups if these differ systematically in the amount of head motion present during the scan (Van Dijk et al., 2012). There are several data-cleaning approaches available, and most toolboxes for rs-fMRI image analysis have built-in options. Nevertheless, minimising head motion during acquisition of an rs-fMRI scan and obtaining enough data are key to ensure stability of the BOLD signal. While stable functional connectivity measures can be derived from only a few minutes of scanning (Van Dijk et al., 2010), evidence shows that optimal balance between reliability of the BOLD signal and minimisation of noise is achieved in a scan length of around 10 minutes (Birn et al., 2013).

Other sources of noise include physiological signals of heart rate and respiration. These physiological processes induce small amounts of head motion due to breathing and cardiac pulsation, and moreover movements of brain tissue itself due to intracranial fluid pulsation, as well as influencing the BOLD signal itself. Physiological noise is widespread throughout the brain and has been shown to account for up to 15% of variance in the measured BOLD fluctuations in an rs-fMRI scan (Murphy et al., 2013). Many scanners will be equipped with tools for measuring cardiac and respiratory fluctuations, which allows for the quantification and regression of these confounds from the raw data.

In Chapter 4, data was collected in a single 5 minute scan run. Physiological noise was regressed out and to ensure that motion did not influence group differences, head motion parameters were collected and compared between the two groups of interest. In Chapter 5 data we were able to increase the scan length to 10 minutes. However we were unable to collect physiological noise measurements. We therefore consider the potential influence of these confounds, and how they may be balanced against the increased signal from longer scans, when interpreting the results of this chapter. However, both chapters used the independent component analysis (ICA) approach, described below, which aims to separate noise from signal.

2.3.1.4 Analysis

There are a range of approaches for analysing rs-fMRI data, depending on the research question being asked. In this thesis rs-fMRI has been used to study functional connectivity between brain regions, and the two most common methods for this purpose are ICA and Seed-based Connectivity Analysis (SCA).

Both ICA and SCA are based on the concept of identifying regions with correlated BOLD time courses and are able to provide information about the network structure of the brain. However, they are suited to different research questions. ICA is data-driven and aims to identify the structure in the data, and is therefore best suited for identifying the RSNs present in the dataset. In contrast, SCA requires definition of one or several regions of interest and identifies the functional connections between those regions, or between a region and the rest of the brain. Thus it is best suited for studies with hypotheses about specific spatial region(s), such as those attempting to find where in the brain a region connects to. Used in this way SCA produces results which are straightforward to interpret. SCA can also be used to identify RSNs, however, the spatial extent of the network will vary depending on which region of interest is used as the seed, how it is defined, and how a threshold for a connection is set (Bijsterbosch et al., 2017) and thus it is susceptible to bias. For instance, if only one seed is selected, the resulting connectivity map will show every region it connects to. This can be problematic when looking for RSNs because some regions, particularly the network 'hubs' (i.e. those regions that are highly interconnected and so of pivotal importance for the flow of information in the brain), are heavily interconnected, so the results will likely show more than one network. Similarly, it is challenging to select two or more regions of a network to overcome this first challenge, because there is no established consensus of which regions make up any given network.

In contrast, ICA is entirely data-driven. It takes the full rs-fMRI dataset as the input and performs multivariate decomposition to separate the BOLD-signal into independent components (ICs). Each component represents the voxels of the data with synchronised BOLD signal. As such, each component is considered to be a separate network. ICA can be applied to rs-fMRI data without any a priori hypotheses about specific regions of interest. It is commonly used to obtain the network structure of the brain and then further analysis techniques can be applied to ask questions about those networks. However, it can be used even in instances where a study makes hypotheses about specific networks, as ICA has been shown to consistently extract common RSNs, including those investigated in this thesis (Beckmann et al., 2005; Damoiseaux et al.,

2006). One benefit of identifying RSNs in this way is that the spatial extent of the RSN is determined by the presence of correlations between voxels in the data, not on the definition of a seed region. ICA shows high reliability across subjects (Damoiseaux et al., 2006), and further high test-retest reliability in individuals, from as short as 45 min to as long as 16 months (Seeley et al., 2007). Another advantage of ICA is that it is able to decompose non-neuronal signal into ICs, and so it can separate signal from noise. It is commonly used in this way as a pre-processing step for single participants. Noise components are identified and regressed out, leaving only components thought to have a neural basis to run the analysis on. However, even when applied to group-level data ICA can achieve some level of separation between signal and noise by decomposing structured noise, such as physiological, into their own ICs (Cole et al., 2010).

The data-driven nature of ICA does however present some challenges. First, a decision needs to be made about the number of ICs to decompose the data into. This presents a risk of underfitting the data and obtaining components which reflect several networks bundled together, or overfitting it and fragmenting single networks into several components. Most software today will have an option to estimate this based on the variance in the data, reducing the risk of fitting the data incorrectly. Even so, there is no single best fit for an ICA solution, because the brain network structure is complex and there are several levels of organisation. Therefore, even when a software estimates the number of ICs to extract some networks may be split or combined across ICs and this must be considered when interpreting the components (Bijsterbosch et al., 2017). Related to this, a further challenge is interpreting what each component is. Many RSNs, such as the default mode network, are easily recognisable and can be identified manually or through matching to templates of the network. Less common networks or networks split across several components are harder to identify. Further, network components need to be separated from noise components. To date there is no consensus on how to distinguish RSNs from noise and label them, and while atlases exist (e.g. Yeo et al., 2011; Joliot et al., 2015), their use is not widespread and limited by the lack of consensus on how RSNs should be defined. Data-driven labelling approaches exist, and generally require training a machine learning algorithm to perform this function, yet to date the gold standard is manual labelling, which is labour intensive and can be susceptible to error (Griffanti et al., 2017). In this thesis manual labelling has been performed by first carefully inspecting components to ensure they are unlikely to be noise, and then comparing them to published descriptions of networks of interest. Relatively common RSNs are of interest to this thesis, including

the default mode, frontoparietal and salience networks, so there are ample published descriptions of these networks to aid identification.

2.3.1.5 Interpretation

A range of metrics can be obtained from rs-fMRI analyses, of which functional connectivity (FC) is one of the most common and that which is used in this thesis. Changes and abnormalities in rs-fMRI FC have been investigated in MS and, as outlined in Chapter 3, found to be associated with impaired cognitive function across most of the literature.

However, interpretation of FC abnormalities in a sample of people with MS relative to healthy controls, or in cognitively impaired patients compared to non-impaired, is complex. As well as the consideration of methodological choices outlined in the previous section, it must be remembered that the BOLD signal on which the FC metric is based is a surrogate marker of neuronal activity. FC, in turn, reflects the correlation of spontaneous BOLD fluctuations between different regions of the brain. Thus, FC is a statistical rather than a biological metric. Yet, a strong argument can be made that FC reflects neural activity and that changes in FC can tell us something about the health of neuronal populations. Because RSNs are localised in the grey matter, reproducible across subjects and methods, and can be 'activated' by task fMRI, it is generally accepted that their signal is neuronal and that they reflect the inherent functional organisation of the brain (Bijsterbosch et al., 2017).

A key question is how to interpret disease-related change in FC. Because FC reflects synchronous neural activity between functionally connected regions, an increase or decrease in the FC of a region reflects increased or reduced connectivity between that region and the rest of the network, respectively. However, the pathology and biological processes underlying altered connectivity remain to be elucidated. It is logical to assume that decreases in FC associated with worse cognition reflect damage throughout the brain, for example atrophy of network regions, or damage in the white matter connecting those regions. Yet an equally common finding is increased FC in patients with cognitive impairment. In a systematic review of the FC literature on cognition in MS (Chapter 3) 18 of the 57 studies reviewed found associations between increased FC and impaired cognitive function, and an additional 9 found patterns of both increased and decreased FC. A common interpretation in the literature is that of 'functional reorganisation,' which suggests that functional networks are capable of compensatory activity to preserve functioning in the face of structural damage

(Schoonheim et al., 2010). This interpretation is speculative and moreover flawed as it fails to account for results where FC decreases are related to poor cognitive function.

A more recent attempt to explain FC changes is the 'network collapse' model, which predicts three stages of network changes. In the first stage, early in MS, structural damage is low and so network functioning remains high. However, as structural damage accumulates in the second stage, network functioning will start to reduce. In the third and final stage the structural damage is too great for any compensatory activity and the network fails (Schoonheim et al., 2015). Multimodal studies which have investigated the link between white matter damage and functional network connectivity have found support for this model (Patel et al., 2018; Tewarie et al., 2018). This model is therefore a plausible explanation of the relationship between structural damage in the brain and FC, but needs further testing in multimodal studies. Other theories of what altered FC reflects have also been proposed and are outlined in Chapter 1. Common to all these theories is that further testing is needed to support or refute them. In this thesis theories around the energy state of neurons in network hub regions are tested in Chapters 4 and 5, to understand the role of metabolic function as a potential mechanism of FC abnormalities.

The interpretation of FC results, including increases and decreases associated with a disease state, is considered in the discussion section of each chapter that used this method, and a more in depth discussion of interpretative challenges is outlined in the general discussion of this thesis.

2.3.2 Diffusion MRI

Another way to probe brain networks is to investigate the anatomical connectivity of the brain, which can be achieved with diffusion MRI. Diffusion MRI (dMRI) is based on the principle of Brownian, or random, motion of water molecules, and the idea that this movement is altered in biological tissue. For instance, in the white matter of the brain, diffusion is restricted by axonal membranes and average diffusivity is low, or anisotropic. Anisotropy is caused by the orientational preference of restrictions, such as neuronal axons. In contrast, in the CSF difussion is completely unrestricted and average diffusivity is therefore high, or isotropic. This sensitivity to tissue microstructure makes dMRI suitable for investigating a range of neurological disorders, including stroke and tumours, which cause changes in tissue composition. Due to its sensitivity to microscopic changes in the white matter, it is also used as a research tool in multiple sclerosis to uncover

microstructural changes in both lesioned tissue and normal-appearing white matter (NAWM).

2.3.2.1 Diffusion Tensor Imaging

By fitting mathematical models to dMRI data a range of measurements can be obtained. A common such model is the diffusion tensor, the basis of diffusion tensor imaging (DTI). Tensors are 3D ellipsoids fitted at each voxel that provide information about the degree and direction of diffusivity, see Figure 3. For DTI, dMRI data needs to be acquired from several different diffusion encoding directions. A minimum of six encoding directions is advised (Soares et al., 2013), but with more directions the signal-to-noise ratio (SNR) in the data increases (Jenkinson and Chappell, 2017). As with all neuroimaging, there is a trade-off between SNR and scan length.

The tensor shape is most elongated along the direction of the fibre where water moves easiest, and most narrow where diffusion is most hindered. The axes of the tensor showing length, width and depth of the tensor are called eigenvectors, and their length is expressed by eigenvalues. The longest eigenvector, V1, and its corresponding eigenvalue, λ_1, represent the degree of anisotropy in the tissue, i.e. the diffusivity of water along this axis compared to the other two (Mori and Zhang, 2006; Jenkinson and Chappell, 2017). From the measurement of the three eigenvalues, several metrics of tissue microstructure can be obtained, including, but not limited to the following:

- *Fractional anisotropy (FA)* a measure of degree of anisotropy across the tensor. It is expressed in values from 0 to 1, where lower values reflect less anisotropy and higher values more anisotropy. In white matter FA values tend to be high. In contrast, in CSF, where diffusion is isotropic, FA values tend to be close to 0.
- *Mean diffusivity (MD)* is the average of all three eigenvalues of the tensor and reflects average diffusion in all directions. MD tends to be high when FA is low.
- *Axial diffusivity (AD)* reflects the amount of diffusion along the main eigenvector and is thought to be parallel to axons (Beaulieu, 2014).
- *Radial diffusivity (RD)* is the amount of diffusion perpendicular to the principal axis of the tensor.

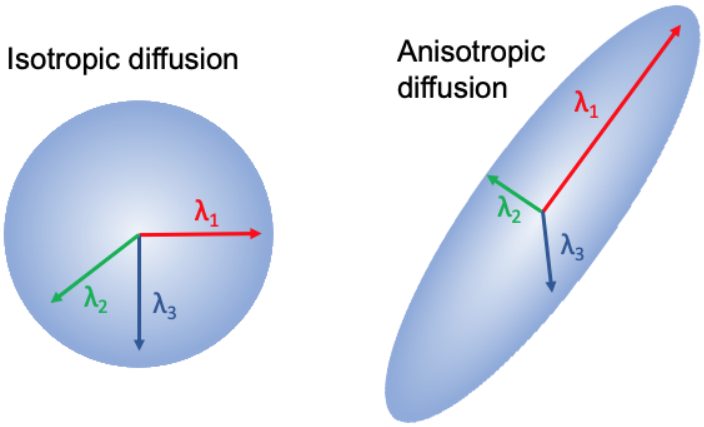

Figure 2.3: Illustration of the diffusion tensor and its three eigenvectors in isotropic and anisotropic environments
In isotropic environments, like the cerebrospinal fluid, water can diffuse freely in all directions and all eigenvalues are the same. In anisotropic environments diffusion is constrained in some directions and moves more freely in one direction, the principal eigenvalue, λ_1, than the other two directions.

2.3.2.2 Analysis

A number of analysis approaches exist for dMRI data. The simplest approach is perhaps to extract an average value of a diffusion metric from parametric maps for statistical analysis. This approach is convenient but may fail when there is heterogeneity in the data, such as was found in chapter 4. Another common approach is the use of voxelwise analysis of parametric maps, of e.g. FA, to identify spatial regions where two groups have different values of the metric of interest. However, this approach faces the difficulty of achieving accurate registration of the white matter to a standard space template, due to lack of sufficient features in the white matter to drive registration, and is therefore susceptible to partial volume effects (Soares et al., 2013).

A solution to this problem was developed that achieves registration by using the centre of white matter tracts based on FA to produce an FA 'skeleton' (Smith et al., 2006). This tract-based spatial statistics (TBSS) method is fully automated and easy to run. However, it suffers from a number of limitations including that it is based purely on FA, ignoring other diffusion metrics in the construction of the WM skeleton. Moreover, TBSS does not reconstruct individual white tracts, but rather the white matter skeleton of the whole brain. Further, as it uses an average of the centre of all participant's tracts, individual variability can be lost. Perhaps even more importantly, the anatomical accuracy of TBSS has been shown to be limited in healthy volunteers (Bach et al., 2014), and an important question to consider is therefore what effect MS lesions may have

on the skeleton. Finally, because it considers only the centre of tracts, it does not capture white matter neighbouring grey matter, which can be important when trying to understand local tissue characteristics of network hubs in the grey matter. For this reason, chapter 4 used both TBSS and a voxel-based analysis of FA maps to probe the tissue characteristics of RSN regions of interest to improve our confidence in the results across the two methods.

For the investigation of connectivity throughout the brain, not just in the centre of white matter tracts, and also for the study of how non-lesional damage in individual white matter tracts relates to clinical symptoms, tractography can be a more attractive option than TBSS, see Figure 4. Tractography relies on the directional information of diffusion tensors, or alternative models, across the voxels of the brain, which are pieced together to reconstruct white matter pathways through the brain (Mori et al., 1999; Basser et al., 2000; Catani et al., 2002; Jeurissen et al., 2019). There are two broad types of tractography, deterministic and probabilistic. Deterministic tractography is based on the assumption that fibres within a tract can be identified by neighbouring voxels with the same orientation of diffusion ellipsoids and uses seed and termination points to identify the streamline through the voxels of these points. However, this approach is susceptible to errors due to the presence of noise in the data and fibre orientation model inaccuracies, and struggles when there are crossing fibers within a voxel. In contrast, probabilistic tractography takes into account noise and uncertainty by generating a distribution of possible streamlines from a seed point. The resulting spatial maps show higher intensities in voxels which have a higher probability of being connected to the seed point. Both approaches use certain information to facilitate fibre tracking and reconstruction of individual tracts, including seed and termination points. To reduce uncertainty waypoint and exclusion points can be added, all of which help to determine areas that tracking algorithms have to pass to and may not pass to, respectively. Moreover, limits need to be made on the FA values of streamlines to avoid tracking outside the white matter or in regions of high directional uncertainty. Similarly, a limit is set on the maximum curvature of streamlines. White matter tracts rarely have large bends, so a high curvature likely reflects errors in the tracking algorithm or fibre orientation model. Finally, limits can be set on other factors such as the tract length, to avoid spurious streamlines (Jenkinson and Chappell, 2017; Jeurissen et al., 2019).

Because many older tractography algorithms use FA limits, they may not work well in the presence of MS lesions in which FA is low, and as a result few studies use tractography in MS (Filippi et al., 2001; Inglese and Bester, 2010). One way to overcome

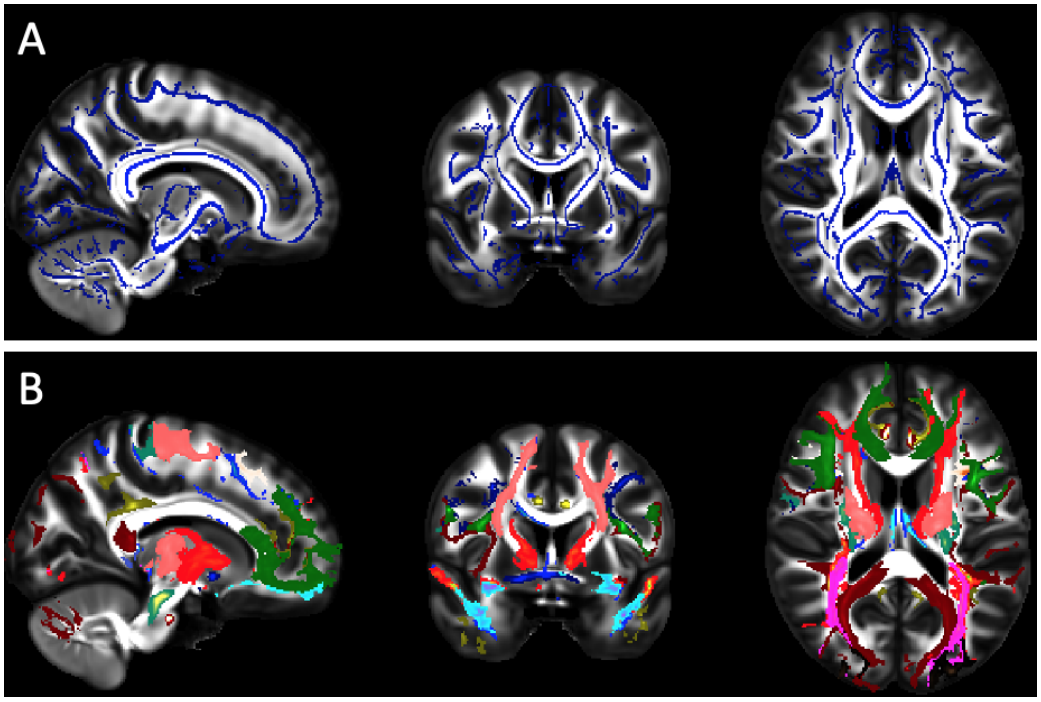

Figure 2.4: Examples of Tract-Based Spatial Statistics and tractography outputs
Figure A shows a mean Tract-Based Spatial Statistics (TBSS) skeleton from 129 participants, which captures only the centre of each white matter tract and is based on high fractional anisotropy values. Figure B shows 42 white matter tracts, including bilateral pairs, from one participant, reconstructed using a probabilistic tractography approach and thresholded. From each tract a range of information can be gained, including metrics for tractometry analyses. This data is from individual example MS patients from Chapters 4 and 6.

this problem is to use probability maps of white matter tracts based on data from healthy subjects, however, such approaches lack detail about individual variability in tract size and shape. More recent tracking algorithms do not rely as heavily on FA and have been found to be able to reconstruct white matter tracts even in the presence of high lesion volume (Lipp et al., 2020), indicating the suitability of tractography in MS research.

In this thesis probabilistic tractography, based on crossing fibre models that can estimate multiple fibre orientations (Behrens et al., 2007; Jbabdi et al., 2012), has been used in chapters 4, 5 and 6 to investigate pathological features of normal appearing white matter. Anatomical Connectivity Mapping (ACM), a probabilistic tractography method that initiates streamlines from every voxel of the brain parenchyma was used in chapters 4 and 5. By determining how many streamlines pass though each voxel of the brain it can be calculated how connected each voxel is to the rest of the brain. The outputs are spatial maps, ACM maps, in which the magnitude of the ACM value in each voxel shows the number of streamlines passing through that voxel, and thus the relative degree of connectivity of that voxel to any other voxel (Embleton et al., 2007; Bozzali et al., 2011; Cercignani et al., 2012). The strength of this method is that it does not require a priori assumptions about the location of white matter damage.

Instead it shows where in the brain anatomical connectivity is altered in one group relative to another, regardless of where in the brain the white matter damage is. This method can also be combined with a region-of-interest approach to query whether the anatomical connectivity of a certain region is affected in any way, without prior knowledge or hypotheses of the spatial source of white matter damage, such as has been done in chapter 4. In chapter 6 tractography was performed using an approach which automates individual tractography, maintaining individual variability in white matter tracts while being feasible in a large sample (Warrington et al., 2020). Further analyses were conducted using a tractometry approach in which diffusion metrics are extracted from tracts of interest (Bells et al., 2011).

2.3.2.3 Interpretation

Clinical neuroimaging research often aims to identify changes in diffusion measures in a clinical sample of interest relative to a control sample, or correlations between the diffusion measures and measures of clinical function. However, while such findings point to abnormalities in white matter integrity, the interpretation in terms of more specific biological mechanisms, i.e. what aspects of white matter integrity are altered, is not straight-forward. A range of things could influence measures of anisotropy, including number, size and density of axons, and the presence and thickness of myelin. In demyelinating diseases like MS it is conceivable that reductions in anisotropy in patients relative to controls reflect loss of myelin. However, research on anisotropy has found anisotropy in non-myelinated axons of fish, sea creatures, rodents, rabbits and human infants (suggesting a prominent role for other features, such as axonal membranes). Interestingly, in premature human infants anisotropy increased in the final weeks of gestational age, before myelination starts to occur, pointing to increased parallel organisation and packing of maturing axons as being the driver of increased anisotropy (Partridge et al., 2004, 2005; Berman et al., 2005). Overall, this evidence highlights the influence of axonal properties independent of myelin on anisotropy values. Nevertheless, there is evidence from both animal research and human histological studies to demonstrate that myelin also influences anisotropy values (Beaulieu, 2014).

Further information can be gained by assessing AD and RD measurements in addition to FA. There is evidence from both animal and human research that AD and RD are markers of axonal and myelin damage, respectively. In a series of studies in mouse models Song et al. (2002, 2003, 2005; Sun et al., 2006) have shown that RD is related to myelin, while AD is not affected by changes in myelination, but instead relates to presence of axons. There is support for this idea in human research. For instance, in

humans with corpus callosum resection, FA was reduced both 1 week and 2 months after the procedure, but FA changes were accompanied with AD reduction at the first timepoint, when axons were damaged, and RD reduction at 2 months, suggesting demyelination of the damaged axons (Concha et al., 2006). However, this evidence is not conclusive and these measures seem to be influenced by both technical and pathological factors. Using simulated data, Wheeler-Kingshott and Cercignani (2009) showed that measurements of AD and RD are affected by the presence of crossing fibres, partial volume effects and a more oblate shape of the diffusion tensor due to a decrease in anisotropy. These are factors which could be caused by pathology. Inflammation, a key feature of several neurological disorders, including multiple sclerosis, has been shown to influence measures of AD and RD. In an animal model with demyelination of the corpus callosum, RD was increased in demyelinated voxels, but not if inflammation in the form of astrogliosis was present, in which case there was no change in RD (Xie et al., 2010). Therefore, interpretations of results from diffusion MRI studies in terms of biological mechanisms, particularly in disease states, must be made carefully.

2.3.3 Arterial Spin Labelling

The brain is reliant on blood flow for the delivery of nutrients and removal of waste. The delivery of blood to the capillary bed is called perfusion and is a key physiological mechanism in healthy brain function which can provide indirect information about the brain's energy demands and thus activity. Therefore, studying how it is altered in disease can be very informative in understanding pathological changes to brain physiology.

2.3.3.1 Acquisition and analysis

Most neuroimaging techniques for studying perfusion require an injectable contrast agent which acts like a tracer in the blood. Arterial spin labelling (ASL) MRI is unique in that it is non-invasive; it uses an endogenous tracer created by the MRI scanner (Chappell et al., 2017). The process relies on collecting two sets of images, one labelled with the endogenous tracer, the label image, and one without, the control image. To acquire the label image hydrogen atoms in blood-water in the neck are subjected to a radiofrequency field that inverts their magnetisation and thus creates a tracer. This process is known as labelling and is followed by a post-label delay in which there is a wait for the labelled water to flow into brain tissue before the image is acquired. The

signal difference between the label and control images provides a measure of blood delivery to the brain.

The signal-to-noise ratio (SNR) in ASL imaging is poor due to the small signal difference between label and control images of 1-2% (Chappell et al., 2017). In addition, the half-life of the endogenous tracer is very short, less than 2 seconds, which is similar to the time it takes labelled blood to reach brain tissue, called the arterial transit time. As a result, a decision needs to be made about the duration of the post-label delay; a short delay may result in incomplete delivery of labelled blood, while a long delay will result in tracer decay and worsen the SNR further. Therefore, a set of recommendations have been developed by Alsop et al 2015 for optimal implementation of ASL in clinical research. This consensus paper is often called the 'White Paper,' (Alsop et al., 2015).

Several metrics can be obtained from ASL data, but regardless of metric, quantification analysis starts with the subtraction of label and control images. The difference image is perfusion-weighted in that it is sensitive to the perfusion in each voxel. Multiple difference images are averaged to reduce the effects of noise. However, they lack information about absolute perfusion in each voxel. To obtain absolute perfusion a kinetic model is applied that accounts for the delivery of labelled blood-water over the course of the label duration, and calibration is performed using a calibration PD-weighted image to obtain a measure of magnetization of arterial blood (Chappell et al., 2017).

2.3.3.2 Metrics and interpretation

ASL is thought to be able to measure both the blood flow to the capillary bed throughout the brain, called cerebral blood flow (CBF), and the arrival of the blood, called perfusion. CBF is measured as the volume of blood per tissue volume per unit of time, usually as ml/ml/min. Perfusion, on the other hand, is measured as the volume of blood delivered to a volume of brain tissue per unit time, as ml/g/min (Chappell et al., 2017). In this thesis only the measure of CBF has been used, so the use of the term 'perfusion' refers to the general umbrella term of measures derived from the ASL method of measuring perfusion in the brain.

Perfusion abnormalities are common in MS reviewed in Lapointe et al., 2018. It is generally accepted that increases in these measures in active lesions reflects vasodilation following inflammatory activity in the affected tissue. However, reductions in perfusion measures are also reported in normal appearing grey and white matter, and less well understood (Lapointe et al., 2018). CBF at rest in healthy tissue is relatively

constant, through a process known as autoregulation, but can also change in response to neuronal metabolic demand (Duffin et al., 2018). Thus hypoperfusion in normal appearing tissue could reflect decreased energy demand. While there is evidence in support for this, there is also evidence to suggest that hypoperfusion could reflect primary vascular insult (Lapointe et al., 2018). It is therefore likely that hypoperfusion in normal appearing tissue reflects a combination of inflammatory, metabolic and vascular activities and this must be considered when interpreting the results of Chapter 4.

2.3.4 Sodium (23Na) MRI

It has been proposed that energy failure occurs in demyelinated axons and is responsible for axonal dysfunction. As outlined in the introduction of this thesis, axonal function is dependent on a chemical balance between the intra- and extracellular space, particularly in sodium and potassium concentrations. Axonal dysfunction can result in accumulation of intracellular sodium, and by measuring sodium concentrations in brain tissue information can be gained about the energy state of the axons in that tissue (Paling et al., 2011).

After hydrogen protons, sodium nuclei give the second strongest nuclear MR signal in biological tissue, and so changes in sodium concentration in the brain can be imaged in vivo with sodium, or 23Na, MRI, using a head coil sensitive to this signal. Sodium MRI is a more challenging technique than proton MRI, due to the lower concentration and shorter relaxation time of sodium, resulting in a lower SNR (Ouwerkerk, 2011). However, technological advances, including use of high-field ($\geq$3 Tesla) scanners, have improved both the speed and sensitivity of sodium MRI (Zaaraoui et al., 2012).

Sodium nuclei have a short T2 relaxation and so sodium MRI acquisition is done with ultrashort TE sequences. Phantoms containing known sodium concentrations are required for the acquisition to enable quantification of sodium concentration in the biological tissue during analysis. These are placed near the research volunteer's head, within the field of view (Madelin et al., 2014).

At present most sodium MR protocols do not have the resolution to provide information about intra- and extracellular sodium concentrations separately, but provide an output which is a combination of the two. There are currently two techniques suitable for in vivo measurement of intra- and extra-cellular sodium accumulation in humans. Inversion recovery (IR) T1 weighting relies on the difference in T1 relaxation in different compartments, to distinguish the signal from intra- versus extracellular space. The multiple quantum filtering (MQF) technique on the other hand relies on

different T2 relaxation properties in the different compartments (Ouwerkerk, 2011). These techniques have low signal-to-noise ratio and should therefore be performed on an ultra-high-field scanner, such as 7T (Petracca et al., 2016). Data collection for chapter 5 was performed on a 3T scanner, and therefore neither of these techniques were used.

2.3.4.1 Metrics and interpretation

The measurement most commonly obtained from sodium MRI, including in chapter 5 of this thesis, is the total sodium concentration (TSC), the combined concentration from intra- and extracellular sodium (Madelin et al., 2014), mainly from neuronal axons. An increase in TSC between two groups or time points could be a result of either increased intracellular sodium in the axon following demyelination, or increased extracellular sodium following an increase in extracellular space and fluid due to axonal loss. Thus it can reflect either disruption of axonal energy metabolism or damage to the cell membrane integrity (Paling et al., 2011). Without the measurement of intra- and extracellular sodium concentration separately it is not possible to differentiate between these two causes. Nevertheless, TSC can be a surrogate marker of axonal injury in normal appearing tissue, before more widespread tissue damage, and so hold promise for identifying pathological changes underlying cognitive symptoms in early MS.

A note should be made here that both ASL and sodium MRI were used in this thesis to gain insights into potential metabolic changes in the MS brain. However, neither is a direct measure of metabolic function. To probe neural metabolism directly a measure like fluorodeoxyglucose (FDG) positron emission tomography (PET) is required. Nevertheless, within the MR modality, ASL and 23Na can give important insights about blood flow and sodium accumulation non-invasively, which can both be related to neural metabolism, and can be used to inform the direction of future research.

2.3.5 Myelin Water Fraction and Magnetisation Transfer Ratio

Finally, this thesis uses two additional MR metrics in chapter 6, myelin water fraction (MWF) and magnetisation transfer ratio (MTR). As they are not a big part of the thesis they have not been described in detail here. Yet, to understand the composite diffusion metric used in chapter 6 it is important to briefly outline what these measures are and what additional information they add to the FA and RD metrics used for the composite metric.

MWF is calculated from the MR signal from water within myelin sheaths. This trapped water has a short T2 relaxation time and can therefore be separated from other water compartments with longer T2 relaxation. The MWF is the ratio of this short T2 signal to the total T2 relaxation signal (Beaulieu et al., 1998). It has been shown to correlate with histological markers of myelin (Moore et al., 2000).

Magnetisation transfer is a measure of the transfer of magnetisation of macromolecules, such as those in myelin, to surrounding water following the application of an off-resonance radiofrequency pulse. The MT ratio is the difference between measurements obtained with and without the off-resonance radiofrequency. Similarly to MWF, MTR shows a reasonable correlation with myelin in post mortem multiple sclerosis brains (alongside other histological features) (Schmierer et al., 2008).

Thus, MWF and MTR may provide additional information about myelin, which can be combined with FA and RD to provide a composite diffusion metric, such as in tractometry approaches (Chamberland et al., 2019). Such an approach has been used in Chapter 6 to assess the integrity of normal appearing white matter.

2.3.6 A multimodal approach

MS is a complex neurodegenerative disorder and evidence is increasingly showing that many aspects of brain structure and function are affected. As a result, models such as the 'network collapse' model make predictions about the relationship between pathology and cognition by considering both structure and function. To test such models effectively and understand pathological changes in the brain a multimodal MRI approach is essential. This has been shown in chapter 4, where we found that FC abnormalities co-occur with anatomical and CBF abnormalities, highlighting the possibility of a shared mechanism, and in chapter 5, where we found altered sodium concentrations in functional network regions compared to the rest of the brain. Moreover, in chapter 6 we used several diffusion metrics together in a dimensionality reduction approach to get an overall measure of white matter health (i.e. tractometry; (Chamberland et al., 2019), which we explored in relation to cognitive function. Overall, our findings support the use of a multimodal approach for understanding the pathological mechanisms of cognitive impairment in MS.

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Chapter 3

A Systematic Review of Resting-State Functional MRI Connectivity Changes and Cognitive Impairment in Multiple sclerosis

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This is an Accepted Manuscript version of the following article, accepted for publication in *Brain Connectivity:* Jandric D, Doshi A, Scott R, Paling D, Rog D, Chataway J, et al. A Systematic Review of Resting-State Functional MRI Connectivity Changes and Cognitive Impairment in Multiple Sclerosis. Brain Connect 2021; Online Ahead of Print, DOI:10.1089/brain.2021.0104.

Abstract

Introduction: Cognitive impairment in multiple sclerosis (MS) is increasingly being investigated with resting-state functional MRI (rs-fMRI) functional connectivity (FC). However, results remain difficult to interpret, showing both high and low FC associated with cognitive impairment. We conducted a systematic review of rs-fMRI studies in MS to understand whether the direction of FC change relates to cognitive dysfunction, and how this may be influenced by the choice of methodology.

Methods: Embase, Medline, and PsycINFO were searched for studies assessing cognitive function and rs-fMRI FC in adults with MS.

Results: Fifty-seven studies were included in a narrative synthesis. Of these, 50 found an association between cognitive impairment and FC abnormalities. Worse cognition was linked to high FC in 18 studies, and to low FC in 17 studies. Nine studies found patterns of both high and low FC related to poor cognitive performance, in different regions or for different magnetic resonance (MR) metrics. There was no clear link to increased FC during the early stages of MS and reduced FC in later stages, as predicted by common models of MS pathology. Throughout, we found substantial heterogeneity in study methodology, and carefully consider how this may impact on the observed findings.

Discussion: These results indicate an urgent need for greater standardization in the field—in terms of the choice of MRI analysis and the definition of cognitive impairment. This will allow us to use rs-fMRI FC as a biomarker in future clinical studies, and as a tool to understand mechanisms underpinning cognitive symptoms in MS.

3.1 Introduction

Multiple sclerosis (MS) is a chronic immune mediated disorder of the central nervous system that predominantly affects young adults (Dobson and Giovannoni, 2019; Filippi et al., 2018; Thompson et al., 2018). Inflammatory demyelination is pathognomonic with neurodegeneration insidiously dominating over time (Lassmann, 2018).

Cognitive impairment is common in all MS phenotypes (Benedict, 2020; Benedict et al., 2020; Charcot, 1888) with an estimated prevalence of 43-70% dependent on factors including phenotype and the cognitive diagnostic criteria used (Fischer et al., 2014; Sumowski et al., 2018). Cognitive impairment is associated with several adverse outcomes including a higher risk of depression, unemployment and reduced quality of life (Ruet et al., 2013b; Strober et al., 2014; Sumowski et al., 2018). A more progressive MS phenotype and longer disease duration have been shown to be associated with greater cognitive impairment (Baird et al., 2019; Connick et al., 2013; Johnen et al., 2019, 2017; Patti et al., 2010). There are currently no licensed treatments for cognitive symptoms in MS, however exercise (Motl and Sandroff, 2020) and behavioural therapy show promise (Sandroff and DeLuca, 2020). Disease modifying therapies show positive outcomes on cognitive dysfunction in MS, despite no routine evaluation in phase 3 clinical trials currently. However, effects are small and at present understudied, and there are to date no approved pharmaceutical treatments for cognitive symptoms (Benedict et al., 2020; Landmeyer et al., 2020).

Gaining an understanding of the underlying pathophysiology of cognitive dysfunction is essential for diagnosing, monitoring and developing treatments for this debilitating aspect of MS. The ‘clinico-radiological’ paradox highlights the mismatch of MS cognitive symptoms and conventional Magnetic Resonance Imaging (MRI) measures, such as lesion volumes (Rocca et al., 2015). It is widely accepted that cognitive function is supported by a complex network of structurally interconnected brain regions supporting a highly dynamic functional network, which is researched with advanced MRI tools such as resting state functional MRI (rs-fMRI), in MS and other neurodegenerative diseases (Battle et al., 2017; Castellazzi et al., 2014; Mori et al., 2011; Rocca et al., 2015; Schoonheim et al., 2015b).

The main measure derived from rs-fMRI is the functional connectivity (FC) metric. It is a measure of the statistical correlation of blood-oxygenation-level-dependent (BOLD) signal time course between any selection of voxels. The underlying assumption is that voxels with similar BOLD time courses are connected in the performance of a function (Bijsterbosch et al., 2017), see Figure 1. FC has the potential to be an imaging biomarker

of cognitive performance in neurodegenerative disease (Hohenfeld et al., 2018) and is the subject of a growing research field in MS (Benedict et al., 2020). Such a marker could offer a fast, non-invasive way to detect imminent cognitive decline, which is often underdiagnosed on routine neurological examinations (Romero et al., 2015). For a measure to be suitable as a clinical biomarker, it needs to be able to identify those with cognitive dysfunction from those without it, and to show acceptable repeatability and reproducibility across studies. In some diseases, like Alzheimer's disease, the rs-fMRI literature shows consistently low FC in the default mode network (DMN) (Badhwar et al., 2017), yet a recent review of rs-fMRI studies in several neurodegenerative diseases, including Alzheimer's, argued that the evidence is not yet strong enough for rs-fMRI FC measures to be suitable biomarkers (Hohenfeld et al., 2018). This review cited a lack of standardised protocols as a challenge in the field.

The rs-fMRI FC literature on cognition in MS has not yet been subject to systematic review, and so the specificity and reliability of FC as a marker of cognitive dysfunction has not been established. Correlations between FC metrics and cognition have been frequently reported (Hawellek et al., 2011; Lin et al., 2020; Schoonheim et al., 2012; Tona et al., 2014), but in studies comparing FC between cognitively impaired (CI) and cognitively preserved (CP) patients, results have shown both high and low FC linked with worse cognitive function (Basile et al., 2014; Bonavita et al., 2011; Cruz-Gómez et al., 2014; Faivre et al., 2012; Rocca et al., 2018). A common interpretation of increases in any type of brain function is that of functional "reorganisation": a compensatory mechanism that enables the functioning of networks in the presence of structural damage, hence delaying clinical progression. This compensatory mechanism is thought to be sustainable only up to a critical point, at which the structural damage becomes too great to compensate for, leading to the hypothesized "network collapse", manifested as decreases in FC and clinical progression (Schoonheim et al., 2015b; Schoonheim et al., 2017). In support of this, several studies indicate different patterns of FC changes at different disease stages, such as high FC in clinically isolated syndrome (CIS), the earliest stage of MS, and low FC in progressive MS (Basile et al., 2014; Cocozza et al., 2018; Rocca et al., 2010; Roosendaal et al., 2010a; Roosendaal et al., 2010b). However, high FC has also been related to the severity of impairment (Hawellek et al., 2011), casting doubt on the beneficial nature of these changes. As such, it is not yet clear whether the pattern of results from rs-fMRI studies consistently fits the predictions of this model. This may be complicated by the heterogeneity in methodological aspects of studies which could influence the direction of findings (Tewarie et al., 2018).

In this study we carry out a systematic review of rs-fMRI FC studies of cognitive dysfunction in MS to outline the state of the field and provide a critical analysis of findings to date. We considered directionality of results and the influence of methodological aspects on findings of FC alterations. Through doing so we offer key points that need to be addressed in order to develop a parsimonious account of why FC may change in MS and what it may mean for clinical practice.

3.2 Method

3.2.1 Procotol and Registration

The design of the systematic review and manuscript preparation were based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Moher et al., 2015). The systematic review protocol was developed in advance and, in accordance with PRISMA guidelines, registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 18 May 2020, and last updated on 31/8/2020 (registration number CRD42020154415).

3.2.2 Information sources and search strategy

Literature searches were conducted in Embase (accessed through the Ovid interface, 1974 onwards), Medline (accessed through Ovid, 1946 onwards), and PsycINFO (accessed through Ovid, 1806 onwards) on 31st October 2019, with no limits imposed on the searches. The search strategy used terms for 'multiple sclerosis' 'functional connectivity' and 'cognition' and was tailored for each database to use both controlled terms where available and uncontrolled keywords in order to capture any synonym, abbreviation and related term of the keywords of interest. The searches were repeated on 22nd October 2020 to capture any studies published since the original searches. The same search strategy was used, but limits were added to capture only results which had been added or updated in the period 1st November 2019 – 22nd October 2020. The full search strategy used in each database is available in Supplementary Table 1.

3.2.3 Study eligibility and selection

Records returned by each search were imported into the Mendeley reference management software v 1.19.4, and duplicates were removed using the tool's de-

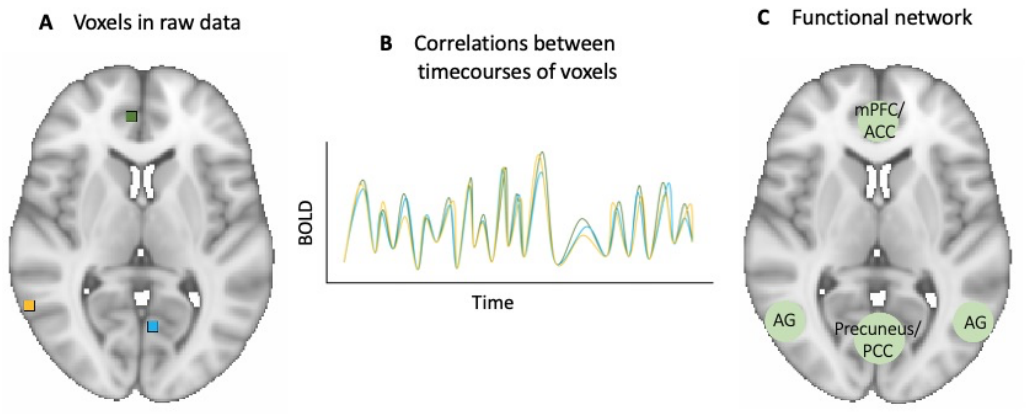

Figure 3.1: Schematic of functional connectivity and a functional network
Functional connectivity is a measure of the statistical correlation of blood-oxygenation-level-dependent signal timecourses (part B) between any selection of voxels (part A). Voxels or voxel clusters showing high correlations are considered functionally connected, and can be used to identify functional networks such as the default mode network (part C). Abbreviations: ACC = anterior cingulate cortex; AG = angular gyrus; BOLD = Blood-oxygenation-level-dependent signal; mPFC = medial prefrontal cortex; PCC = posterior cingulate cortex

duplication function. Titles and abstracts were then manually screened by two independent reviewers (DJ and RS). Full text publications were obtained for all papers chosen for full text review by one or both reviewers and assessed for inclusion in the review against pre-defined eligibility criteria. Any disagreements about study inclusion were resolved through discussion and reasons for study exclusion were recorded. This process was then repeated for the search conducted on 22nd October 2020. The results at each stage, for the combined two searches, are presented in Figure 2.

Eligibility criteria were: original peer-reviewed research studies reporting on cognitive function and FC metrics derived from rs-fMRI in adult MS patients. Review articles, book chapters and conference abstracts were excluded, as were any original research studies in a paediatric population, on diseases other than MS, studies which had not measured cognitive function and/or functional connectivity, studies focusing on cognitive rehabilitation, studies which had assessed social cognition only, and any articles which were not available in English.

3.2.4 Data collection and synthesis

Data extraction was performed by DJ and RS and the following data items were recorded: 1) study characteristics (authors, year of publication, journal); 2) aims of the study; 3) Participant details (MS subtype, control group, sample size, disease duration of MS sample, Expanded Disability Status Scale (EDSS) score of MS sample); 4) MR methodology (scanner field strength, MR metrics); 5) FC analysis (data pre-processing,

method for analysis, whether analysis was global or regional [and if so, which regions], use of covariates); 6) cognitive testing (cognitive test(s) used, definition of cognitive impairment, number of cognitively impaired/preserved patients if applicable); 7) results from FC analysis and from other MR metrics).

To understand whether there might be a link between methodological aspects and FC results, we examined whether a particular feature was commonly present in studies that report links between worse cognition and either high or low FC. The features we examined were the MS subgroup studied, the average disease duration of patient samples, the rs-fMRI analysis method and the brain region or resting state network (RSN) investigated. Because the studies included were too heterogeneous for a meta-analysis, data synthesis was done by tallying the number of studies sharing a specific methodological feature or FC result.

3.2.5 Assessment of study quality

A quality assessment approach was chosen over a risk of bias tool because most articles for inclusion in this review were expected to be cross-sectional. The AXIS tool was designed for cross-sectional studies across a range of scientific disciplines (Downes et al., 2016) and was therefore selected to judge the quality of the evidence included in the review. The AXIS tool is a 20 item checklist which asks ‘yes/no’ questions about important elements of a study. Three of the 20 items in the tool were not relevant for the studies selected for this review, as they refer to responding to an intervention, so quality assessment was based on the remaining 17 items. The items of the AXIS tool are not scored, but instead recorded in a similar way to the Cochrane risk of bias tool (Higgins et al., 2011), allowing review authors to make an overall assessment of the quality of the study based on the presence or absence of reporting of the items covered by the tool.

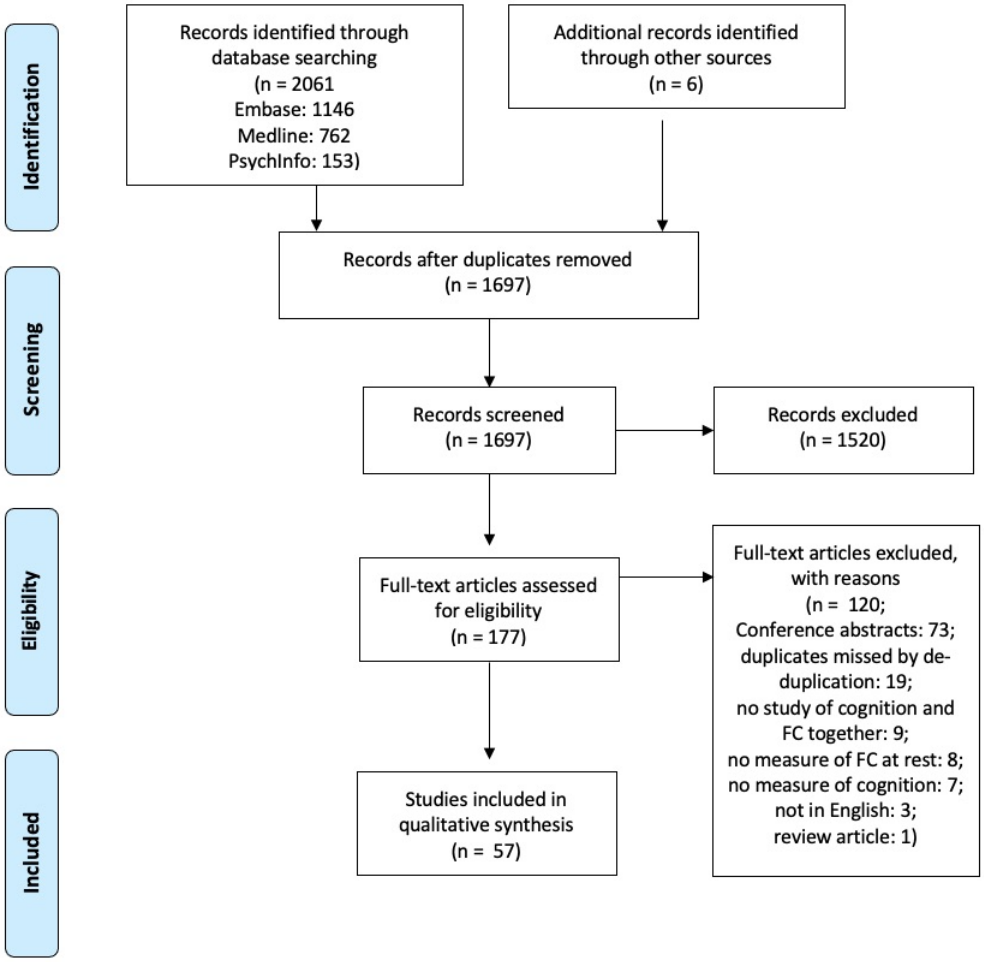

Figure 3.2: Flow diagram showing identification, screening and selection of records
Figure 2 outlines combined database searches conducted on the 31st October 2019 and on the 22nd October 2020 using the PRISMA protocol for studies of rs-fMRI and cognitive function in MS. Template from Moher et al. (2015)

3.3 Results

3.3.1 Study selection and quality assessment

The systematic review process is outlined in Figure 2. The database searches yielded 2061 results, and in addition 6 were identified from other sources. After removal of duplicates 1697 remained, which were screened for eligibility until 177 remained for full-text assessment. At this point 120 records were excluded, most of which were conference abstracts (see Figure 1 for reasons for exclusion). Fifty-seven studies met eligibility criteria and were included in the review. These studies are summarised in Table 1. All studies were of high quality, as measured by the AXIS tool (Downes et al., 2016). Eighteen studies did not include clear details of where participants were recruited from for the study, and very few studies (5/57) had a justification for the sample size used.

3.3.2 Participant characteristics

The studies that were included differed in the clinical and demographic details of the MS samples used. The majority of studies used a mixed sample of different MS phenotypes (29/57 studies), and slightly over a third used a sample of relapsing-remitting MS (RRMS) patients only (22/57 studies). The remaining six studies used either a primary progressive MS (PPMS) sample (1/57), CIS sample (2/57), a benign MS (BMS) sample (1/57) or did not specify the MS subtype (2/57). See Table 1 for details on the cohort of each study.

The average disease duration ranged from as little as 4.2 months (Koubiyr et al., 2019) to 21.9 years (Lin et al., 2019) from either time from first symptom or from diagnosis, and median EDSS ranging from 1 (Faivre et al., 2012; Koubiyr et al., 2019) to 6.5 (Manca et al., 2019).

Most studies (54/57) used healthy volunteers as a control group. In one study normative data from age-matched healthy controls was used for neuropsychological assessments, but no control group was used for comparisons of MRI metrics (Manca et al., 2019). In one study no control group was specified (Leavitt et al., 2014), and in one longitudinal study no control group was used (Petsas et al., 2019). Out of the studies using healthy controls, many did not report matching groups on any demographic variables (18/54) while some reported matching groups but not on which variables (3/54) and one reported not matching the groups. Of the studies reporting the variables

groups were matched on, most were on age and sex (15/54), followed by age, sex and education (10/54), age only (2/54), sex only (2/54) or age, sex, education and premorbid IQ (1/54). In this review we have interpreted the words ‘sex’ and ‘gender’ to both refer to sex, given that MS is a disease characterised by sex differences in prevalence (Krysko et al., 2020; Thompson et al., 2018).

3.3.3 Neuropsychological assessment

Most studies (34/57) looked at relationships between cognitive test performance and MR metrics through correlations or regressions, and 19 studies examined group differences in MR metrics between patients who met criteria for cognitive impairment and those who did not. Of the remaining four studies, one looked at FC only in MS patients with intact spatial memory (Roosendaal et al., 2010b), and three did not directly assess the relationship between cognition and FC. Despite this, they were included in the review for the following reasons: the authors of one study expressed intentions to correlate FC measures with clinical measures, but did not because the FC measure did not show any abnormalities in MS patients (Romascano et al., 2015); two studies indirectly explored the relationship between FC and cognition and did not meet any exclusion criteria (van Geest et al., 2018, 2017).

To assess cognitive function most studies used either the Brief Repeatable Battery of Neuropsychological tests (BRB-N), which has been validated for use in MS (Amato et al., 2006), alone or in combination with other tests (20/57), or a collection of individual tests (22/57). The remaining studies used either another cognitive battery; Brief International Cognitive Assessment for MS (BICAMS) n=2 (Langdon et al., 2012), Minimal Assessment of Cognitive Function in MS (MACFIMS) n=2 (Benedict et al., 2002), or a single test; Paced Auditory Serial Addition Test (PASAT) n=6, Symbol Digit Modalities Test (SDMT) n=1, Location Learning Test n=1, Short test of mental status n=1, the computerised test of information processing n=1) or a cognitive reserve index (n=1). See figure 3A for an overview of the tests used across the reviewed studies. The specific battery or tests used by each study are summarised in Table 1.

Within the 19 studies that split the MS sample into cognitively impaired and cognitively preserved sub-samples, there were 12 different definitions of cognitive impairment. Some definitions are likely guided by the test(s) used to assess cognition, but even amongst studies using the BRB-N, there were five different definitions of cognitive impairment (see Figure 3B). These include: $\geq$1.5 SD below normative values on $\geq$1 test (n=1); $\geq$1.5 SD below controls scores on $\geq$2 tests (n=5, but note that four

used this definition of a mildly cognitively impaired group), ≥2 SD below normative values on ≥1 test (n=1); ≥2 SD below normative values on ≥2 tests (n=9); performance in the 5th percentile of scores on either the Selective Reminding Test or Spatial Recall Test compared to normative data (n=1).

3.3.4 Functional connectivity analysis

Half of all studies (28/57) used a seed-based connectivity analysis (SCA) method for assessing FC. In this category we have included studies which used one or a few specific regions of interests (ROIs; regional SCA) or divided the whole brain into ROIs and created a connectivity matrix (global SCA). The second most common method was independent component analysis (ICA) (14/57), and the remaining studies either calculated graph theory metrics (7/45), used a principal component analysis (1/45) or used a combination of SCA and graph theory (1/45) or ICA and graph theory (1/45). See Table 1 for the design and rs-fMRI analysis method of each study.

A wide range of regions and RSNs were investigated, either as a priori defined areas of interest, or as patterns emerging from a data-driven analysis, of which the most common were the DMN (21/57), thalamus and thalamic networks (9/57), the fronto-parietal network (FPN), including the right, left, dorsal and ventral FPNs (7/57). Other RSNs and regions investigated include the attentional network including left, right, dorsal, ventral variants, the salience network, the executive network, the working memory network, the motor network, the sensorimotor network, the visual processing network, the auditory network, the auditory and language processing network, visual processing networks, including medial and lateral variants, the cerebellar network, the medial prefrontal cortex, anterior cingulate cortex, posterior cingulate cortex, precuneus, basal ganglia, hippocampus and cerebellum. Ten studies conducted a whole-brain analysis and did not report regional FC changes.

3.3.5 Functional connectivity results

The main result of the relationship between FC and cognition of each study is summarised in Table 1 and Figure 4A. Overall, 18 studies found worse cognition to be linked with high FC and 17 found it to be associated with low FC. Nine studies found patterns of both high and low FC to be associated with cognitive impairment, in different regions or for different MR metrics, and seven studies found no significant relationship between cognitive and FC measures. Six studies had a methodology which did not measure the direction of FC change in relation to cognitive impairment.

When grouping studies based on methodological and clinical features to assess whether one direction of FC change associated with worse cognition is more commonly seen in studies with that feature, we found no trend to suggest that one FC direction change associated with worse cognition is more commonly seen in studies using a specific method or studying a specific type of sample. This includes grouping studies based on the RSN or network assessed. For example, of the 21 studies measuring FC in the DMN, 10 found worse cognition to be associated with low FC, 6 with high FC, 1 with both high and low FC, 3 obtained a negative result, and one study did not test the relationship directly. See Figure 4B and Supplementary Table 2 for a full overview of study results by regions investigated.

We also considered the role of disease phenotype, however, most studies used either a mixed sample consisting of several phenotypes or a sample of RRMS patients only. Of the 22 studies which used a RRMS sample, eleven reported worse cognition to be associated with high FC and ten with low FC. Three studies reported a negative result and one had a study method which does not inform about the direction of FC changes. Similarly, within the mixed sample studies almost half of studies reported worse cognition to be associated with high FC (13/29) and more than half with low FC (16/29). Some studies reported both high and low FC to be associated with worse cognitive function and have therefore been counted twice. See Figure 4C for an overview. In seven of the studies with mixed phenotype samples subgroup analyses were conducted to compare FC changes between different MS phenotypes in the sample, but only two included cognition in these analyses. One found a stronger positive correlation between FC in the DMN and errors on the PASAT in secondary progressive MS (SPMS) compared to RRMS, while another found differences between RRMS and SPMS in the spatial location of FC abnormalities that corelated with cognitive test performance.

Finally, we ordered studies by the average reported disease duration of the sample used, to see if patterns of FC changes differ from early to late in the disease and found no such trend, see Figure 5 and Supplementary Table 3.

Table 3.1: Study characteristics, cognitive assessment and relationship between cognition and functional connectivity

Study	Cohort (n)	Design	Cognitive measures	FC-cognition analysis outcome	Direction of FC result
Rocca et al. 2010 (Rocca et al., 2010)	SPMS (33) PPMS (24) HC (24)	Cross-sectional ICA	PASAT3, TMT, SST, WLT, RCFT, VFT	Lower ACC FC within the DMN in MS patients compared to HC, but more pronounced reductions in cognitively impaired MS patients.	↓
Roosendaal et al. 2010 (Roosendaal et al., 2010a)	CIS (14) RRMS (31) HC (41)	Cross-sectional ICA	Stroop, LLT, LDST	No correlations between FC metrics and cognitive measures.	-
Roosendaal et al. 2010 (Roosendaal et al., 2010b)	CIS (5) RRMS (18) SPMS (2) HC (30)	Cross-sectional Seed	LLT	Lower FC in hippocampus bilaterally in MS patients with intact spatial memory compared to HC.	-
Bonavita et al. 2011 (Bonavita et al., 2011)	RRMS (36) HC (18)	Cross-sectional ICA	BRB-N, Stroop	Lower ACC and PCC FC in cognitively impaired and cognitively preserved RRMS compared to HC. Lower PCC FC in cognitively impaired patients compared to cognitively preserved.	↓
Hawellak et al 2011 (Hawellek et al., 2011)	CIS (2) RRMS (12) MS* (2) HC (16)	Cross-sectional PCA	PASAT, SDMT, TMT, Digitspan, Verbal Intelligence Test 'Mehrfachwortschatztest-B," COWAT, subtests of TAP	High FC in DMN correlated with low cognitive efficiency.	↑

	*Subtypes not specified.				
Jones et al 2011 (Jones et al., 2011)	MS NOS (1) HC (10)	Case study ICA	The short test of mental status	Single patient with cognitive symptoms showed lower FC in PCC, precuneus and left inferior parietal lobe of DMN compared to HC.	↓
Faivre et al. 2012 (Faivre et al., 2012)	RRMS (13) HC (14)	Cross-sectional ICA	BRB-N	High FC in DMN correlated with decreased performance in semantic fluency task. High FC in dorsal FPN and right ventral FPN correlated with worse PASAT scores.	↑
Loitfelder et al. 2012 (Loitfelder et al., 2012)	CIS (10) RRMS (16) SPMS (5) HC (31)	Cross-sectional Seed	BRB-N, WCST	Better cognitive performance correlated with high FC from ACC to cerebellum, middle temporal gyrus, occipital pole and angular gyrus.	↓
Schoonheim et al. 2012 (Schoonheim et al., 2012)	RRMS (26) SPMS (4) HC (30)	Cross-sectional SCA GT	LLT, LDST	Low FC and network efficiency in male MS correlated with visuospatial memory.	↓
Janssen et al. 2013 (Janssen et al., 2013)	RRMS (28) HC (28)	Cross-sectional ICA	PASAT3, letter comparison and pattern comparison tasks	No correlations between FC in any network and processing speed measure.	-
Koenig et al 2013 (Koenig et al., 2013)	RRMS (30) SPMS (2) HC (32)	Cross-sectional SCA	CVLT-II, BVMT-R, PASAT, SDMT, COWAT	No correlations between FC metrics and cognitive measures.	-

Basile et al. 2014 (Basile et al., 2014)	RRMS (34) SPMS (14) HC (25)	Cross-sectional ICA	PASAT3, SDMT, RCFT	Positive correlation between ACC FC and PASAT3 mistakes in patients.	↑
Cruz-Gomez et al. 2014 (Cruz-Gómez et al., 2014)	RRMS (60) HC (18)	Cross-sectional ICA	BRB-N	Lower FC in DMN, LFPN, RFPN and SN in cognitively impaired MS compared to cognitively preserved patients.	↓
Leavitt et al 2014 (Leavitt et al., 2014)	RRMS (33) PPMS (4) SPMS (6)	Cross-sectional SCA	HVLT-R, BVMT-R, Digitspan, COWAT, PASAT, SDMT, JoLO, WTAR, Stroop	Higher FC in DMN in memory intact compared to memory impaired patients. Higher FC correlated with better memory performance.	↓
Louapre et al 2014 (Louapre et al., 2014)	RRMS (35) HC (20)	Cross-sectional ICA	Mattis Dementia Rating Scale, PASAT, TMT, verbal fluency, Digitspan, SPART	Lower FC in cognitively impaired compared to cognitively preserved in DMN and ATT.	↓
Schoonheim et al. 2014 (Schoonheim et al., 2014)	RRMS (112) PPMS (7) SPMS (9) HC (50)	Cross-sectional GT	BRB-N, CST, Stroop, MCT	Low eigenvector centrality mapping values in the ventral stream correlated with worse cognition.	↓
Tona et al. 2014 (Tona et al.,	RRMS (48) HC (24)	Cross-sectional SCA	PASAT3	Inverse correlation of thalamo-cortical resting state functional connections with	↑

2014)				PASAT3 score.	
Wojtowicz et al 2014 (Wojtowicz et al., 2014)	RRMS (18) HC (16)	Cross-sectional SCA	The computerised test of information processing	Better cognitive task performance associated with high FC in DMN regions.	↓
Hulst et al 2015 (Hulst et al., 2015)	RRMS (40) SPMS (17) HC (28)	Cross-sectional SCA	Dutch equivalent of CVLT, LLT, Digit Span, WLG, LDST	Memory impairment was predicted by (among other variables) high hippocampal FC.	↑
Romascano et al 2015 (Romascano et al., 2015)	RRMS (28) HC (16)	Cross-sectional GT	BRB-N	Relationship between FC and cognition not assessed.	-
Sbardella et al 2015 (Sbardella et al., 2015)	RRMS (30) HC (24)	Cross-sectional ICA	Mini Mental State Examination, PASAT	FC of executive control and medial visual networks correlated inversely with PASAT scores.	↑
Schoonheim et al. 2015 (Schoonheim et al., 2015a)	RRMS (133) PPMS (15) SPMS (9) HC (47)	Cross-sectional SCA	BRB-N, Stroop, CST, MCT	Higher thalamic FC in severely cognitively impaired patients compared to cognitively preserved patients.	↑
Rocca et al. 2016 (Rocca et al., 2016)	RRMS (121) BMS (45) SPMS (80) HC (55)	Cross-sectional GT	PASAT3	Abnormal network properties in cognitively impaired compared to cognitively preserved patients: lower mean network degree, global efficiency and hierarchy, higher path length, fewer hubs in left frontal cortex and thalamus.	↓

Sanchis-Segura et al 2016 (Sanchis-Segura et al., 2016)	RRMS (56) HC (63)	Cross-sectional SCA	BRB-N	Positive correlation between FC and cognitive performance.	↓
Zhou et al 2016 (Zhou et al., 2016)	RRMS (20) HC (20)	Cross-sectional SCA	PASAT	No correlations between FC metrics and cognitive measures.	-
D'Ambrosio et al 2017 (d'Ambrosio et al., 2017)	RRMS (136) PPMS (9) SPMS (42) HC (94)	Cross-sectional SCA	BRB-N	Higher thalamic FC in cognitively impaired compared to cognitively preserved patients.	↑
Eijlers et al. 2017 (Eijlers et al., 2017)	RRMS (243) SPMS (53) PPMS 36) HC (96)	Cross-sectional GT	BRB-N, SRT, WLG, SDMT, Stroop, MCT	Widespread high DMN network centrality in cognitively impaired compared to cognitively preserved patients. Some low centrality in CI, in occipital and sensorimotor areas.	↓↑
Gabilondo et al 2017 (Gabilondo et al., 2017)	RRMS (22) PPMS (1) SPMS (7) HC (28)	Cross-sectional SCA	TMT, Salthouse Perceptual Comparison Test, SDMT	Low visual processing speed correlated with both low and high FC, in the medial visual component.	↓↑
Meijer et al 2017 (Meijer et al., 2017)	RRMS (243) PPMS (36) SPMS (53) HC (96)	Cross-sectional ICA	BRB-N	Higher FC in cognitively impaired compared to cognitively preserved patients in DMN and FPN.	↑

Petracca et al 2017 (Petracca et al., 2017)	PPMS (25) HC (20)	Cross-sectional SCA	MACFIMS	Pattern of both lower and higher FC in cognitively impaired compared to cognitively preserved patients.	↓↑
Sbardella et al 2017 (Sbardella et al., 2017)	RRMS (54) HC (24)	Cross-sectional SCA	PASAT	Positive correlation between FC of dentate nuclei and PASAT performance.	↓
Van Geest et al 2017 (van Geest et al., 2017)	RRMS (52) SPMS (18) HC (40)	Cross-sectional SCA	Dutch equivalent of CVLT, LLT, Digitspan, WLG, LDST	Lower FC in sleep disturbed patients, but sleep disturbed patients did not differ from normally sleeping in cognitive test performance. (No direct analysis of FC at rest and cognition.)	-
Cocozza et al 2018 (Cocozza et al., 2018)	Progressive MS* (29) HC (22) *Number of PPMS relative to SPMS not reported	Cross-sectional SCA	BICAMS	Inverse relationship between cerebellar FC and BVMT scores.	↑
Cruz-Gomez et al 2018 (Cruz-Gómez et al., 2018)	RRMS (36) HC (18)	Cross-sectional SCA	BRB-N	Higher FC in cognitively impaired compared to cognitively preserved patients between right caudate and bilateral orbitofrontal cortex.	↑
Eijlers et al 2018 (Eijlers et al.,	RRMS (239)	Cross-sectional	BRB-N	Higher network centrality in PCC in cognitively impaired compared to	↑

2018)	PPMS (35) SPMS (53) HC (96)	GT		cognitively preserved patients regardless of presence of GM atrophy.	
Gao et al 2018 (Gao et al., 2018)	RRMS (29) HC (29)	Cross-sectional SCA	Auditory verbal learning test, RCFT, SDMT, TMT	No correlations between FC metrics and cognitive measures in MS group.	-
Lin et al 2018 (Lin et al., 2018)	RRMS (27) HC (15)	Cross-sectional SCA	Digit span, arithmetic, letter-numbering sequencing, symbol search, coding subtests from the WAIS IV, VFT, WCST, TMT.	Better executive functions and processing speed correlated with higher dynamic and stationary FC.	↓
Meijer et al. 2018 (Meijer et al., 2018a)	RRMS (241) SPMS (53) HC (96)	Cross-sectional SCA	BRB-N, CST, MCT, Stroop	High within-DGM and DGM-cortex FC correlated to worse cognition.	↑
Meijer et al 2018 (Meijer et al., 2018b)	RRMS (243) PPMS (36) SPMS (51) HC (96)	Cross-sectional SCA	BRB-N	Higher FC in patients with impaired information processing speed compared to those with preserved.	↑
Rocca et al. 2018 (Rocca et al., 2018)	RRMS (119) PPMS (13) SPMS (41) BMS (29) CIS (13) HC (98)	Cross-sectional SCA	BRB-N	Lower FC in DMN and DAN in cognitively impaired compared to cognitively preserved patients. Higher FC in thalamic network in cognitively impaired compared to cognitively preserved patients.	↓↑

Van Geest et al. 2018 (van Geest et al., 2018)	MS* (29) HC (19) *Subtypes not specified.	Cross-sectional SCA	LDST, SDMT, Stroop	Information processing task performance predicted by difference in dynamic FC between task and rest states. (No direct analysis of FC at rest and cognition.)	-
D'Ambrosio et al 2019 (D'Ambrosio et al., 2019)	RRMS (62) HC (65)	Cross-sectional ICA	BRB-N, WCST	Lower dynamic FC in the subcortical and default mode networks in cognitively impaired compared to cognitively preserved patients. Static FC showed pattern of both lower and higher FC in cognitively impaired compared to cognitively preserved patients.	↓↑
Eijlers et al 2019 (Eijlers et al., 2019)	RRMS (197) PPMS (23) SPMS (47) HC (96)	Cross-sectional GT	BRB-N	Lower dynamic FC in cognitively impaired compared to cognitively preserved patients in DMN regions.	↓
Fuchs et al 2019 (Fuchs et al., 2019)	RRMS (48) PPMS (2) SPMS (24) HC (29)	Cross-sectional ICA	BICAMS, North American Adult Reading Test	Cognitive reserve predicted preservation of functional connectivity describe accumulation of GM atrophy and additionally attenuated structural network disruption.	-
Karavasilis et al 2019 (Karavasilis et al., 2019)	CIS (16) RRMS (15) HC (16)	Cross-sectional SCA	BRB-N	Compared to memory impaired patients, memory preserved patients showed higher FC between left hippocampus and right	↓↑

				temporo-occipital fusiform/lingual gyrus, and lower FC between left hippocampus and right supramarginal gyrus.	
Koubiyr et al 2019 (Koubiyr et al., 2019)	CIS (52) HC (20)	Longitudinal GT	TAP, PASAT3, SRT, BVMT-R, Stroop test, WLG, computerised speed cognitive test, SDMT alertness test	No significant correlations between structural-functional coupling and neuropsychological variables at either baseline or 1 year follow up.	-
Lin et al 2019 (Lin et al., 2019)	RRMS (37) PPMS (3) SPMS (24) HC (26)	Cross-sectional ICA	SDMT, CVLT, BVMT-R, PASAT	Negative correlation between SDMT scores and FC in MS group. No other cognitive measures correlated with FC.	↑
Manca et al 2019 (Manca et al., 2019)	RRMS (40) SPMS (25)	Cross-sectional ICA	Mini Mental State Examination, Raven's Coloured Progressive Matrices, TMT, Stroop, Semantic and Phonemic Fluency Tests	FC correlated positively with cognitive test performance in LFPN, and negatively in SN and DMN. No correlations in RFPN.	↓↑
Petsas et al 2019 (Petsas et al., 2019)	RRMS (32)	Longitudinal SCA	PASAT 2 and 3 sec	Low resting state FC before a task (baseline FC) over a 6 month period was inversely related to PASAT 3 performance, but not PASAT 2. No relationships were found for the resting state FC metric obtained after a task.	↑

Bizzo et al 2020 (Bizzo et al., 2020)	RRMS (28) HC (28)	Cross-sectional SCA	Cognitive reserve index created by combining premorbid IQ measured with the Test of Premorbid Function, leisure activities, and education level	Intrinsic FC within the left dorsal anterior insula and left occipital cluster was inversely correlated with cognitive reserve index values.	↑
Carotenuto et al 2020 (Carotenuto et al., 2020)	RRMS (29) HC (24)	Cross-sectional SCA, GT	SDMT	Both positive and negative correlations between SDMT scores and FC and graph theory metrics in neuromodulatory networks.	↓↑
Lin et al 2020 (Lin et al., 2020)	RRMS (25) HC (41)	Cross-sectional SCA	SDMT, PASAT 3 sec	Static FC analysis showed that high interhemispheric connectivity across homologous regions predicts performance on the SDMT and PASAT. Dynamic FC analysis showed a negative correlation between interhemispheric connectivity changes and PASAT scores.	↓↑
Pasqua et al 2020 (Pasqua et al., 2020)	RRMS (91) SPMS (28) HC (42)	Cross-sectional SCA	PASAT 2 and 3 sec	FC of cerebellar ROIs correlated positively with PASAT score.	↓
Riccitelli et al 2020 (Riccitelli et al., 2020)	BMS (37) HC (50)	Cross-sectional ICA	BRB-N	No significant correlations between cognitive impairment index and FC abnormalities.	-

Has Simelek et al 2020 (Has Silemek et al., 2020)	RRMS (33) HC (29)	Cross-sectional GT	PASAT3, SDMT, Verbal Learning and Memory task, Block Tapping Task of the WMS, BVMT, Regensburger Word Fluency Task	No relationship between global functional graph metrics and cognitive tests. Some significant associations between cognitive tests and nodal functional graph measures, predominantly negative.	↑
Soares et al 2020 (Soares et al., 2020)	RRMS (21) HC (17)	Cross-sectional ICA, GT	MACFIMS, PASAT	Whole brain connectome FC correlated positively with information processing efficiency composite. For specific RSNs, there were positive correlations between information processing efficiency and FC of the DMN, precuneus, sensorimotor and ventral attentional networks.	↓
Welton et al 2020 (Welton et al., 2020)	RRMS (22) SPMS (15) HC (23)	Longitudinal, GT	PASAT 3 sec, SDMT, attention network test	FC graph theory network metrics were significantly predictive for the PASAT3 and SDMT, but not for the attention network test. Worse performance on the PASAT was predicted by increased clustering and modulatory, longer average path lengths and less small worldness. Worse performance on the SDMT was predicted by less small worldness, lower global efficiency and longer average path	-

				lengths.	

↑arrow up indicates that high FC is associated with worse cognition, ↓ arrow down indicates that low FC is associated with worse cognition, - dash indicates negative result or that the study did not assess directionality in the relationship between FC and cognition

Abbreviations: FC=functional connectivity, ICA=independent component analysis, SCA=Seed based connectivity analysis, GT=graph theory, MS=multiple sclerosis, RRMS=relapsing-remitting multiple sclerosis, PPMS=primary progressive multiple sclerosis, SPMS=secondary progressive multiple sclerosis, CIS=clinically isolated syndrome, BMS=benign multiple sclerosis, HC=healthy controls, ACC=anterior cingulate cortex, PCC=posterior cingulate cortex, DGM=deep grey matter, DMN=default mode network, FPN=frontoparietal network, LFPN=left FPN, RFPN=right FPN, SN=salience network, ATT=attentional network, DAN=dorsal attention network

Abbreviations and references of cognitive measures: Attention network test (Fan et al., 2002); Auditory verbal learning test (Zhao et al., 2012); BICAMS=Brief International Cognitive Assessment for MS (Langdon et al., 2012); BRB-N=Brief Repeatable Battery of Neuropsychological tests (Rao, 1990); BVMT-R=Brief Visuospatial Memory Test-Revised (Benedict, 1997); Computerised speed cognitive test (Ruet et al., 2013c); COWAT=Controlled Oral Words Association Test (Benton et al., 1983a); CST=Concept Shifting Test (Van der Elst et al., 2006a); CVLT=California Verbal Learning Test (Delis et al., 2000); Digitspan (Kaufman and Lichtenberger, 2005); HVLT-R=Hopkins Verbal Learning Test-revised (Benedict et al., 1998); JoLO=Judgement of Line Orientation (Benton et al., 1983b); Letter comparison and pattern comparison tasks (Salthouse, 1995); LLT=Location Learning Test (Bucks and Willison, 1997); LDST=letter digit substitution test (van der Elst et al., 2006b); MACFIMS=Minimal Assessment of Cognitive Function in Multiple Sclerosis (Benedict et al., 2002); Mattis Dementia Rating Scale (Hugonot-Diener et al., 2008); MCT=Memory Comparison Test; Mini Mental State Examination (Folstein et al., 1975); North American Reading Test (Blair and Spreen,

1989); PASAT=Paced Auditory Serial Additions Test (Fischer et al., 1999); Raven's Coloured Progressive Matrices (Basso et al., 1987); RCFT=Rey-Osterrieth Complex Figure Test (Caffarra et al., 2002); Regensburger Word Fluency Task (Aschenbrenner et al., 2000); Salthouse Perceptual Comparison Test (Salthouse et al., 1991); SDMT=symbol digit modalities test (Benedict et al., 2017); Semantic and Phonemic Fluency Tests (Lezak, 2004); SPART = 10/36 Spatial Recall Test (Rao, 1990); SRT=Selective Reminding Test (Rao, 1990); SST=Short Story Test; Stroop=Stroop Interference Test (Stroop, 1935); TAP=Test of Attentional Performance (Zimmermann and Fimm, 2002); Test of Premorbid Function (Wechsler, 2011); The computerised test of information processing (Tombaugh and Rees, 2008); The short test of mental status (Kokmen et al., 1991); TMT-trail making test (Tombaugh, 2004); Verbal Intelligence Test Mehrfachwortschatztest-B (Lehrl, 1991); VFT=Verbal Fluency Test (Patterson, 2011); Verbal Learning and Memory task (Helmstaedter and Durwen, 1990); WCST=Wisconsin card sorting test (Robinson et al., 1980); WAIS=Wechsler Adult Intelligence Scale (Kaufman and Lichtenberger, 2005); WMS=Wechsler Memory Scale (Wechsler, 1997); WLG=word list generation (Rao, 1990); WLT=word learning test; WTAR=Wechsler Test of Adult Reading (Holdnack, 2001)

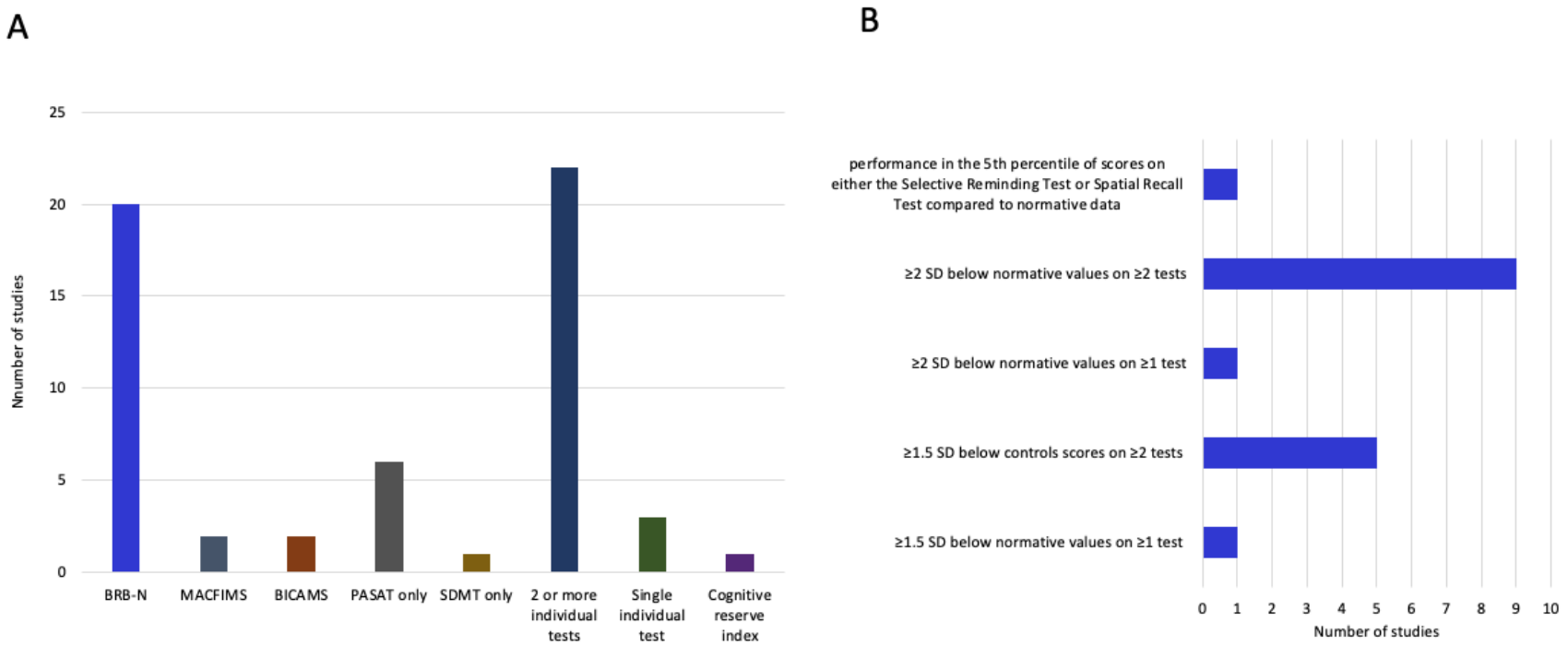

Figure 3.3: Neuropsychological tests used in the reviewed studies
Figure A shows the number of each neuropsychological test used in the 57 reviewed studies. The tally has been simplified for visualisation purposes. When the BRB-N, MACFIMS or BICAMS have been used in combination with other tests, only the battery has been counted in this figure. The PASAT and SDMT have only been counted when they have been used without other tests. Full details of tests used in each study are provided in Table 1. Figure B shows the definitions for cognitive impairment in the studies that used the BRB-N, and the number of studies that used that definition. Note that four of the five studies that used the definition of≥1.5 SD below controls scores on≥2 tests used it to define a mildly cognitively impaired group.

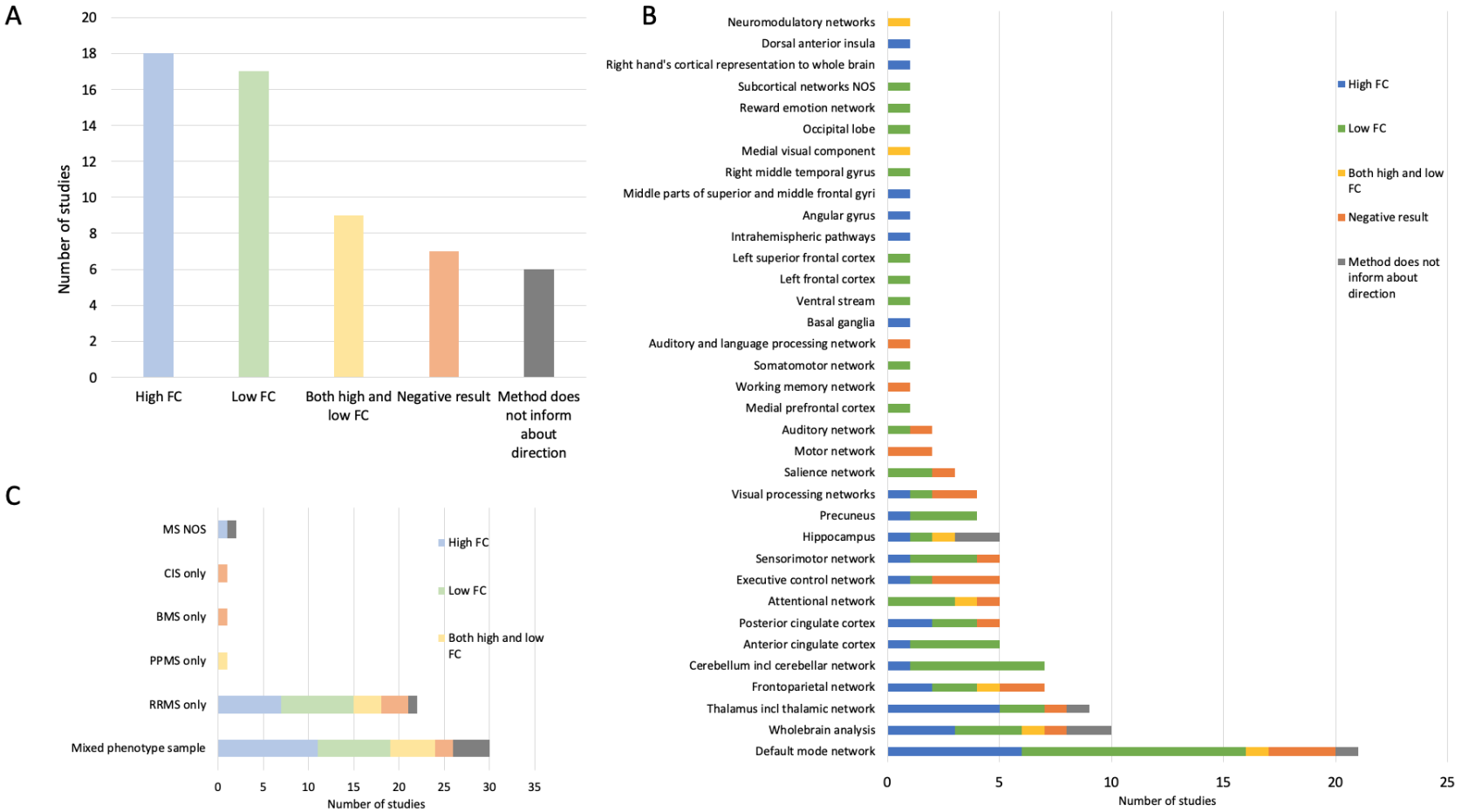

Figure 3.4: Number of studies reporting an association between poor cognitive test performance and high or low functional connectivity
A) Eighteen studies reported worse cognition to be associated with high functional connectivity (FC), seventeen with low FC and nine studies with both high and low FC. Seven studies did not find a link between FC abnormalities and cognitive function. Six studies used a method that does not provide information about directional changes in FC in relation to cognitive test performance. B) Number of studies showing directional FC findings associated with worse cognition, sorted by the brain region or network investigated. Studies which used different sub-networks of the same network have been grouped together, for example, the left, right, doral and ventral frontoparietal networks have been grouped into one 'Frontoparietal network' label. Otherwise labels have been kept as consistent with the wording used in original studies as possible. The label 'Neuromodulatory networks' refers to the serotonergic, noradrenergic, cholinergic and dopaminergic networks. The label 'Interhemispheric pathways' refers to right olfactory cortex to right amygdala, right middle temporal pole to right inferior frontal gyrus, and left parahippocampalgyrus to left inferior frontal gyrus. References are provided in Supplementary Table 2. C) Number of studies showing directional FC findings associated with worse cognition, sorted by the MS phenotype in the sample of each study. Abbreviations: BMS = Benign Multiple Sclerosis, CIS = Clinically Isolated Syndrome, FC = functional connectivity, NOS = Not Otherwise Specified, PPMS = Primary Progressive Multiple Sclerosis, RRMS = Relapsing Remitting Multiple Sclerosis.

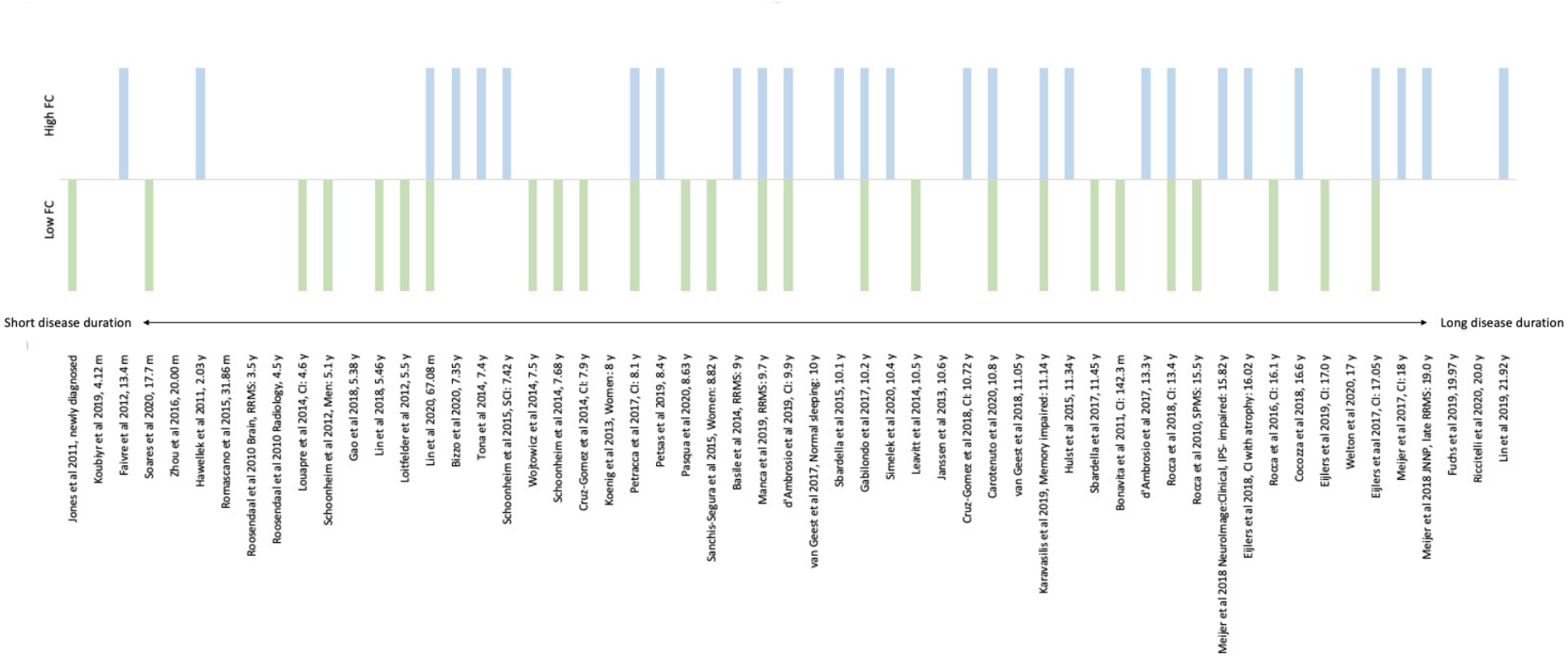

Figure 3.5: Direction of functional connectivity abnormalities sorted by average disease duration
Direction of functional connectivity abnormalities associated with worse cognition, sorted by average disease duration of the sample in each study. Disease durations reported in months in the original study have been converted to years by dividing by 12. Because several studies used samples of mixed phenotypes and different disease durations, the following decisions were taken when ordering studies by the disease duration: 1) studies were ordered by the overall disease duration of the sample, when given; 2) studies were ordered by the disease duration of the cognitively impaired group; 3) if there were two cognitively impaired groups, studies were ordered by the disease duration of the more impaired group, or the cognitively impaired group with atrophy, in one case; 4) when the disease duration was only reported for each MS phenotype, or sex, studies were ordered by the disease duration of the larger sample; 5) for a study which had equal numbers of males and females, the study was ordered by the sex with the longer disease duration; 6) for one study that used a subset of MS patients that were matched to HC, the study was ordered by the disease duration of the matched subset. References are provided in Supplementary Table 3.

3.4 Discussion

In this systematic review we examined the consistency and direction of findings of studies investigating associations between rs-fMRI FC measures and cognition in MS. Overall, the studies reviewed support the notion of FC alterations associated with cognitive dysfunction in MS (Filippi and Rocca, 2013). Although most changes were related to cognitive dysfunction, the direction of FC changes varied considerably between studies and was not clearly linked to any methodological factors. There was substantial heterogeneity in clinical and rs-fMRI methodology, as has previously been noted in non-imaging cognition studies in MS (Benedict et al., 2020; Sumowski et al., 2018). We therefore consider ways in which the field can reflect on what has been learned to date and improve future study designs to more clearly understand the mechanisms and consequences of changes in rs-fMRI FC. Specifically, we propose that future studies should consider the following points which are the source of much heterogeneity identified in this review: 1) the possibility of different network degeneration patterns in different MS clinical and cognitive phenotypes; 2) the role of disease duration and aging processes; 3) the definition and measurement of cognitive impairment; 4) the spatial topography of brain regions of resting state networks of interest; 5) the investigation of the mechanisms of FC abnormalities. A discussion of each follows below.

3.4.1 Models of network changes in MS

To consider how FC should relate to cognitive function in MS, and what results to expect from rs-fMRI studies, a model of the relationship is useful. The most commonly used model for understanding FC changes in MS is the ‘network collapse’ model, which postulates three main stages (Schoonheim et al., 2015b). In the first, early stage network efficiency remains normal, at this point structural damage can be compensated by increases in local activation. This predicts early increases in FC, reflecting these compensatory processes. The second stage is where structural damage accrues to a critical point, at which compensatory processes become less effective. Finally, in the third stage structural damage exceeds the critical point with associated ‘network collapse’, and concomitant decreases in FC. Computational modelling of empirical data on FC in MS supports this model (Tewarie et al., 2018). Similarly, longitudinal studies demonstrate a reorganisation of structural and functional networks in early stages of MS (i.e. CIS) despite intact cognitive performance, suggesting compensatory processes are at work (Koubiyr et al., 2019). Cross-sectional task-related fMRI studies also indicate

increasing deviation from healthy control patterns of brain activation during cognitive tasks, consistent with functional reorganization, as patients progress from CIS to RRMS to secondary progressive MS (Loitfelder et al., 2011). Together, these theories predict early adaptive reorganization of functional networks, followed by a failure of effective network organization in MS over time (see also Chard et al., 2021).

3.4.2 Role of clinical phenotype, disease duration and age

In our review, when ordering studies by the average disease duration of the sample, we did not observe a trend in the direction of FC findings from early to advanced MS, as predicted by the network collapse model and as observed in some studies (e.g. Castellazzi et al., 2018). We therefore consider whether the lack of fit to the model relates to the particular samples or methods of analysis employed. Many of the studies included in this review used samples of mixed clinical phenotypes. MS phenotype has previously been reported to influence resting network FC alterations, so the inclusion of mixed MS samples could contribute to the lack of consistency in findings. However, in our review only two studies assessed the relationship between FC, phenotype and cognition, and these found both abnormally increased (Meijer et al., 2018a) and abnormally decreased (Rocca et al., 2018) FC in patients with progressive MS. This suggests that even in specific MS subgroups, there remains considerable variability in the direction of findings. More evidence is needed in order to determine whether FC changes vary between phenotypes, and whether any model of network changes has different explanatory power for the different phenotypes. A further important consideration is the effect of disease duration and how it may mediate the relationship between FC, phenotype and cognition. Longer disease duration in RRMS is associated with FC changes in attentional, executive, and default mode networks (Castellazzi et al., 2018). This suggests that disease duration may have an important influence on FC changes associated with cognitive impairment, possibly due to increased structural damage with longer disease duration. While we did not find such a trend in our review, our analysis of disease duration was confounded by samples of mixed phenotypes, the study of many different spatial regions of the brain, and the vast number of definitions of cognition. Therefore, the effect of disease duration should be formally tested in studies in which other variables, such as neuropsychological tests and spatial regions, are kept constant. Those studying patients with longer disease duration (such as those with SPMS) will also have to account for age-related atrophy in these samples (Azevedo et al., 2019), which will be exacerbated when studying those patients with relapsing as well as progressive subtypes of MS.

3.4.3 Cognitive tests and definition of cognitive impairment

We also considered whether the direction of FC change relates to definitions of cognitive impairment and choice of FC analysis. Studies of cognition in MS use a vast array of definitions of cognitive impairment (Benedict et al., 2020; Fischer et al., 2014; Sumowski et al., 2018), as reflected in this review. For example, of the studies using the BRB-N to assess cognitive function, most use a more conservative definition of cognitive impairment of at least 2 SDs below controls on 2 or more tests (Bonavita et al., 2011; d'Ambrosio et al., 2017; d'Ambrosio et al., 2020; Eijlers et al., 2017; Eijlers et al., 2019; Meijer et al., 2018a; Meijer et al., 2017; Rocca et al., 2018; Schoonheim et al., 2015a), but other, less conservative definitions are used too (Cruz-Gómez et al., 2018, 2014; Eijlers et al., 2018). The definition of cognitive impairment has been shown to have effects on underlying FC alterations of MS CI by the classification used (Doshi et al., 2019). A few studies have compared different thresholds of cognitive impairment and found the greatest FC abnormalities in those participants meeting the more conservative thresholds (i.e., more than 2 standard deviations from controls on 2 or more tests). In contrast, less clear FC abnormalities were observed in samples performing between 1.5 and 2 SDs below controls on 2 tests ("mild cognitive impairment") (Doshi et al., 2019; Eijlers et al., 2017; Meijer et al., 2017; Schoonheim et al., 2015a). This demonstrates the possible effect of the definition of cognitive impairment on FC findings and the arbitrary nature of these thresholds. Such findings highlight the importance of using a consistent measure of cognitive dysfunction and definition of impairment across studies. As a further challenge there is no consistency in use of specific cognitive tests or batteries for defining cognitive dysfunction in MS, with many studies using impairments on multiple separate tests to assess global cognitive function. There are documented phenotypic differences in impairments by test and domain (Chan et al., 2017; Connick et al., 2013; Johnen et al., 2017; Ruet et al., 2013a), yet very few studies have looked at network alternations associated with deficits in specific cognitive domains, such as information processing speed or memory, and those that have used a range of cognitive tests to probe the same domain, further complicating comparisons. The use of consistent measures of cognition and definitions of cognitive impairment, and possibly conducting sub-group analyses of different cognitive domains, should therefore be an aim for future studies.

3.4.4 Spatial topography

Separately, we found scant evidence to support a consistent direction of FC change in cognitively impaired patients when using model-based (e.g., seed) or data driven (e.g., ICA) approaches, or when considering specific resting state networks. Indeed, the default mode network, the most commonly studied RSN across the literature, showed both increases and decreases in cognitively impaired MS patients. One explanation of increases in FC is that processing moves from local networks to hub regions when the former accumulate structural damage (Meijer et al., 2017; Stam, 2014; Tahedl et al., 2018), but this explanation fails to account for the findings in this review. Attempting to understand these findings is complex. The role of disease stage in the samples studied could influence the FC directions reported, in line with the network collapse model. Another consideration is the spatial location of the regions investigated. It must be remembered that the default mode network consists of several key 'hub' regions, which are heavily interconnected and involved in several additional networks. For example, the anterior cingulate cortex is also a key hub in the salience network. Moreover, the regions making up a network can vary between studies, often depending on the analysis method used. In a seed-based connectivity study the extent of the network of interest will depend on how and where the seed is defined. The idea that different networks or even subregions of a network hub have different patterns of connectivity is evidenced by the thalamus, a network hub which has shown both hypo- and hyperconnectivity in MS, depending on the thalamic nucleus and pathways investigated (Lin et al., 2019). Despite this, the topography of a network might not be the full explanation of the inconsistent results observed. A meta-analysis of mild cognitive impairment prodromal to Alzheimer's disease did not find consistent FC abnormalities even when using a voxel-wise analysis to assess the same spatial regions, suggesting that directional inconsistencies of FC findings cannot be fully explained by the spatial extent of the region(s) studied (Eyler et al., 2019). Nevertheless, to rule out the potential influence of topography, and enable comparisons between studies, care should be taken to define a specific region consistently with previous research.

3.4.5 Mechanisms of FC changes

There also needs to be a greater understanding of the mechanisms through which FC changes in MS. The 'network collapse' model suggests that network efficiency reduction is a function of accumulation of structural damage. In support of this, work focusing on structural connectivity in MS has found consistent evidence for structural network

alterations associated with cognitive dysfunction (Llufriu et al., 2019, 2017; Solana et al., 2018). However, these studies have considered white matter in isolation, so conclusions about the effect of anatomical network changes including grey matter on functional connectivity cannot be drawn. In contrast, multimodal MRI studies of diffusion-weighted MRI (DWI) and rs-fMRI can assess the relationship between changes in structural and functional connectivity. Those that have been conducted support the influence of alterations in white matter linked to FC abnormalities in MS, and fit the predictions of the 'network collapse' model (Enzinger et al., 2016; Lowe et al., 2008; Patel et al., 2018; Tewarie et al., 2018, 2014). Future multimodal studies using DWI and rs-fMRI can test the predictions of the 'network collapse' model further and to develop this or new models as needed to better characterise progression and the influence of pathology in MS brains, in order to develop clinically useful disease markers. In addition, there is evidence of physiological abnormalities in MS that are associated with cognitive dysfunction, such as cerebral hypoperfusion and sodium accumulation in the grey and white matter (Lapointe et al., 2018; Maarouf et al., 2017; Paling et al., 2013), and additional proton spectroscopic changes (Solanky et al., 2020). Considering how these are related to network changes can help us understand the mechanisms of network abnormalities and aid in the search for a biomarker of cognitive impairment.

3.4.6 FC as a biomarker of cognitive impairment in MS?

This systematic review provides a call to arms for the need to standardize the study of cognitive impairment in MS, but also the use of specific rs-fMRI methodology and interpretations of results. Eleven years ago Fox and Greicius (2010) identified inconsistent results of FC changes across rs-fMRI studies as a barrier to the clinical applicability of this modality, and suggested a set of guidelines for rs-fMRI studies of clinical populations (Fox and Greicius, 2010). Despite this, heterogeneity in study methodology seems to be a challenge across neurodegenerative diseases investigated by rs-fMRI, and the rs-fMRI derived FC measure is not yet suitable as a biomarker of disease (reviewed by Hohenfeld et al., 2018). Even in Alzheimer's disease, where there is evidence of consistent hypoconnectivity compared to controls, there is a problem of inconsistent directional results in the prodromal stages (i.e. mild cognitive impairment) of this disease (Badhwar et al., 2017; Eyler et al., 2019). A recent systematic review and meta-analysis found inconsistent results across 56 studies in mild cognitive impairment and concluded that while FC changes may be a marker of Alzheimer's disease, at present the evidence for FC to be a biomarker of the risk of developing Alzheimer's disease is limited (Eyler et al., 2019). In this review we have shown that, similarly, the FC measure

is not yet a suitable biomarker for cognitive impairment in MS. Unlike Alzheimer's disease, the use of rs-fMRI in MS has not been the subject of many systematic reviews, and so we do not at present know whether FC results become more consistent at a certain stage of the disease. In this review we found considerable variability in the study of cognitive impairment in MS by rs-fMRI, both in study methods and findings, which pose a challenge for the interpretation of results.

3.4.7 Standardisation of FC studies of cognition in MS and future directions

The FC measure shows promise; most studies suggest that FC alterations are a key pathological feature. Therefore, we argue that standardisation of study methods and more model-driven research would lay a clearer path towards understanding directional FC changes, and thereby clinical utility of the FC metric and the potential use as a biomarker of MS disease state. First, clinical studies using the rs-fMRI method should ensure that the guidelines suggested by Fox and Greicius (2010) are followed: "(1) A priori hypotheses regarding a region or network with abnormal [rs-fMRI FC] and clear criteria for selecting this region or network; (2) A priori hypothesis and demonstration of a region or network with normal [rs-fMRI FC] to serve as a control; (3) Correlation with clinical variables whenever possible; (4) Stringent correction for multiple comparisons; (5) An analysis of movement in patients and control subjects; (6) An analysis of the differential impact of pre-processing in patients and control subjects; (7) A discussion of how current findings relate to prior [rs-fMRI FC] findings." In the studies considered in this systematic review, point 3 is necessarily met. Points 4, 5 and 6 are typically met. Points 1, 2 and 7 are occasionally met.

Going forward, research using FC as a marker of cognitive impairment in MS should consider the following to meet points 1, 2 and 7: 1) studying different clinical and cognitive phenotypes of a disease separately to identify phenotype specific influences; 2) controlling for age and disease duration, where this is known to have an influence on the clinical symptom of interest; 3) using well-established and validated measures of the symptom of interest for the disease being investigated; 4) defining regions of interest consistently with previous research; 5) conducting model-led research to understand the underlying pathophysiological basis of any alterations in FC, for example in MS this might involve multimodal diffusion MRI and rs-fMRI studies to test the network collapse model and its prediction of FC being driven by structural changes.

The studies so far have been useful to establish that effects do exist and that there is an association with cognitive impairment, but what is needed now is the equivalent of a well powered multi-site phase 3 trial to establish that the effect is robust. This will help to determine whether functional connectivity measures can indeed be used as biomarkers of cognitively relevant network degeneration in MS.

3.4.8 Limitations

This review is the first to systematically summarise the rs-fMRI functional connectivity literature on cognitive impairment in MS. However, there are some limitations to consider. First, rs-fMRI is not the only imaging modality for studying functional connectivity. While they were outside the scope of this review, electroencephalography and magnetoencephalography studies may offer additional insights into FC changes associated with cognitive impairment in MS. Similarly, there are other network measures that can be derived from rs-fMRI in addition to FC, such as dynamic FC and graph theory measures. At present the number of studies reporting these measures is small and so we did not consider them separately, but rather grouped them with the FC measure for the purposes of the review. Nevertheless, these metrics provide somewhat different information to the FC metric, which has not been captured in detail in this review. In addition, we compared results from studies which looked at the same networks or regions of interest, but using different analysis methods, and vice versa. It could be argued that differences in methods and spatial topography of networks limit the information that can be gained from this approach, however, grouping studies which shared similarities on several methodological variables, such as networks studied and analysis method, would have created very small groups from which it would have been difficult to infer anything with confidence. Previous systematic reviews of rs-fMRI FC changes in mild cognitive impairment find inconsistent directions of altered FC in patient groups even when using a voxel-wise analysis (Eyler et al., 2019). This suggests that the findings of inconsistency in FC results are not entirely due to variation in networks studied or spatial topography. Finally, we did not carry out a formal statistical meta-analysis of the studies in this review. Instead, due to low numbers of homogeneous studies we were limited to tallying the number of studies with a specific feature. As studies start to become more consistent in their use of methods it will become easier to determine across the field whether the hypotheses including disease-specific effects, such as the ‘network collapse’ model, can suitably explain the patterns of associations that are observed.

3.4.9 Conclusion

In conclusion, this systematic review shows that cognitive impairment in MS is associated with both high and low FC, indicating that any network change seems related to poorer functioning. This is an important finding that shows that rs-fMRI FC is sensitive to cognitively relevant brain changes. However, because of the inconsistencies in the direction of FC results this measure needs further exploration in consistently designed studies in order to become a suitable biomarker of cognitive impairment in MS. To better understand the relationship between worsened cognitive function and FC abnormalities, including directional FC changes, there must be standardisation in the field of the definition and measurement of CI, rs-fMRI methodology, and correction and allowances for MS phenotype, and non-MS related pathology from ageing. We have outlined five recommendations to this effect for future research, based on the sources of heterogeneity we have identified in literature, and welcome a discussion of these with our colleagues in this field.

3.5 Acknowledgements

This article has been published on preprint server medRxiv, DOI 10.1101/2021.03.18.21253878, and is available on the following weblink: https://www.medrxiv.org/content/10.1101/2021.03.18.21253878v1

3.6 Author Contributions

AD and DJ contributed to the conception and design of the study. The data was acquired and analysed by AD, DJ and RS. All authors contributed to drafting and reviewing the text and figures.

3.7 Declarations of Interest

The authors report no potential competing interests relating to this work.

3.8 Funding Sources

This work was funded by grants held by DJ and AD. DJ was supported by a Medical Research Council Doctoral Training Partnership grant (MR/N013751/11). AD was

supported by the Multiple Sclerosis Progressive Multi-arm Randomisation Trial, an investigator-led project sponsored by University College London.

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3.10 Supplementary material

Table 3.2: Search strategies for literature searches in Embase, Medline and PsychINFO. Searches were conducted on the 31st October 2019 and updated on 22nd October 2020.

Database: Embase <1974 to 2019 Week 43> Search Strategy:	Database: Ovid MEDLINE(R) <1946 to October Week 4 2019> Search Strategy:	Database: PsycINFO <1806 to October Week 3 2019> Search Strategy:
1 multiple sclerosis/ (122811) 2 functional magnetic resonance imaging/ (74477) 3 functional neuroimaging/ (11826) 4 functional connectivity/ (12717) 5 resting state network/ (5850) 6 rsfmri.mp. (703) 7 rs fmri.mp. (2131) 8 rs-fmri.mp. (2131) 9 resting state fmri.mp. (6119) 10. resting state functional magnetic resonance imaging.mp. (3664) 11 network.mp. (488049) 12 connectivity.mp. (69254) 13 cognition/ (227236) 14 cognitive defect/ (157776) 15 cogniti*.mp. (649243) 16 symptom*.mp. (1750241) 17 impairment*.mp. (584165) 18 dysfunction*.mp. (784129) 19 decline.mp. (264441) 20 defect*.mp. (830373) 21 deficit*.mp. (347374) 22 disabilit*.mp. (317502)	1 Multiple Sclerosis/ (50628) 2 Magnetic Resonance Imaging/ (385453) 3 Functional Neuroimaging/ (3309) 4 rsfmri.mp. (225) 5 rs fmri.mp. (801) 6 rs-fmri.mp. (801) 7 resting state fmri.mp. (2674) 8 resting state functional magnetic resonance imaging.mp. (2035) 9 network.mp. (256415) 10 connectivity.mp. (39575) 11 Cognition/ (92646) 12 Cognition Disorders/ (63435) 13 Cognitive Dysfunction/ (13910) 14 cogniti*.mp. (355865) 15 symptom*.mp. (954357) 16 impairment*.mp. (285360) 17 dysfunction*.mp. (413051) 18 decline.mp. (169699) 19 defect*.mp. (474313) 20 deficit*.mp. (215585) 21 disabilit*.mp. (231078) 22 problem*.mp. (889159)	1 exp Multiple Sclerosis/ (12466) 2 exp Functional Magnetic Resonance Imaging/ (21226) 3 brain connectivity/ (4435) 4 rsfmri.mp. (188) 5 rs fmri.mp. (614) 6 rs-fmri.mp. (614) 7 resting state fmri.mp. (2198) 8 resting state functional magnetic resonance imaging.mp. (1687) 9 network.mp. (91298) 10 connectivity.mp. (24043) 11 exp Cognition/ (36363) 12 exp Cognitive Impairment/ (35048) 13 cogniti*.mp. (576040) 14 symptom*.mp. (352302) 15 impairment*.mp. (146444) 16 dysfunction*.mp. (82048) 17 decline.mp. (47882) 18 defect*.mp. (26404) 19 deficit*.mp. (140889) 20 disabilit*.mp. (144151) 21 problem*.mp. (564617) 22 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 (118304)

23 problem*.mp. (1262696) 24 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 (575026) 25 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 (5023274) 26 15 and 25 (404197) 27 13 or 14 or 26 (516348) 28 1 and 24 and 27 (1002) Limits added to search conducted 22nd October 2020: 29 limit 28 to dd=20191101-20201022 (61) 30 limit 28 to rd=20191101-20201022 (83) 31 29 or 30 (144)	23 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 (647967) 24 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 (3126890) 25 14 and 24 (196009) 26 11 or 12 or 13 or 25 (263948) 27 1 and 23 and 26 (639) Limits added to search conducted 22nd October 2020: 28 limit 27 to dt=20191101-20201022 (11) 29 limit 27 to rd=20191101-20201022 (123) 30 28 or 29 (123)	23 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 (1204462) 24 13 and 23 (234956) 25 11 or 12 or 24 (259865) 26 1 and 22 and 25 (132) Limits added to search conducted 22nd October 2020: 27 limit 26 to ch=20191101-20201022 (11) 28 limit 26 to up=20191101-20201022 (11) 29 27 or 28 (21)

Table 3.3: Study findings sorted by the brain region or network investigated

Study*/ Brain region or network	Wholebrain analysis, regional changes not specified	mPFC	ACC	PCC	Precuneus	DMN	FPN incl left, right, dorsal, ventral	Attentional network incl dorsal, ventral, right, left	SN	EC	Working memory network	Motor network	Sensorimotor	Somatomotor network	Visual processing networks, incl medial and lateral	Auditory and language processing network	Auditory network	Hippocampus	Thalamus incl thalamic network	Basal ganglia	Cerebellum	Cerebellar network	Other	Other, additional (if other pattern reported)
Rocca 2010		↓	↓			↓																		
Roosendaal et al 2010 Brain						-	-	-		-			-		-	-								
Roosendaal et al 2010 Radiology																								
Bonavita et al 2011 MSJ			↓	↓		↓																		
Hawellek et al 2011 PNAS						↑																		
Jones et al 2011 Arch Neurol				↓	↓	↓																		
Faivre et al 2012 MSJ						↑	↑																	
Loitfelder et al 2012 PLoS ONE			↓																					
Schoonheim et al 2012 MSJ	↓																							
Janssen et al 2013 Neurpsychologia						-	-			-		-			-		-					-		
Koenig et al 2013 American Journal of Neuroradiology				-																				
Basile et al 2014 MSJ			↑			↑																		

Cruz-Gomez et al 2014 MSJ						↓	↓		↓														
Leavitt et al 2014 Journal of the International Neuropsychological Society						↓																	
Louapre et al 2014 HBM						↓		↓				-											
Schoonheim et al 2014 MSJ																						Ventral stream ↓	
Tona et al 2014 Radiology																		↑					
Wojtowicz et al 2014 MSJ						↓																	
Hulst et al 2015 MSJ																	↑						
Romascano et al 2015 HBM																				-			
Sbardella et al 2015 MSJ										↑				↑									
Schoonheim et al 2015 Neurology																		↑					
Rocca et al 2016 Brain Struct Func			↓		↓													↓		↓		Left frontal cortex, left superior frontal gyrus ↓	
Sanchis-Segura et al 2015 Neuroscience Letters																						Three intra-hemispheric pathways: right olfactory cortex to right amygdala, right middle temporal pole to right inferior frontal gyrus, left parahippocampal	

																							gyrus to left inferior frontal gyrus ↑	
Zhou et al 2016 Frontiers in Human Neuroscience																		-						
d'Ambrosio et al 2017 HBM																		↑						
Eijlers et al 2017 Neurology				↑	↑	↑																	Angular gyrus, middle parts of superior and middle frontal gyri ↑	Right middle temporal gyrus ↓
Gabilondo et al 2017 MSJ																							Medial visual component ↓↑	
Meijer et al 2017 Neurology						↑	↑																	
Petracca et al 2017 Scientific Reports								↓ ↑		↓														
Sbardella et al 2017 MSJ																				↓				
van Geest et al 2017 Journal of Neurology																								
Cocozza et al 2018 Journal of Neurology																				↑				
Cruz-Gomez et al 2018 Neuroreport																			↑					
Eijlers et al 2018 Radiology				↑																			Occipital lobe ↓	

Gao et al 2018 Hippocampus																		-						
Lin et al 2018 HBM	↓																							
Meijer et al 2018 JNNP	↑																							
Meijer et al 2018 NeuroImage : Clinical	↑																							
Rocca et al 2018 MSJ						↓		↓					↓						↑			↓	Reward emotion network ↓	
Van Geest et al 2018 NeuroImage : Clinical																								
d'Ambrosio et al 2019 MSJ						↓ ↑							↓	↓	↓		↓						Subcortical networks NOS ↓	
Eijlers et al 2019 Radiology						↓	↓												↓					
Fuchs et al 2019 Human Brain Mapping																								
Karavasilis et al 2019 Brain Imaging and Behaviour																		↓ ↑						
Koubiyr et al 2019 Brain	-																							
Lin et al 2019 MSJ																			↑					
Manca et al 2019 Postgraduate Medicine						↑	↓ ↑		↓				↑											
Petsas et al 2019 Frontiers in Neurology																							Right hand's cortical representation to whole brain ↑	
Bizzo et al 2020 Journal of Neuroimaging																							Dorsal anterior insula ↑	

Carotenuto et al 2020 Journal of Neurology																							The serotonergic, the noradrenergic, the cholinergic, and the dopaminergic networks ↓↑	
Lin et al 2020 Frontiers in Neurology	↓ ↑																							
Pasqua et al 2020 MSJ																					↓			
Riccitelli et al 2020 Journal of Neurology						-			-	-	-													
Simelek et al 2020 NeuroImage : Clinical	↑																							
Soares et al 2020 Brain Imaging and Behavior	↓				↓	↓		↓					↓											
Welton et al 2020 Brain Connectivity																								

* See main manuscript for full citations of studies.

Table presents the directional FC result for each region investigated, either as a priori defined areas of interest, or as patterns emerging from a data-driven analysis.Key: High FC is denoted by ↑ and a blue colour cell; low FC by ↓ and a green colour cell; studies demonstrating both high and low FC associated with worse cognition are denoted by ↑↓ and a yellow colour cell; studies which did not find support for a relationship between FC alterations and worse cognition are denoted by – and a pink colour cell; "something else," denoted by a grey colour cell, refers to studies which used a methodology that does not fit into the groupings presented in this table . Abbreviations: ACC = Anterior Cingulate Cortex, DMN = Default Mode Network, EC = Executive Control network, mPFC = medial Prefrontal Cortex, NOS = Not Otherwise Specified, PCC = Posterior Cingulate Cortex, SN = Salience Network

Table 3.4: Study findings sorted by the average disease duration of the sample

Study*	**Average disease duration†**	**Direction of FC result‡ associated with worse cognitive function**
Jones et al 2011 Arch Neurol	Not specified, patient newly diagnosed	↓
Koubiyr et al 2019 Brain	4.12 m	-
Faivre et al 2012 MSJ	13.4 m	↑
Soares et al 2020 Brain Imaging and Behavior	17.7 m	↓
Zhou et al 2016 Frontiers in Human Neuroscience	20.00 m	-
Hawellek et al 2011 PNAS	2.03 y	↑
Romascano et al 2015 HBM	31.86 m	something else
Roosendaal et al 2010 Brain	CIS: 1.4 y, RRMS: 3.5 y	-
Roosendaal et al 2010 Radiology	4.5 y	something else
Louapre et al 2014 HBM	CI: 4.6 y, CP: 4.5 y	↓
Schoonheim et al 2012 MSJ	Men: 5.1 y, Women: 4.9 y	↓
Gao et al 2018 Hippocampus	5.38 y	-
Lin et al 2018 HBM	9.85 y in overall MS group, 5.46 y in those matched to HC	↓
Loitfelder et al 2012 PLoS ONE	5.5 y	↓
Cruz-Gomez et al 2014 MSJ	CP 5.5 y , CI 7.9 y	↓
Lin et al 2020 Frontiers in Neurology	67.08 m	↑↓
Bizzo et al 2020 Journal of Neuroimaging	7.35 y	↑
Tona et al 2014 Radiology	7.4 y	↑

Schoonheim et al 2015 Neurology	CP: 7.49 y, MCI: 7.57 y, SCI: 7.42 y	↑
Wojtowicz et al 2014 MSJ	7.5 y	↓
Schoonheim et al 2014 MSJ	7.68 y	↓
Koenig et al 2013 American Journal of Neuroradiology	Men: 6.5 y, Women: 8 y	-
Petracca et al 2017 Scientific Reports	8 y for whole group, 9.7 y for CP, 8.1 y for CI	↑↓
Petsas et al 2019 Frontiers in Neurology	8.4 y	↑
Pasqua et al 2020 MSJ	8.63 y	↓
Sanchis-Segura et al 2015 Neuroscience Letters	Females: 8.82 y, Males: 6.45 y	↓
Basile et al 2014 MSJ	RRMS: 9 y, SPMS: 13 y	↑
Manca et al 2019 Postgraduate Medicine	RRMS: 9.7 y, SPMS: 15.5 y	↑↓
d'Ambrosio et al 2019 MSJ	8.2 y (7.1 y for CP, 9.9 y for CI)	↑↓
van Geest et al 2017 Journal of Neurology	Normal sleeping: 10 y, sleep disturbed: 12 y	something else
Sbardella et al 2015 MSJ	10.1 y	↑
Gabilondo et al 2017 MSJ	10.2 y	↑↓
Simelek et al 2020 NeuroImage: Clinical	10.4 y	↑
Leavitt et al 2014 Journal of the International Neuropsychological Society	10.5 y	↓
Janssen et al 2013 Neurpsychologia	10.6 y	-
Cruz-Gomez et al 2018 Neuroreport	CP: 5.94 y, CI: 10.72 y	↑
Carotenuto et al 2020 Journal of Neurology	10.8 y	↑↓

Van Geest et al 2018 NeuroImage: Clinical	11.05 y	something else
Karavasilis et al 2019 Brain Imaging and Behaviour	9.75 y for memory preserved, 11.14 y for memomry impaired	↑↓
Hulst et al 2015 MSJ	11.34 y	↑
Sbardella et al 2017 MSJ	11.45 y	↓
Bonavita et al 2011 MSJ	CI: 142.3 m, CP: 130.9 m	↓
d'Ambrosio et al 2017 HBM	13.3 y	↑
Rocca et al 2018 MSJ	12.1 y for whole group (11.5 y for CP, 13.4 y for CI)	↑↓
Rocca et al 2010 Neurology	SPMS: 15.5 y, PPMS: 12.7 y	↓
Meijer et al 2018 NeuroImage: Clinical	(symptom duration reported) IPS- impaired; 15.82 y, IPS-preserved; 9.8 y	↑
Eijlers et al 2018 Radiology	(symptom duration reported) CP with no atrophy 13.47 y, CI with no atrophy 13.73 y, CP with atrophy 13.61 y, CI with atrophy 16.02 y	↑
Rocca et al 2016 Brain Struct Func	MS: 13.7 y, CI: 16.1 y, CP: 12.4 y, RRMS: 9.1 y, BMS: 20.2 y, SPMS: 17.1 y	↓
Cocozza et al 2018 Journal of Neurology	16.6 y	↑
Eijlers et al 2019 Radiology	(symptom duration reported) CP: 13.6 y, CI 17.0 y	↓
Welton et al 2020 Brain Connectivity	17 y	something else
Eijlers et al 2017 Neurology	(symptom duration reported) CP: 13.58 y, MCI: 14.15 y,, CI: 17.05 y	↑↓
Meijer et al 2017 Neurology	(symptom duration reported) CP: 10 y, MCI: 13 y, CI: 18 y	↑

Meijer et al 2018 JNNP	(symptom duration reported) early RRMS; 6.6 yyears, late RRMS; 19.0 y, SPMS; 21.8 y	↑
Fuchs et al 2019 Human Brain Mapping	19.97 y	Something else
Riccitelli et al 2020 Journal of Neurology	20.0 y	-
Lin et al 2019 MSJ	21.92 y +/- 10.49	↑

* See main manuscript for full citations of studies.

†Because several studies used samples of mixed phenotypes and different disease durations, the following decisions were taken when ordering studies by the disease duration: 1) studies were ordered by the overall disease duration of the sample, when given; 2) studies were ordered by the disease duration of the cognitively impaired group; 3) if there were two cognitively impaired groups, studies were ordered by the disease duration of the more impaired group, or the cognitively impaired group with atrophy, in one case; 4) when the disease duration was only reported for each MS phenotype, or sex, studies were ordered by the disease duration of the larger sample; 5) for a study which had equal numbers of males and females, the study was ordered by the sex with the longer disease duration; 6) for one study that used a subset of MS patients that were matched to HC, the study was ordered by the disease duration of the matched subset.

‡Key: High FC is denoted by ↑; low FC by ↓; studies demonstrating both high and low FC associated with worse cognition are denoted by ↑↓; studies which did not find support for a relationship between FC alterations and worse cognition are denoted by -; “something else” refers to studies which used a methodology that does not fit into the groupings presented in this table

Chapter 4

Mechanisms of Network Changes in Cognitive Impairment in Multiple Sclerosis

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This is an Accepted Manuscript version of the following article, accepted for publication in *Neurology:* Jandric D, Lipp I, Paling D, Rog D, Castellazzi G, Haroon H, et al. Mechanisms of Network Changes in Cognitive Impairment in Multiple Sclerosis. Neurology 2021; 97: e1886 LP-e1897. DOI: 10.1212/WNL.0000000000012834

Abstract

Background and Objectives Cognitive impairment in multiple sclerosis (MS) is associated with functional connectivity abnormalities. While there have been calls to use functional connectivity measures as biomarkers, there remains to be a full understanding of why they are affected in MS. In this cross-sectional study, we tested the hypothesis that functional network regions may be susceptible to disease-related "wear and tear" and that this can be observable on co-occurring abnormalities on other magnetic resonance metrics. We tested whether functional connectivity abnormalities in cognitively impaired patients with MS co-occur with (1) overlapping, (2) local, or (3) distal changes in anatomic connectivity and cerebral blood flow abnormalities.

Methods Multimodal 3T MRI and assessment with the Brief Repeatable Battery of Neuropsychological tests were performed in 102 patients with relapsing-remitting MS and 27 healthy controls. Patients with MS were classified as cognitively impaired if they scored$\geq$1.5 SDs below the control mean on$\geq$2 tests (n = 55) or as cognitively preserved (n = 47). Functional connectivity was assessed with Independent Component Analysis and dual regression of resting-state fMRI images. Cerebral blood flow maps were estimated, and anatomic connectivity was assessed with anatomic connectivity mapping and fractional anisotropy of diffusion-weighted MRI. Changes in cerebral blood flow and anatomic connectivity were assessed within resting-state networks that showed functional connectivity abnormalities in cognitively impaired patients with MS.

Results Functional connectivity was significantly decreased in the anterior and posterior default mode networks and significantly increased in the right and left frontoparietal networks in cognitively impaired relative to cognitively preserved patients with MS (threshold-free cluster enhancement corrected at p 0.05, 2 sided). Networks showing functional abnormalities showed altered cerebral blood flow and anatomic connectivity locally and distally but not in overlapping locations.

Discussion We provide the first evidence that functional connectivity abnormalities are accompanied by local cerebral blood flow and structural connectivity abnormalities but also demonstrate that these effects do not occur in exactly the same location. Our findings suggest a possibly shared pathologic mechanism for altered functional connectivity in brain networks in MS.

4.1 Introduction

Cognitive impairment affects about half of people with multiple sclerosis (MS) (Sumowski et al., 2018). Although the disease mechanisms responsible are not fully elucidated, resting-state functional MRI (rs-fMRI) studies have shown differences in functional connectivity (FC) between cognitively impaired and non-impaired patients (Benedict, 2020). However, a shortcoming of rs-fMRI, which limits the ability to interpret findings, is the lack of information about pathological mechanisms underlying FC abnormalities.

It has been proposed, in the 'nodal stress' hypothesis, that the high activity of network regions with high connectivity, so called 'hubs' or 'nodes,' makes them susceptible to pathological 'wear-and-tear,' possibly due to high metabolic demands, which could accelerate neurodegeneration, leading to network dysfunction (Buckner et al., 2009, Zhou et al., 2012).

If 'wear-and-tear' changes are responsible for FC abnormalities, we would expect to see abnormalities also on other MR metrics. Network hubs are heavily interconnected within both functional and structural networks, and activity-related damage can be expected to affect anatomical connectivity. In addition, if nodal damage is caused by unmet metabolic demands, this could affect cerebral blood flow (CBF) (Lapointe et al., 2018). By collecting diffusion MRI and CBF data alongside rs-fMRI images we can establish whether FC abnormalities co-occur with white matter and perfusion changes, as would be expected under the 'nodal stress' hypothesis. Such co-occurring abnormalities can point to shared underlying mechanisms and thus inform the direction of future research.

In this study we tested the 'nodal stress' hypothesis in a cohort of relapsing-remitting multiple sclerosis patients (RRMS) to test whether FC abnormalities in cognitively impaired patients co-occur with anatomical connectivity and CBF abnormalities in 1) spatially overlapping regions within networks, 2) the same networks, or 3) distal areas from resting state networks.

4.2 Methods

4.2.1 Participants

One hundred two patients with a diagnosis of RRMS were recruited through the Helen Durham Centre for Neuroinflammation at the University Hospital of Wales, and

twenty-seven healthy controls (HC) from the community. All participants were aged between 18 and 60 years, right-handed and had no contraindications for MR scanning. Patients had no comorbid neurological or psychiatric disease, were relapse-free and had no change to treatment for 3 months prior to the MRI scan. All participants underwent MRI scanning and assessment of clinical and cognitive function in one study session.

4.2.2 Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the NHS South-West Ethics and the Cardiff and Vale University Health Board R&D committees. All participants provided written informed consent to participate in the study.

4.2.3 Clinical and neuropsychological assessment

Clinical functioning was assessed with the Multiple Sclerosis Functional Composite (MSFC), a standardised measure of upper and lower limb and cognitive function (Cutter et al., 1999).

All participants underwent neuropsychological assessment with the Brief Repeatable Battery of Neuropsychological Tests (BRB-N), a validated battery with demonstrated sensitivity to cognitive impairment in MS (Amato et al., 2006). Patients' scores on each test were converted to Z scores based on means and standard deviations from the 27 HCs. Patients who scored$\geq$1.5 standard deviations below the control mean, on$\geq$2 tests were considered cognitively impaired (CI), a medium stringency definition of cognitive impairment (Sepulcre et al., 2006). Remaining patients were considered cognitively preserved (CP). Scores for each of the four cognitive domains of verbal memory, visual memory, attention, information processing and executive function, and verbal fluency, were calculated by averaging the scores for each test in that domain, as described by Sepulcre et al. (2006).

4.2.4 MRI acquisition

All participants underwent MRI examination on a 3T MR scanner (General Electric HDx MRI System, GE Medical Devices, Milwaukee, WI) using an eight channel receive-only head RF coil. A high-resolution 3D T1-weighted (3DT1) sequence was acquired for identification of T1-hypointense MS lesions, segmentation, registration and volumetric measurements [resolution 1x1x1 mm, TE = 3.0 ms, TR = 7.8 ms, matrix = 256x256x172,

FOV = 256 x 256 mm, flip angle = 20°]. A T2/proton-density (PD)-weighted sequence (voxel size = 0.94x0.94x4.5 mm, TE = 9.0/80.6 ms, TR = 3000 ms, FOV = 240 x 240 mm, 36 slices) and a fluid-attenuated inversion recovery (FLAIR) sequence (voxel size = 0.86x0.86x4.5 mm, TE = 122.3 ms, TR = 9502 ms, FOV = 220 x 220 mm, 36 slices) were acquired for identification and segmentation of T2-hyperintense MS lesions. Rs-fMRI was acquired using a T2* weighted gradient-echo echo-planar (GE-EPI) imaging sequence (voxel resolution = 3.4x3.4x3 mm, TE = 35 ms, TR = 3000 ms, FOV = 220 x 220 mm, 100 volumes, 46 axial slices each in an interleaved order), during which all participants were instructed to relax with their eyes closed. Diffusion MRI (dMRI) was acquired with a twice refocused diffusion-weighted spin echo echo-planar (SE-EPI) sequence with 6 volumes with no diffusion weighting and 40 volumes with diffusion gradients applied in uniformly distributed directions (Camino 40), b = 1200 s/mm2, voxel size=1.8x1.8x2.4 mm, TE = 94.5 ms, TR = 16000 ms, FOV = 230 x 230 mm, 57 slices. CBF was quantified using multi-inversion time pulsed arterial spin labelling (ASL). A PICORE QUIPSS II sequence with a dual-echo gradient-echo readout and spiral k-space acquisition was employed (voxel size=3x3x8 mm, 22 slices) (Warnert et al., 2015). Sixteen tag-control pairs each for short inversion times, TI (400, 500, 600, 700 ms) and 8 tag-control pairs for long TI (1100, 1400, 1700 and 2000 ms) were acquired with QUIPSS II cut-off at 700 ms. A calibration (M0) image was acquired to obtain the equilibrium magnetization of cerebrospinal fluid, needed for the quantification of CBF. A minimal contrast image was acquired with TE=11ms, TR=2000 ms to correct for coil inhomogeneities.

4.2.5 3DT1 image analysis

Structural 3DT1 images from patients were lesion filled, as described by Lipp et al., 2019, to allow better segmentation of brain tissue (Lipp et al., 2019), then segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using FSL's Automated Segmentation Tool (FAST) (Zhang et al., 2001). The quality of segmentation was manually assessed. Binary masks of intracranial brain tissue excluding CSF was created from the GM and WM images, for use in dMRI analyses. Brain volumes, including whole brain volume, GM volume and WM volume were quantified from lesion-filled 3DT1 images with FSL's SIENAX tool (Smith et al., 2002). Lesion volume was calculated from binary lesion masks created as part of lesion filling.

4.2.6 Rs-fMRI analysis

Rs-fMRI BOLD time-series were corrected for physiological noise in MATLAB (The MathWorks, n.d.) using a previously established pipeline (Lipp et al., 2014). Rs-fMRI images were pre-processed with FSL's MELODIC pipeline (Beckmann et al., 2009), which included motion correction, spatial smoothing with a 3 mm full width at half-maximum Gaussian kernel, high-pass temporal filtering equivalent to 0.01 Hz , non-linear registration to Montreal Neurological Institute (MNI) standard space, and resampling to a resolution of 4 mm isotropic. Head motion parameter estimates of absolute and relative displacement values did not differ between any groups (HC-RRMS p=0.58 (absolute), p=0.27 (relative); CP-CI p=0.11 (absolute), p=0.52 (relative)).

Independent Component Analysis (ICA), part of the MELODIC pipeline, decomposed the concatenated dataset into 82 components. Four resting state networks (RSNs) which have been found to be important for cognitive function in MS were manually identified and selected for further analyses: the default mode network (DMN) (Cruz-Gómez et al., 2014; Meijer et al., 2017), left and right frontoparietal networks (LFPN, RFPN) (Cruz-Gómez et al., 2014; Louapre et al., 2014; Meijer et al., 2017) and the salience network (SN) (Rocca et al., 2012; Cruz-Gómez et al., 2014). The anterior and posterior parts of the DMN (DMNa and DMNp, respectively) (Xu et al., 2016), were identified in two additional components. The primary visual network was used as a non-cognitive control network. Dual regression (Beckmann et al., 2009) was used to generate subject-specific versions of the group-average components.

4.2.7 dMRI analysis

Preprocessing of dMRI data was carried out in ExploreDTI (v 4.8.3 (Leemans et al., 2009)) and included motion correction and corrections for eddy current and EPI-induced geometrical distortions by registering each diffusion image to its respective (skull-stripped and downsampled to 1.5 mm) 3DT1 image (Irfanoglu et al., 2012) using Elastix (Klein et al., 2010), with appropriate reorientation of the diffusion-encoding vectors (Leemans and Jones, 2009). FSL's FDT tool was used to fit diffusion tensors, generate fractional anisotropy (FA) maps and fit the probabilistic diffusion model (Behrens et al., 2003, 2007). Processed diffusion data was quality checked manually. Anatomical connectivity maps (ACMs) were generated using FSL's Probtrackx2 tool (Behrens et al., 2003, 2007) by seeding tractography with 50 initiated streamlines per voxel in the binary parenchymal mask. The resulting ACM maps show anatomical connectivity across

the whole brain, where the magnitude of the ACM value in each voxel represents the number of probabilistic streamlines passing through that voxel(Bozzali et al., 2011), thus assessing the degree of anatomical interconnection of every voxel in the brain (Embleton et al., 2007; Cercignani et al., 2012). Each participant's ACM image was divided by the number of voxels in the brain parenchymal mask to normalise for intracranial volume. To normalise to MNI space, the downsampled 3DT1 image of each participant was non-linearly registered to MNI space, and the warps were applied to the ACM images.

4.2.8 ASL analysis

The two sets of ASL tag-control images were motion corrected to the M0 image using FSL's McFLIRT tool (Jenkinson et al., 2002), control-tag subtracted, averaged across pairs, and combined into a single multi-TI series that was fed to oxford_asl (BASIL) (Chappell et al., 2009) for CBF quantification. CBF was estimated with partial volume correction (Chappell et al., 2011), coil sensitivity correction (bias field calculated using the SPM12 (Debernard et al., 2014) segmentation on the minimum contrast image) and calibration with the M0 signal from subject-specific ventricle masks. CBF maps were then registered to the T1 structural scan following 6 DOF affine registration of the M0 scan. T1-weighted images were non-linearly normalised to the Montreal Neurological Institute (MNI) 152 template space, using ANTs SyN (Avants et al., 2008) and the obtained warp was applied to the CBF maps. Full CBF maps could not be obtained for all participants due to technical problems with the MR acquisition or due to failed qualitative quality checks of the data. CBF analyses were therefore conducted on data from 49 CI and 43 CP patients. The excluded patients did not differ substantially on demographic and clinical variables from the remaining CI and CP groups.

4.2.9 Statistical analyses

Statistical analyses of the demographic, clinical, global MRI and median ACM, FA and CBF values were performed in SPSS version 23.0 (IMB Corp., 2015). The distributions of all variables were checked with Kolmogorov-Smirnov tests and visual inspection of histograms and Q-Q plots. Variables showing a skew were analysed with non-parametric tests. To test the hypothesis that RSNs that show FC abnormalities also show ACM, FA and CBF abnormalities, we considered that ACM, FA and CBF changes could either be in the same voxel clusters that showed FC abnormalities, or elsewhere in the affected network. This was tested in analysis steps 1 and 2. In addition, we conducted an exploratory analysis of ACM, FA and CBF changes throughout the brain to understand

how widespread these are in CI compared to CP patients. The data was analysed as follows:

1. **Assessment of spatially overlapping changes**

   Binary masks of the RSN voxels clusters that showed significant FC differences between CI and CP groups were created and used to extract local median ACM, FA and CBF values of these regions, which were then compared between CI and CP groups.

2. **Assessment of local changes within RSNs**

   Second, we determined whether there were more diffuse changes in anatomical connectivity and CBF throughout each RSN. A binary mask of each RSN was created, and, for dMRI analyses, dilated by one voxel to include the white matter surrounding RSN regions. Voxelwise analyses of ACM, FA and CBF maps were conducted to look for abnormalities within the RSN regions. For FA, this was done both with skeletonised FA maps in a tract-based spatial statistics (TBSS) analysis (Smith et al., 2006), and with non-skeletonised FA maps. TBSS overcomes the difficulties of achieving accurate registration of the white matter by projecting all subjects' FA data onto a mean FA tract skeleton, before applying voxelwise cross-subject statistics. However, the FA skeleton includes only the centre of white matter tracts (Smith et al., 2015) and may not capture white matter local to grey matter network regions, hence we conducted both in an exploratory analysis to determine which is most sensitive to FA changes in and around RSNs. Next, we extracted median ACM, FA and CBF values from the RSNs and compared between CI and CP patients. The voxelwise analysis approach can show the spatial location of any abnormalities in the metrics studied, but requires the abnormalities to be in the same spatial location in most subjects in a group for a group difference to be detected. If this is not the case a group difference could be missed, hence we also extracted median values from our regions of interest in an exploratory analysis. Medians, rather than means, were extracted because ACM, FA and CBF values were not normally distributed in RSN regions.

3. **Diffuse changes in anatomical connectivity and CBF throughout the brain**

   Last, we checked whether CI and CP groups showed differences in ACM, FA and CBF throughout the brain, by running voxelwise analysis on the ACM, FA and CBF maps of the whole brain. This was an exploratory analysis to understand the spatial extent of ACM, FA and CBF abnormalities.

4.2.9.1 Comparisons, thresholding and multiple comparison correction

Comparisons of FC were conducted for both the whole RRMS group with HC, and the CI and CP patient groups to each other, to determine whether FC abnormalities are present in our RRMS cohort, and to assess how they differ between the two patient subgroups. Subsequent analyses of anatomical connectivity and cerebral blood flow were conducted only for the two patient groups to limit the number of statistical comparisons and in line with our hypotheses.

Comparisons of median ACM, FA values and CBF values were performed using a two- sample t-test or Mann-Whitney U-test, as appropriate. A Bonferroni correction for multiple comparisons, of a factor of four for the four RSNs of interest, was applied to the results. The corrected threshold was p0.0125.

For all voxelwise analyses, age, sex and education level were included in general linear models as covariates, and all results were threshold-free cluster enhancement (TFCE)-corrected at p0.05, two-sided. For rs-fMRI analyses, we calculated the percentage of network voxels showing abnormal FC between groups, and retained only those RSNs showing the largest proportion of abnormal network voxels for further analyses, in order to reduce the influence of noise. The Harvard-Oxford cortical structural, Harvard-Oxford subcortical structural and JHU white-matter tractography atlases in FSL were used to report anatomical locations.

4.2.10 Data availability

Anonymised data will be shared at the request of other investigators for purposes of replicating procedures and results.

4.3 Results

4.3.1 Demographic, clinical, neuropsychological characteristics and conventional MRI data

Demographic and clinical characteristics of HC, RRMS patients and the CI and CP subgroups are presented in Table 1. RRMS patients and controls showed no significant differences in sex, but the RRMS group was significantly older and less educated than controls and performed worse on all MSFC tests. Fifty-five patients met the

definition for CI, and 47 were considered CP. Compared to CP patients, CI patients did not differ significantly in age, sex, education, disease duration, or lower limb function, as measured by the 25 Foot Walk Test of the MSFC. However, their performance on the 9-Hole Peg Test demonstrated worse upper limb function. CI patients showed impaired cognitive function compared to CP patients and HC on all four cognitive domains assessed by the BRB-N (Table 2). The greatest impairments were observed on the information processing, attention and executive function and verbal memory domains. CP patients did not perform significantly worse than controls on any domain. RRMS patients had significantly lower normalised brain volume (NBV) and normalised GM volume (NGMV) than healthy controls, but showed no significant difference in normalised WM volume (NWMV). CI and CP groups showed no significant differences in any volumetric brain measures (Table 2).

Table 4.1: Demographic and clinical characteristics

	HC (n=27)	RRMS (n=102)	*Inferential test results* *HC-RRMS comparisons*	RRMS CP (n=47)	RRMS CI (n=55)	*Inferential test results* *CI-CP comparisons*
Demographic characteristics						
Age, yr (median, range)	37.00 (23-59)	45.00 (18-60)	$U = 958.00$, $p = .015$	42.00 (18-60)	47.00 (20-60)	$U = 1069.50$, $p = .134$
Male/female, n	12/15	33/69	$\chi^2(1) = 1.37$, $p = .241$	11/36	22/33	$\chi^2(1) = 3.19$, $p = .074$
Education years (median, range)	19.00 (12-30)	15.00 (10-30)	$U = 613.50$, $p < 0.001$	15.00 (10-27)	14.00 (10-30)	$U = 1084.50$, $p = .161$
Disease duration, yr (median, range)	N/A	12.24 (1-39)	N/A	11.50 (2-37)	12 (1-39)	$U = 1232.50$, $p = .803$
MSFC						
25 Foot Walk Test (median, range)	4.35 (3.2-5.4)	5.25 (3.6-26.8)	$U = 572.50$, $p < 0.001$	5.15 (3.7-13.0)	5.43 (3.6-26.8)	$U = 1169.50$, $p = .498$
9-Hole Peg Test (median, range)	18.65 (15.35-23.00)	21.75 (16.35-59.50)	$U = 537.50$, $p < 0.001$	21.45 (17.15-44.85)	21.95 (16.35-59.5)	$U = 956.00$, $p = .024$
PASAT3 (median, range)	51.00 (35-59)	43.50 (0-60)	$U = 715.00$, $p < 0.001$	50.00 (30-60)	34.00 (0-58)	$t(83.10) = 6.50$, $p < 0.001$

Independent samples t-tests were used for group comparisons of variables with a normal distribution. Those variables which were not normally distributed were assessed with Mann-Whitney U tests. Categorical variables were tested with the chi-squared test. Abbreviations: CI = cognitively impaired; CP = cognitively preserved; HC = healthy controls; MSFC = Multiple Sclerosis Functional Composite; PASAT3 = Paced Auditory Serial Addition Test; RRMS = relapsing remitting multiple sclerosis.

Table 4.2: Neuropsychological and MRI volumetric measures

	HC (n=27)	RRMS (n=102)	*Inferential test results* *HC-RRMS comparisons*	RRMS CP (n=47)	RRMS CI (n=55)	*Inferential test results* *CI-CP comparisons* *HC-CI-CP comparison of BRB-N*
BRB-N Z-scores						
Verbal memory (mean, SD)	0.00 (0.92)	*	N/A	0.07 (0.071)	-1.53 (1.09)	$F(2, 125) = 44.82$, $p < .001$ Post hoc: HC-CI $p < .001$, HC-CP $p = .931$, CP-CI $p < .001$
Visual Memory (mean, SD)	0.00 (0.92)	*	N/A	-0.13 (0.93)	-1.20 (1.01)	$F(2, 126) = 22.36$, $p < .001$ Post hoc: HC-CI $p < .001$, HC-CP $p = .883$, CP-CI $p < .001$
Information processing, attention, executive function (mean, SD)	0.00 (0.75)	*	N/A	-0.37 (0.73)	-1.90 (1.26)	$F(2, 126) = 44.58$, $p < .001$ Post hoc: HC-CI $p < .001$, HC-CP $p = .298$, CP-CI $p < .001$
Verbal fluency (mean, SD)	0.00 (1.00)	*	N/A	0.08 (0.72)	-0.51 (0.93)	$F(2, 125) = 6.33$, $p = .002$ Post hoc: HC-CI $p = .04$, HC-CP $p = .932$, CP-CI $p = .003$
MRI volume measures						
NBV, L (median, range)	1.55 (1.42-1.70)	1.55 (1.42-1.70)	$t(41.94) = 3.33$, $p = .002$	1.50 (1.37-1.66)	1.51 (1.30-1.68)	$t(99.83) = 0.36$, $p = .721$
NGMV, L (median, range)	0.81 (0.72-0.89)	0.77 (0.61-0.89)	$U = 755.00$, $p < 0.001$	0.77 (0.61-0.89)	0.76 (0.62-0.88)	$t(99.83) = 1.48$, $p = .142$
NWMV, L (median, range)	0.76 (0.68-0.81)	0.74 (0.66-0.83)	$t(40.43) = 1.56$, $p = .127$	0.74 (0.66-0.81)	0.75 (0.66-0.83)	$t(97.31) = -1.24$, $p = .218$
LV, mL (median, range)	N/A	*		9.73 (0.64-63.32)	9.73 (0.69-59.64)	$U = 1258.00$, $p = .817$

Independent samples t-tests were used for group comparisons of variables with a normal distribution. Those variables which were not normally distributed were assessed with Mann-Whitney U tests. Categorical variables were tested with the chi-squared test. BRB-N Z-scores were tested with a one-way ANOVA and Tukey's post hoc test. *RRMS group averages not calculated.

Abbreviations: BRB-N = Brief Repeatable Battery of Neuropsychological Tests; CI = cognitively impaired; CP = cognitively preserved; HC = healthy controls; LV = lesion volume; NBV = normalised brain volume; NGMV = normalised grey matter volume; NWMV = normalised white matter volume; RRMS = relapsing remitting multiple sclerosis; SD = standard deviation

4.3.2 Functional connectivity

RRMS patients showed FC abnormalities in in all RSNs investigated compared to HC.

CI patients had areas of decreased FC in the DMNa, DMN, DMNp, LFPN and primary visual network, and increased FC in areas of the DMN, SN, RFPN, LFPN and primary visual network relative to CP patients. The DMNa, DMNp, LFPN and RFPN showed the largest proportion of abnormal voxels between groups and were therefore retained for subsequent analyses (Fig 1).

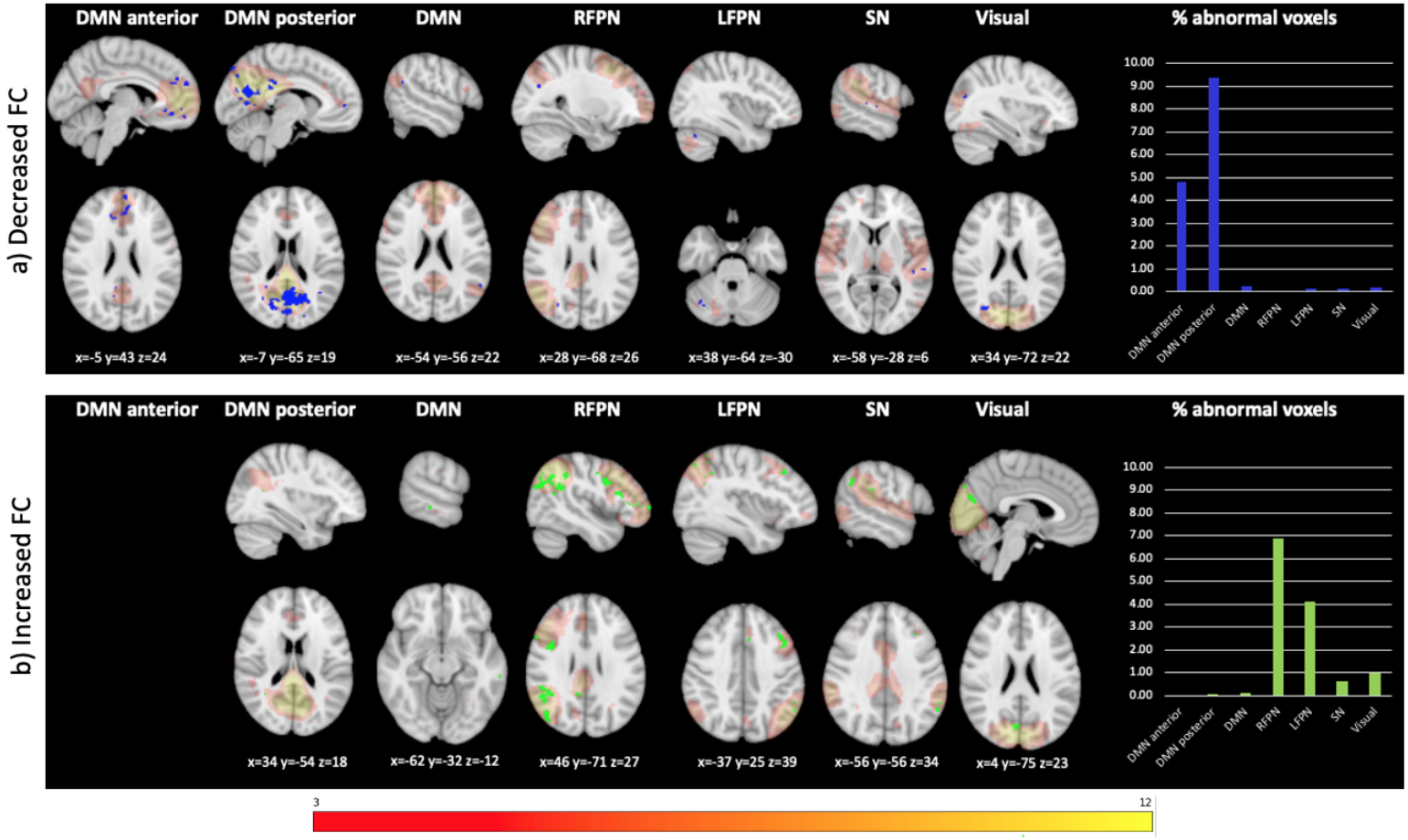

Figure 4.1: Functional connectivity abnormalities in cognitively impaired compared to cognitively preserved patients
Figure shows voxels showing FC abnormalities in CI compared to CP, overlaid onto the group average spatial map of each RSN analysed in red-yellow. First seven columns in each panel show each of the RSNs investigated: DMN anterior, DMN posterior, DMN, RFPN, LFPN, SN and primary visual network. For networks not displayed, no significant group differences were found. The eight column shows graphs indicating the percentage of voxels showing abnormalities, of the total number of voxels in the network. Rows show areas of: a) decreased FC in the CI group vs CP (in blue); b) increased FC in CI group (in green). Results were TFCE-corrected at p0.05, two-sided. MNI coordinates are given for results displayed. Colour bar shows signal intensity of RSNs.

4.3.3 Anatomical connectivity and cerebral blood flow

4.3.3.1 Local changes in ACM, FA and CBF in regions showing FC changes

In RSN regions that showed FC changes in CI patients compared to CP patients, there were no significant differences in median ACM, FA and CBF values between the CI and CP groups, following application of a Bonferroni correction for multiple comparisons (corrected p threshold = 0.0125).

4.3.3.2 Diffuse changes in connectivity within RSNs

Voxelwise analyses of ACM, FA and CBF demonstrated abnormalities in all four RSNs in CI compared to CP patients. ACM was reduced in: DMNa regions that correspond to the forceps minor, left cingulum, left anterior thalamic radiation and right anterior corona radiata; DMNp regions including parts of the splenium of the corpus callosum, left and right cingulum, forceps major and also forceps minor; RFPN white matter corresponding to parts of the right inferior longitudinal fasciculus (ILF) and the right inferior fronto-occipital fasciculus (IFOF); and LFPN regions corresponding to parts of the left superior longitudinal fasciculus, left ILF and left side of forceps major. There were also areas of increased ACM values, including some voxels in the left superior parietal lobe and left occipital lobe in the DMNa, in a part of the left superior longitudinal fasciculus in the DMNp, the right posterior temporal lobe in the RFPN and in regions of the occipital lobe which could be in either the right ILF or right IFOF in the LFPN (Fig 2).

The TBSS analysis showed FA reductions in: the genu of the corpus callosum, forceps minor and cingulum bilaterally in the DMNa; in the splenium of the corpus callosum, posterior parts of the cingulum bilaterally and posterior corona radiata bilaterally in the DMNp; in parts of the right frontal lobe and right parietal lobe in the RFPN; and in the left side of the splenium of the corpus callosum, left side of forceps major and left cingulum in the LFPN (Fig 3A). There were also small areas of FA increases, across the frontal and parietal lobes (Fig 3B). The voxelwise analysis of non-skeletonised FA maps found FA changes in largely the same regions as the TBSS analysis (Fig 3C to D).

There were regions of reduced CBF in all four networks in CI, compared to CP patients (Fig 4). Reductions were seen in the bilateral cingulate gyrus and precuneus in the DMNa; bilateral precuneus, left cuneal cortex, right lateral occipital cortex, left lingual gyrus and left posterior cingulate gyrus in the DMNp; the right occipital cortex, right angular gyrus, right superior supramarginal gyrus and right cingulate gyrus in the RFPN. The same regions but in the left hemisphere showed CBF reductions in the LFPN. We found some individual voxels, likely artefacts, showing increased CBF in CI patients, in the DMNa, DMNp and RFPN (Fig 4).

Comparisons of extracted median values only found reduced ACM in CI patients (Mdn = 0.0039) compared to CP patients (Mdn = 00043) in the anterior DMN (U=897.00, p=0.008), but no other RSNs. There were no differences in median FA or CBF values in RSN regions.

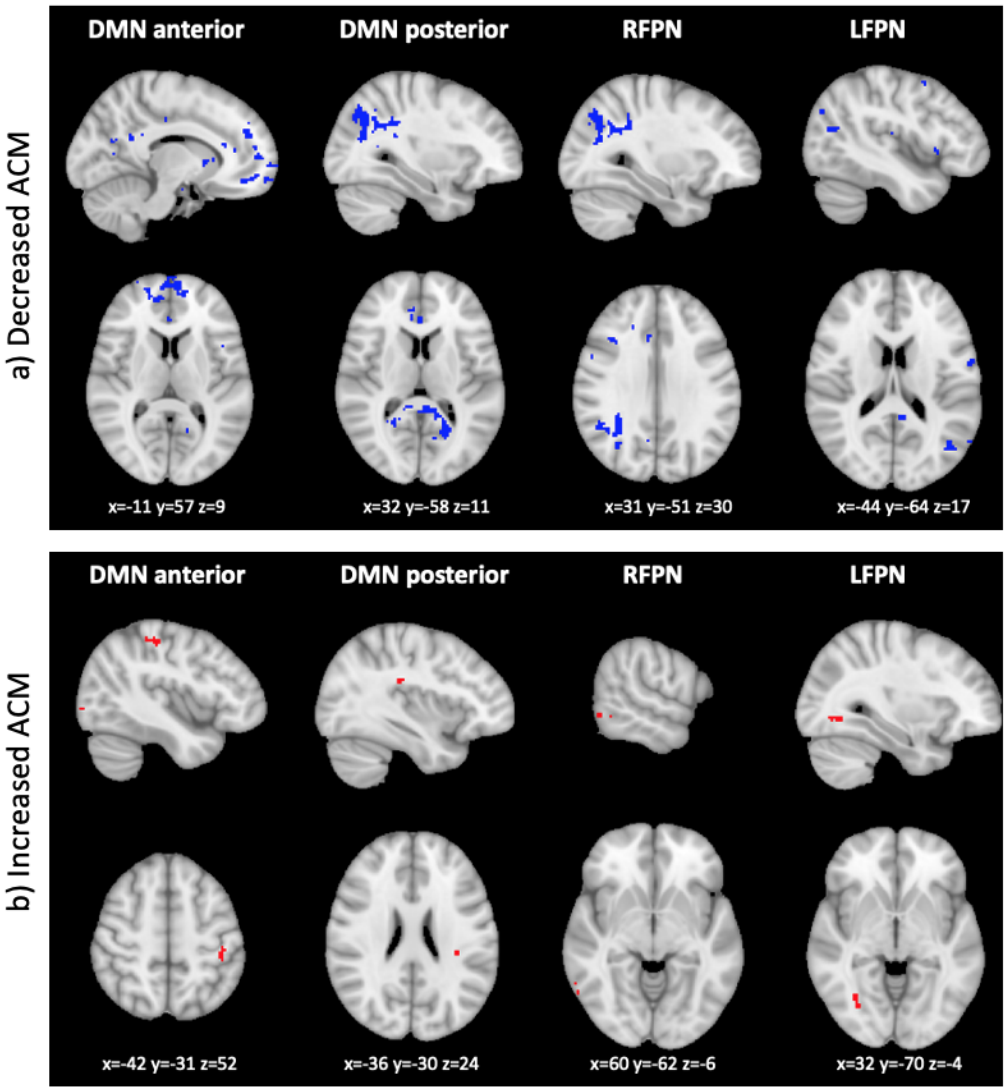

Figure 4.2: Anatomical connectivity changes in CI compared to CP patients, based on a voxelwise analysis of anatomical connectivity maps
Figure shows voxels showing ACM value abnormalities. Columns show each of the RSNs compared. The first row, part a), shows areas of decreased ACM values (in blue), the second row, part b), areas of increased ACM values (in red). MNI coordinates are given for the biggest voxel clusters displayed. Results were TFCE-corrected at p0.05, two-sided

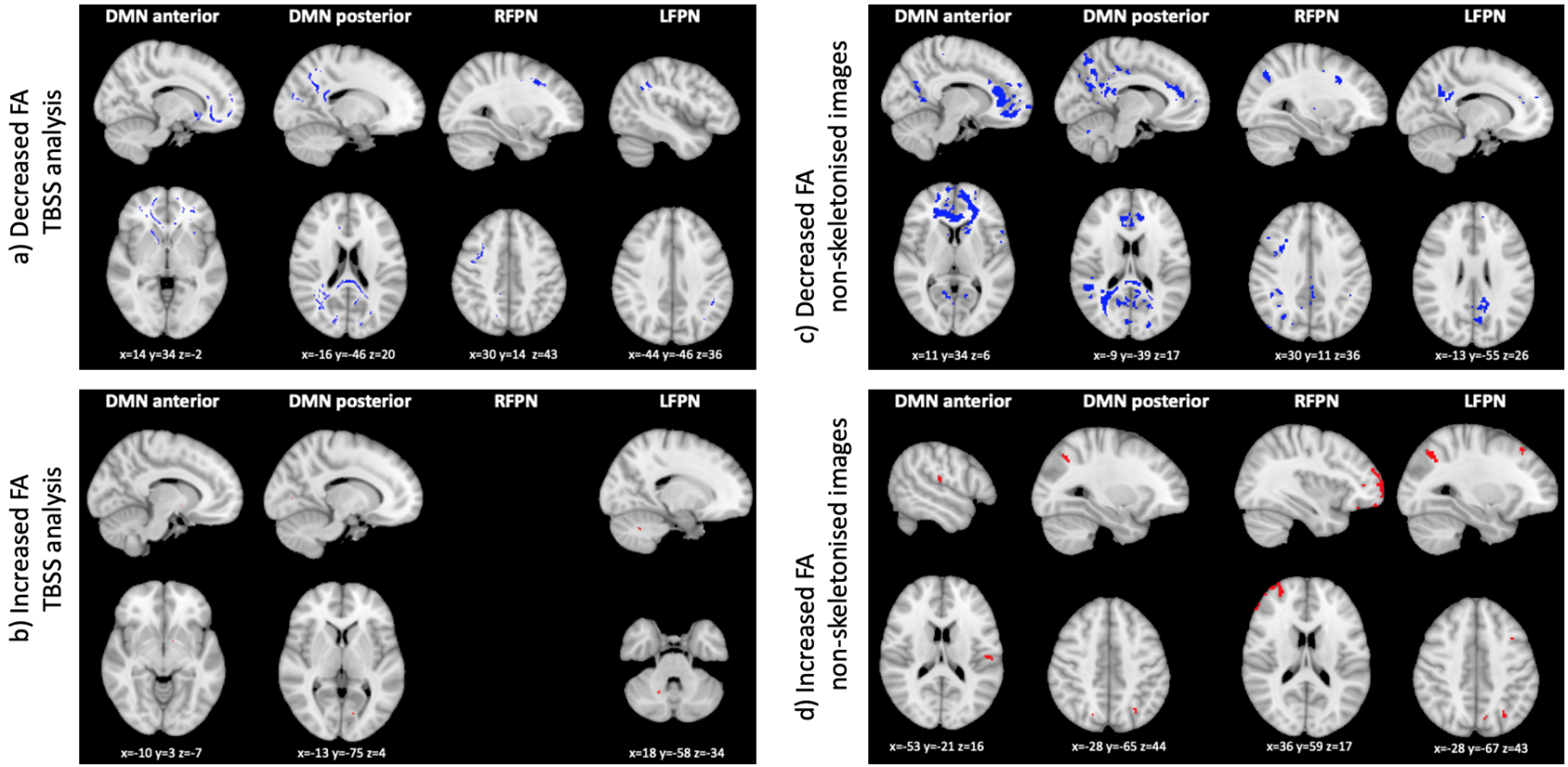

Figure 4.3: FA changes in CI compared to CP patients
Figure shows voxels showing FA abnormalities. Parts a) and b) show results from the TBSS analysis. Parts c) and d) show results from the voxelwise analysis of non-skeletonised FA maps. Columns show each of the RSNs compared. The first row shows areas of decreased FA (in blue), the second row areas of increased FA (in red). MNI coordinates are given for the biggest voxel clusters displayed. For networks not displayed, no significant results were found. Results were TFCE-corrected at p0.05, two-sided.

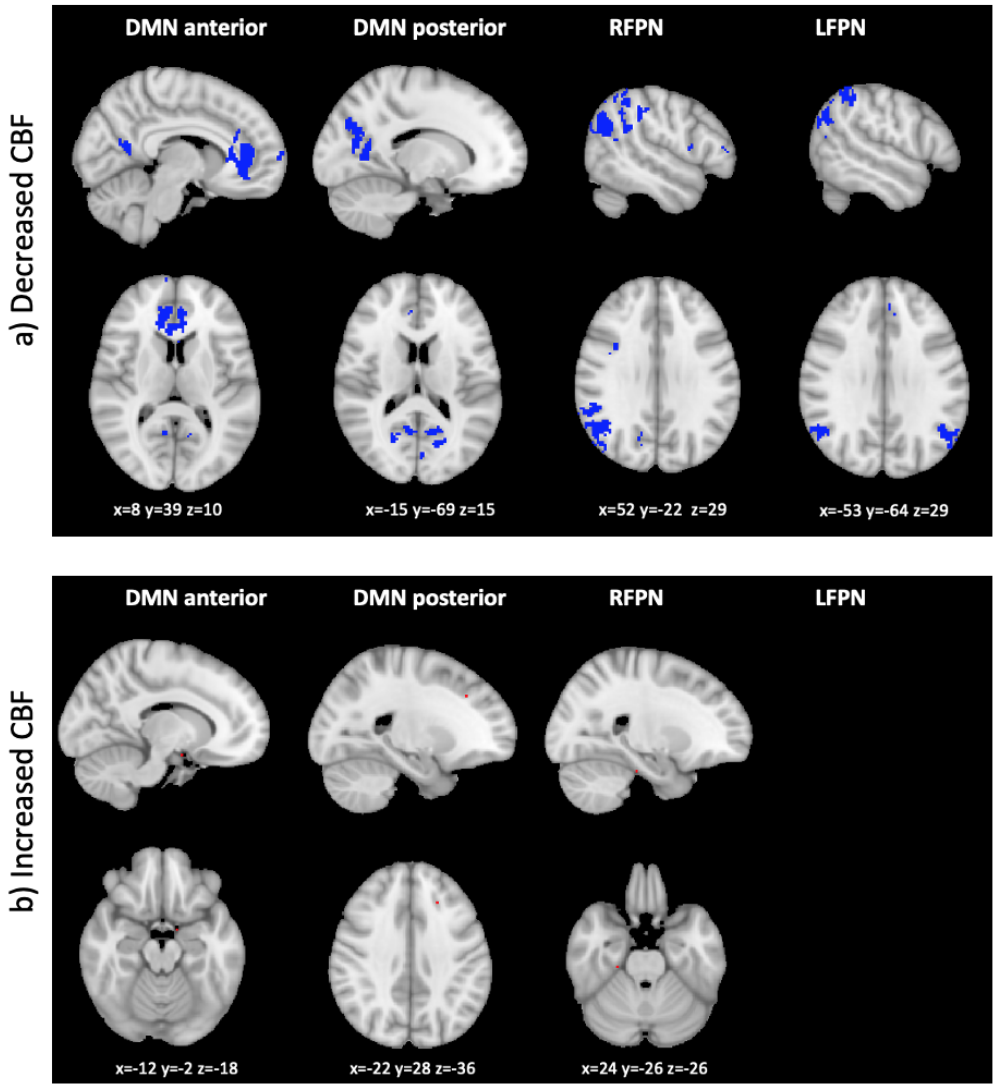

Figure 4.4: CBF changes in CI compared to CP patients, based on a voxelwise analysis of CBF maps

Figure shows voxels showing CBF abnormalities in red. Columns show each of the RSNs compared. The first row, part a), shows areas of decreased CBF (in blue), the second row, part b), areas of increased CBF (in red). MNI coordinates are given for the biggest voxel clusters displayed. Results were TFCE-corrected at p0.05, two-sided.

4.3.3.3 Diffuse changes in connectivity and CBF throughout the brain – rationale and results

CI, compared to CP, had widespread ACM and FC reductions throughout the brain and some small areas of increased ACM and FC at the edges of the brain. CBF was decreased throughout the brain (Fig 5).

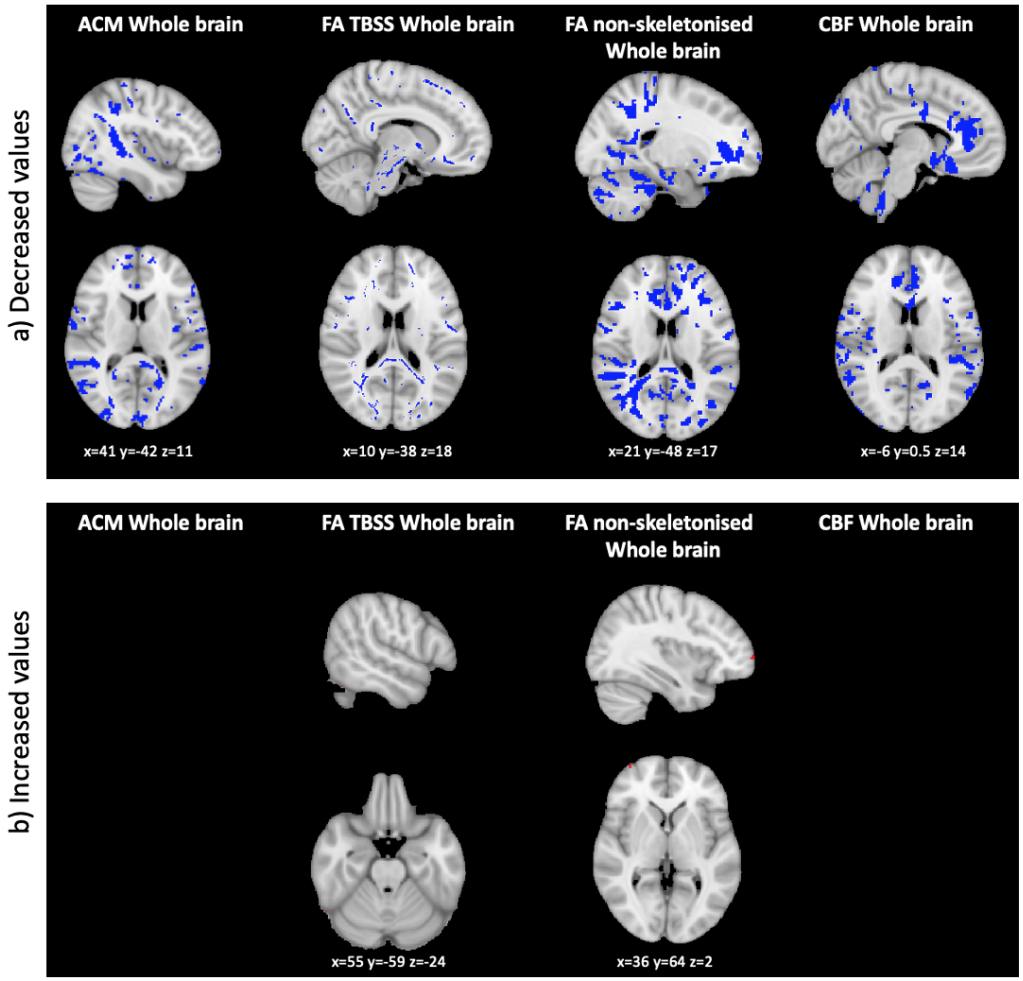

Figure 4.5: Diffuse ACM, FA and CBF changes across the whole brain in CI compared to CP patients
Figure shows ACM, FA and CBF abnormalities throughout the brain. Columns show each of the metrics assessed: ACM, FA from TBSS, FA from analysis of non-skeletonised FA maps, and CBF, in that order. The first row, part a), shows areas of decreased values (in blue), the second row, part b), areas of increased values (in red). MNI coordinates are given for the biggest voxel clusters displayed. Results were TFCE-corrected at p0.05, two-sided.

4.4 Discussion

In this study we provide the first evidence that abnormal FC co-occurs with altered structural connectivity and cerebral blood flow in cognitively impaired MS patients in resting state network regions. At the same time our findings reveal that the exact location of abnormalities differed between metrics. Overall, this indicates that RSNs may be vulnerable to clinically-relevant MS pathology, offering partial support for activity-related 'wear-and-tear' damage of network hubs predicted by the 'nodal stress' hypothesis (Buckner et al., 2009, Zhou et al., 2012).

We found FC abnormalities in our RRMS cohort relative to HC in all RSNs investigated, confirming FC changes as a widespread pathological feature in MS, as per previous studies (Filippi et al., 2013). In CI compared to CP patients, we found FC abnormalities in all networks investigated, with FC decreases in the DMNa and DMNp and increases in the RFPN and LFPN making up the highest proportion of affected network voxels. Increased FC could reflect compensatory mechanisms following structural damage, and decreased FC could be evidence of network breakdown (Schoonheim et al., 2015; Tewarie et al., 2018). However, we did not assess the extent of structural damage and can therefore only speculate about the mechanisms of directional FC change, which is an urgent research priority in this field. Nevertheless, our results are consistent with numerous previous reports of abnormal FC in these networks in patients with cognitive symptoms (Cruz-Gómez et al., 2014; Louapre et al., 2014; Meijer et al., 2017). Importantly, the FC measure distinguished the two patient groups in the absence of significant differences in conventional MR metrics, demonstrating its potential heightened sensitivity to clinically-relevant pathology in MS and highlighting the importance of understanding the mechanisms of FC changes.

As predicted, we found reduced anatomical connectivity of networks showing FC abnormalities in CI patients, with both the ACM and FA metrics. ACM is an anatomical network measure that shows whether the structural connectivity of a region is affected as a result of WM damage, regardless of where in the brain the WM damage is. It is informative of the degree of connectivity of our regions of interests, but not about the WM in and around RSN regions. To understand local tissue characteristics of RSN regions we also tested the FA metric, a measure of the directionality of diffusion within tissue, which is assumed to be largely determined by the presence of aligned axons in WM bundles (Beaulieu, 2002), and can give information about local microstructural integrity in a WM tract. The specific voxels showing FC abnormalities were not those which showed structural changes in CI patients. Instead, other parts of the RSNs were affected. This, combined with widespread ACM and FA changes suggests that more diffuse, as opposed to focal, anatomical changes within RSNs are associated with cognitive impairment, and is in line with previous evidence which shows that FC changes are preceded by a high degree of structural damage (Schoonheim et al., 2015; Tewarie et al., 2018).

As well as reductions, we found small regions of increased ACM and FA in all four RSNs. One possibility is that these are statistical artefacts. ACM increases could reflect an unmasking effect, whereby tracking becomes easier in regions where fibres are lost. However, Bozzali et al. (2011)

found ACM increases in Alzheimer's patients and considered that they may be due to plasticity driven by medication. The mechanism of FA increases is not well understood, but it has been suggested that increased FA reflects changes in axonal structures such as reduced branching, decreased axon diameter, reduced packing density, or increases in myelination (Beaulieu, 2002; Hoeft et al., 2007). In MS, FA increases may be related to inflammatory processes (Calabrese et al., 2011). We cannot conclude which mechanisms are responsible for the ACM and FA increases in our CI group, but acknowledge the findings as important areas for future research.

Finally, we investigated CBF, which may be a response to decreased energy demand in MS (Paling et al., 2011; Lapointe et al., 2018). As with ACM and FA, CBF was reduced in and around RSN regions in CI relative to CP patients, but not within the specific voxel clusters showing FC abnormalities, again pointing to diffuse rather than focal tissue abnormalities in RSNs. CBF reductions may reflect a response to decreased energy demand in the RSNs investigated, demonstrating altered metabolic function of RSN regions. However, there are suggestions that CBF changes could be due to a primary vascular insult (Lapointe et al., 2018), and future studies with more direct measures of metabolism, such as fluorodeoxyglucose (FDG) positron emission tomography (PET), could help elucidate the metabolic status of functional networks.

Overall, our findings show that diffuse ACM, FA and CBF abnormalities co-occur with RSN FC changes in CI MS patients, consistent with the 'nodal stress' hypothesis. The mechanism of nodal 'wear-and-tear' remains to be elucidated, and may relate to unmet metabolic demands (Buckner et al., 2009),(Zhou et al., 2012). There is preliminary evidence that functional networks are susceptible to metabolic changes, recently from a drosophila model where network changes resulted from neuronal metabolism (Mann et al., 2020). Similarly, metabolic changes have been reported in demyelinated axons (Foster et al., 1980; Craner et al., 2004) and if this results in axonal damage or dysfunction that could be reflected in WM metrics in and around RSN regions. Thus, our results are not inconsistent with a role of metabolic changes in RSN regions. However, our methods are indirect measures of metabolic function. Other MR modalities, such as FDG PET, support the role of shared metabolic patterns between regions on RSNs (Savio et al., 2017), and 23Na MRI can show changes in sodium concentration in tissue, which is a measure of the energy state of axons (Paling et al., 2011). If combined with rs-fMRI, these methods may be informative about the metabolic basis of FC changes.

There are limitations to consider when interpreting these results. First, our control group was younger and more educated than our patient cohort. We controlled for this by including age, sex and education as covariates in our analyses. Importantly, the CI and CP groups did not differ significantly on these demographic variables. We also did not investigate separate cognitive domains, but looked at overall cognition. There have been suggestions that domains may be differently affected by pathology (Migliore et al., 2016) and this is an important avenue for future work. Further, we conducted several exploratory analyses to understand how best to explore changes in WM metrics and CBF in and around functional network regions. Comparisons of extracted median ACM, FA and CBF values from RSN regions showed no group differences

between CI and CP patients, pointing to heterogeneity in the metrics across the regions. We conclude that the voxelwise analysis is more sensitive to group differences. The TBSS analysis and the voxelwise analysis of non-skeletonised FA maps showed FA reductions in largely the same regions. The latter additionally showed FA changes at the white-grey matter boundaries, which could reflect FA abnormalities in the grey matter, as has been reported in MS in several studies (reviewed in Inglese and Bester, 2010). However, findings of group differences at the edge of the brain and at the midline points to partial volume effects due to registration problems with non-skeletonised FA images and suggests that results need to be interpreted with caution. Related to this, we assessed metrics which are susceptible to partial volume effects. However, the same MR sequences were used for all participants, so any tissue contamination is unlikely to introduce bias in our group comparisons. Finally, we assessed whether FC, ACM, FA and CBF changes co-occur, but did not test whether these changes are correlated, which should be investigated in future studies with larger samples.

In conclusion, our study provides evidence that functional connectivity changes in cognitively impaired RRMS patients co-occur with abnormal blood flow and anatomical connectivity. This highlights the possibility of a common underlying pathological change in resting state networks, such as altered metabolic state in cognitively impaired patients. The metabolic state of functional networks affected by MS should be further investigated with more direct methods of metabolic brain function to determine the pathological basis of functional connectivity abnormalities and potentially lead to their use as effective biomarkers of disease.

4.5 Acknowledgements

The authors would like to thank Dr Daniele Mascali and Dr Antonio Maria Chiarelli at the Gabriele d'Annunzio University of Cheti and Pescara, Italy, for computing the CBF maps used in this study.

4.6 Funding

This work was funded by a research grant of the MS Society UK and a Medical Research Council Doctoral Training Partnership grant (MR/N013751/11).

4.7 Potential Conflicts of Interest

The authors report no potential conflicts of interests.

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Chapter 5

Increased Sodium Levels in Resting State Network Hubs, and Reductions in Those with Early Multiple Sclerosis

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This is a Prepared Manuscript for submission to *Brain*, with minor editing to position figures and tables where they should appear in the text.

Abstract

Cognitive impairment in multiple sclerosis (MS) is associated with abnormalities in functional brain networks identified with resting state fMRI (rs-fMRI). The mechanisms of functional connectivity abnormalities are poorly understood, but it has been suggested that network 'hub' regions are susceptible to energy failure due to their high metabolic demands. In the present study we combined rs-fMRI with sodium MRI for the first time in MS to assess total sodium concentration (TSC), a marker of axonal energy state, in functional network regions. Here, we tested whether 1) TSC is higher in network hub regions than in the rest of the brain, 2) TSC is abnormal in network hub regions in relapsing remitting MS (RRMS)patients relative to controls, and 3) TSC in network hub regions correlates with cognitive performance in RRMS patients.

Neuropsychological testing was performed in 46 RRMS patients (33 female, mean age 41.3 +/- 10.2 years) with a median disease duration of 2.58 years (range 0.08-6.00 years). Twenty-three patients (16 female, age 41.13 +/- 10.09 years) returned for another neuropsychological assessment and a proton rs-fMRI and sodium MRI scan 2 years later. Independent component analysis was performed on rs-fMRI data to identify four resting state networks (RSN) of interest: the anterior and posterior default mode networks (DMNa, DMNp) and left and right frontoparietal networks (LFPN, RFPN). Ten participants without RRMS that had been imaged using the same sodium MRI acquisition (4 females, age 59.7 +/- 22.37), were included as controls and TSC maps were calculated from sodium MRI data for all participants. TSC maps were masked with RSN masks and median TSC values were extracted for each network.

Eleven RRMS patients (24%) met criteria for cognitive impairment (CI) based on a score of $\geq$1.5 standard deviations below normative values on $\geq$2 tests. In the sub-set of patients who returned for follow-up, cognitive function was stable over 2 years (17% CI at screening, 22% CI at follow-up), with no significant decline on any test. TSC was higher in network regions than the rest of the brain in both RRMS patients and controls. Patients showed lower TSC in network regions than controls, and TSC levels positively correlated with scores on the Paced Auditory Serial Addition Test in RRMS.

These findings suggest that sodium accumulation may occur in functional network hub regions, possibly reflecting higher energy demand than other brain regions. Evidence of change in TSC within these network regions in early MS may reflect a regional vulnerability to pathology, which is associated with cognitive outcomes.

5.1 Introduction

Cognitive impairment is a common and debilitating symptom of multiple sclerosis (MS) (Sumowski et al., 2018). Despite reports of impairment from the earliest stages, including clinically isolated syndrome and early relapsing-remitting MS (RRMS), there is limited understanding of the prevalence and development of cognitive impairment in the first years following diagnosis (McNicholas et al., 2018). Available longitudinal evidence shows decline in cognitive function within five years post-diagnosis (Reuter et al., 2011; Achiron et al., 2013), highlighting the sensitivity of this early stage to the pathological impact on cognition. The pathological factors influencing cognitive outcomes in MS are only starting to become clear (Chard et al., 2021), and likely involve changes in the functional connectivity of the brain (Jandric et al., 2021a), which may be influenced by structural and metabolic factors (Jandric et al., 2021b). A key theory is that functional connectivity hubs may be vulnerable to pathology. Here we test that theory using sodium MRI and rs-fMRI.

We are only starting to understand how changes in the ability of different brain regions to effectively communicate may underpin cognitive impairment in MS. Connectivity abnormalities in the functional networks supporting cognitive function are frequently reported and commonly associated with cognitive impairment, even in the earliest stages of the disease (reviewed in Jandric et al., 2021). However, the mechanisms of network breakdown are not established. The networks which most commonly tend to show abnormal connectivity associated with cognitive impairment, including the default mode network (DMN) and frontoparietal networks (FPN) (Jandric et al., 2021a), are made up of brain regions which are heavily interconnected with the rest of the brain. It has been suggested that such brain 'hubs' are susceptible to pathology due to their high metabolic demands, which, if not met, causes dysfunction in the network (Buckner et al., 2009; Zhou et al., 2012, Jandric et al., 2021b).

There is a growing body of evidence demonstrating whole brain and regional neuronal energy dysfunction in multiple sclerosis, from studies using sodium (^{23}Na) MRI to show that sodium accumulation occurs in MS and is related to clinical symptoms (Inglese et al., 2010, 2013; Zaaraoui et al., 2012; Paling et al., 2013; Petracca et al., 2016; Maarouf et al., 2017; Brownlee et al., 2019; Collorone et al., 2021). In a healthy brain, axonal signal conduction depends on a chemical balance of ions between the intra- and extracellular space. The sodium-potassium ion channel, or Na+/K+ pump, maintains this balance by pumping sodium out of the intracellular space to keep it at 10-15 mM relative to the extracellular concentration of 140-150 mM (Madelin et al., 2015). It has been shown that following demyelination there is an upregulation of sodium-potassium ion channels, or Na+/K+ pumps, along the axon, allowing restoration of signal conduction along the axonal segment (Foster et al., 1980; Craner et al., 2004). This leads to an increased energy demand of the cell to fuel the additional Na+/K+ pumps. If this energy demand is not met, the Na+/K+ pumps cannot pump sodium out of the cell, against the concentration gradient, and sodium accumulates within the axon. This axonal dysfunction eventually leads to axonal death,

which causes an increase in the extracellular space and thereby higher levels of total sodium concentration (**Fig.1**). The main measure obtained with sodium MRI, total sodium concentration (TSC), is a weighted average of the intra- and extracellular space, and therefore TSC could reflect both early metabolic failure and axonal loss. Sodium accumulation can occur shortly after demyelination and thus be an early marker of neuronal energy dysfunction (Paling et al., 2011).

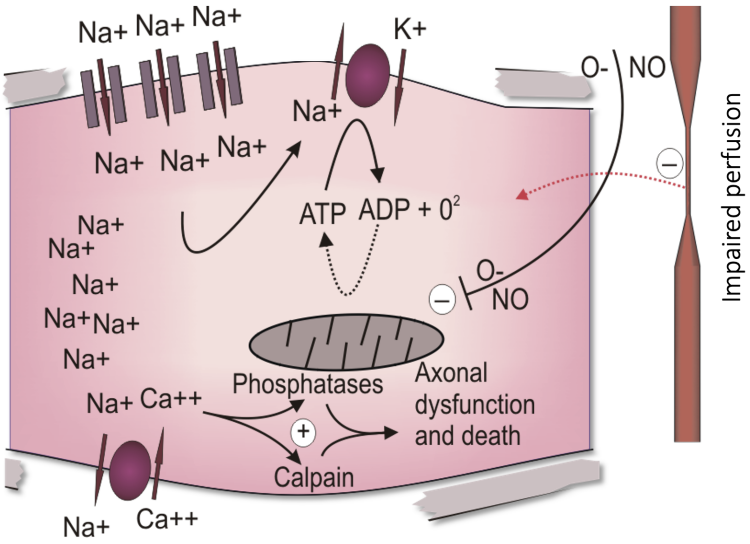

Figure 5.1: Schematic of the energy failure hypothesis
Reproduced, with minor adaptations, with permission from Paling et al. (2011). Schematic shows the chain of events following axonal demyelination leading to axonal dysfunction and death through energy failure: inflow of extracellular sodium down the concentration gradient, placing greater demands on the sodium-potassium pump; impaired mitochondrial function affecting production of adenosine triphosphate (ATP) due to nitric oxide and reactive oxygen species from MS plaques and reduced oxygen delivery as a consequence of impaired perfusion; failure to power sodium-potassium pump resulting in increased intracellular sodium causing reversed activity of the sodium-calcium transporter; increased intracellular calcium causing induction of phosphatases and calpain, resulting in axonal dysfunction and death.

Associations between sodium accumulation and cognitive impairment have been reported in all MS phenotypes, and all brain tissue types, including lesions, normal appearing white matter and normal appearing grey matter (Paling et al., 2013; Maarouf et al., 2017; Brownlee et al., 2019; Collorone et al., 2021). Moreover, sodium accumulation seems to be a better predictor of cognitive impairment than atrophy (Maarouf et al., 2017; Brownlee et al., 2019). One study in early RRMS (average 3.1 year disease duration) found that those patients who met the criteria for cognitive impairment had increased TSC relative to cognitively preserved patients, localised mainly to the neocortex, and that this was a better predictor of cognitive impairment than grey matter atrophy (Maarouf et al., 2017). Such findings highlight sodium accumulation as a key early pathological mechanism and emphasise the need to investigate its role in cognitive impairment further. Given the high metabolic demand of network hub regions (Zhou et al., 2012; Liang et al., 2013; Mann et al., 2020) and the key role of hub regions in brain function, these regions may be 'clinically eloquent' such that alterations (reflected by sodium levels and/or energy failure) may substantially contribute to network breakdown and subsequent cognitive impairment.

In the present study we assessed the association between sodium levels in network hubs and cognitive function in a sample of RRMS patients. People with RRMS of short disease duration were recruited and cognitively assessed over a two year period. We then investigated sodium concentration in network regions to test whether: 1) TSC is higher in network hub regions than in the rest of the brain 2) TSC levels in network hub regions differ between RRMS patients and a group of healthy controls; and 3) TSC levels in network hub regions correlate with cognitive performance in RRMS patients.

5.2 Materials and methods

5.2.1 Standard Protocol Approvals, Registrations and Patient Consents

The study was approved by the NHS North West - Greater Manchester Central Ethics committee (REC 18/NW/0349 for RRMS patients, REC 18/NW/0094 for controls), and written informed consent was received from all study participants, including for later analysis of their data.

5.2.2 Participants

Forty-six participants with a diagnosis of RRMS were recruited through the Salford Royal NHS Foundation Trust. All patients were aged between 18 and 65 years and had received a diagnosis of RRMS no more than six years prior to screening, a limit set to obtain a sample of participants with early MS, before substantial accumulation of disease activity. As conversion to secondary progressive MS typically happens after at least 10-15 years (Compston and Coles, 2008), the first six years are a relatively early stage of the disease. In addition, MS participants had no relapses or change in disease modifying treatment (DMT) in the three months prior to screening, had no contraindications for MR scanning and had no concordant neurological or psychiatric disorders, except mood disorders. Participants with mood disorders were not excluded due to the high lifetime prevalence rates of mood disorders in MS, estimated to up to 54% (Minden et al., 2014). Excluding them may have resulted in a sample unrepresentative of people living with MS.

Upon screening, one patient was excluded due to a disease duration longer than six years. Two patients reported a previous history of head injury, but were included as they reported no neurological conditions or symptoms besides MS.

The COVID-19 pandemic started while this study was ongoing, and the study was paused for 16 months, after which time neuropsychological testing was performed again, on the day of the MRI scan, to ensure up to date test scores. Twenty-three patients returned for an MRI scan on average 2.2 years after their baseline neuropsychological testing, and were subject to the same eligibility criteria for inclusion at this timepoint. The remaining twenty-two patients were lost to follow-up, or had a change to eligibility criteria.

As a control group, data from ten participants without RRMS that had been imaged using the same sodium MRI acquisition were included and analysed. Seven of these control participants had a diagnosis of sporadic vestibular schwannoma or VS (a benign WHO grade I extra-axial tumour that arises from Schwann cells lining the vestibulocochlear or VIIIth cranial nerve), and had been imaged pre-radiotherapy as part of an earlier imaging study performed at our institution (Lewis et al., 2021). VS arise from outside the brain parenchyma and as such are unlikely to affect TSC in the brain, and in these patients pre-treatment TSC values in the grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) had been shown to be consistent with literature values of healthy volunteers (Lewis et al., 2021). Three healthy controls without known pre-existing medical conditions were also included for comparison.

5.2.3 Clinical and neuropsychological assessment

RRMS patients underwent a neuropsychological assessment with the Brief Repeatable Battery of Neuropsychological tests (BRB-N), a validated testing battery with confirmed sensitivity to cognitive impairment in MS (Rao, 1990; Amato et al., 2006), at screening and again at follow-up, immediately after the MRI scan. The BRB-N includes tests of verbal memory (Selective Reminding Test, SRT), visuospatial memory (Spatial Recall Test, SPART), information processing speed, working memory, attention and executive function (Paced Auditory Serial Addition Test, PASAT, and Symbol Digit Modalities Test, SDMT), and verbal fluency (World List Generation Test, WLG) (Rao, 1990). Patients were considered cognitively impaired (CI) if they scored $\geq$1.5 standard deviations (SD) below the normative values provided by Boringa et al., (2001) on $\geq$2 tests (a criterion considered to be of medium stringency, Sepulcre et al., 2006).

Clinical functioning was assessed in RRMS patients with the Multiple Sclerosis Functional Composite (MSFC), a standardised measure of upper and lower limb and cognitive function (Cutter et al., 1999). In addition, physical disability was assessed using Disease Steps, a short ambulation scale which has shown high correlation with Expanded Disability Status Scale (EDSS) scores and good inter-rater reliability (Hohol et al., 1995). Finally, patients were assigned to one of four EDSS bands, a simplified disability assessment used in a large national multi-centre study (Young, n.d.). Clinical and neuropsychological assessment was not performed on control participants.

5.2.4 MRI data acquisition

MRI examination of all participants was performed on a 3T MRI scanner (Philips Achieva, Philips Medical Systems, Best, The Netherlands). For the first part of the protocol, a 32 channel head coil was used. A high-resolution 3D T1-weighted (3DT1) sequence was acquired for identification of T1 hypointense MS lesions, segmentation, registration and volumetric measurements [resolution = 1 mm x 1 mm x 1 mm, TE = 5.6 ms, TR = 11.7 ms, matrix = 240x240x140, FOV = 240 x 240 x 140 mm, flip angle = 9°]. A T2/proton density (PD)-weighted sequence was also acquired for

identification of T2-hyperintense MS lesions [resolution = 0.45 mm x 0.45 mm x 5 mm, TE = 10/80 ms, TR = 2000 ms, matrix = 512x512x30, FOV = 230 x 230 mm, 30 slices, flip angle = 90°]. Resting state-fMRI was acquired in all patients with an echo-planar imaging (EPI) sequence for identification of functional resting state networks [resolution = 3 mm x 3 mm x 4 mm, TE = 35 ms, TR = 2700 ms, matrix = 64x64x34, FOV = 192 x 192 mm, 222 volumes, 34 slices, flip angle = 90°]. Participants were instructed to keep their eyes open for the duration of this sequence, to minimise the chance of them falling asleep.

Finally, participants were briefly removed from the scanner and the 32 channel head coil was replaced with a 1H/^{23}Na dual-tuned birdcage head coil (RAPID Biomedical, Rimpar, Germany). A ^{23}Na MR image was collected using a 3D spiral read-out acquisition [voxel resolution - 4 mm x 4 mm x 4 mm, TE = 0.967 ms, TR = 100 ms, FOV = 240 x 240 x 140 mm number of averages = 7, 35 slices, flip angle = 90°], as described by Lewis et al., (2021). Two sodium calibration phantoms, described by Riemer et al., (2019), containing 60 mM and 120 mM sodium chloride (NaCl), respectively, in water with 4% agar, were placed in the FOV to act as external references for quantification of TSC throughout the brain parenchyma. T1 measurements in each phantom were quantified using inversion recovery experiments and were in good agreement with reported literature values. In addition regular quality assurance scans using the phantoms and commercial 0.9% saline preparations (Baxter Healthcare Inc, Deerfield, IL, USA) as a calibrant demonstrated that the concentrations within both phantoms were stable over the course of the study.

5.2.5 Lesion filling, segmentation and volumetric measurements

MS lesions were semi-automatically delineated on both T1- and T2-weighted images using the Jim software (v.6, Xinapse) and Lesion volume was calculated from T2-weighted lesion maps. Binary masks were created of hypointense lesions in MS patients, marked on T1-weighted images. In order to improve tissue segmentation these masks were then used in FSL's lesion_filling function (Battaglini et al., 2012), to estimate intensities from surrounding white matter to 'fill' the lesions with. Lesion-filled T1-weighted images were segmented with FSL's Automated Segmentation Tool (FAST) (Zhang et al., 2001), and a binary parenchymal mask excluding cerebrospinal fluid (CSF) was created by adding the grey and white matter masks together. FSL's SIENAX tool (Smith et al., 2002) was used to quantify brain volumes, including whole brain volume, grey matter volume, and white matter volume, normalised for intracranial volume. Finally, T1-weighted images were non-linearly registered to Montreal Neurological Institute (MNI) standard space using FSL's fnirt tool (Andersson et al., 2010).

5.2.6 Resting state network identification

Rs-fMRI images from MS patients were pre-processed in FSL's MELODIC tool (Beckmann et al., 2009) which carries out the following steps: motion correction, spatial smoothing with a 5 mm full width at half-maximum Gaussian kernel, high-pass temporal filtering equivalent to 0.01 Hz, non-linear registration to MNI standard space, and resampling to a resolution of 4 mm isotropic. As part of the MELODIC tool, Independent Component Analysis (ICA) was performed on the concatenated dataset and returned 96 components. The default mode network (DMN) and frontoparietal networks (FPN) are the most investigated resting state networks (RSNs) in association with cognitive impairment in MS (Jandric et al., 2021a) consist of the 'hub' regions of the brain. Therefore the components matching the anterior DMN (DMNa), posterior DMN (DMNp), right FPN (RFPN) and left FPN (LFPN) were manually identified and selected for further analyses. The primary visual network was used as a non-cognitively relevant control network for correlations between TSC within RSNs and cognitive test scores. A binary mask of each RSN was created (**Fig.2**) as well as a binary mask of the whole brain except the network, by subtracting the RSN mask from the MNI standard template mask.

Data from one MS patient was excluded due to failed quality checks of the raw data showing substantial signal drop-out, so RSN mask creation was based on data from 22 MS patients.

5.2.7 Total sodium concentration quantification within RSNs

^{23}Na images for both MS patients and controls were reconstructed on the scanner and quantification of sodium concentration was performed offline to generate TSC maps for each participant. TSC maps were generated in Matlab (v. 9.8.0.1323502, R2020a) using an in-house written script, using the method described in Lewis et al., (2021). TSC maps were subsequently co-registered to each participant's T1-weighted image using a rigid affine co-registration in SPM12 (Ashburner and Friston, 1997). As TSC is highest in CSF, the maps were masked with the binary parenchymal mask to remove CSF. To normalise to MNI space, the non-linear registration warps obtained from registration of T1-weighted images were applied. Finally, TSC maps were masked with the RSN binary masks and the binary masks of the brain outside each RSN (**Fig.2**). Histograms of TSC values in each masked map were visually inspected, and because a substantial proportion deviated from normality, median values were extracted from each masked TSC map.

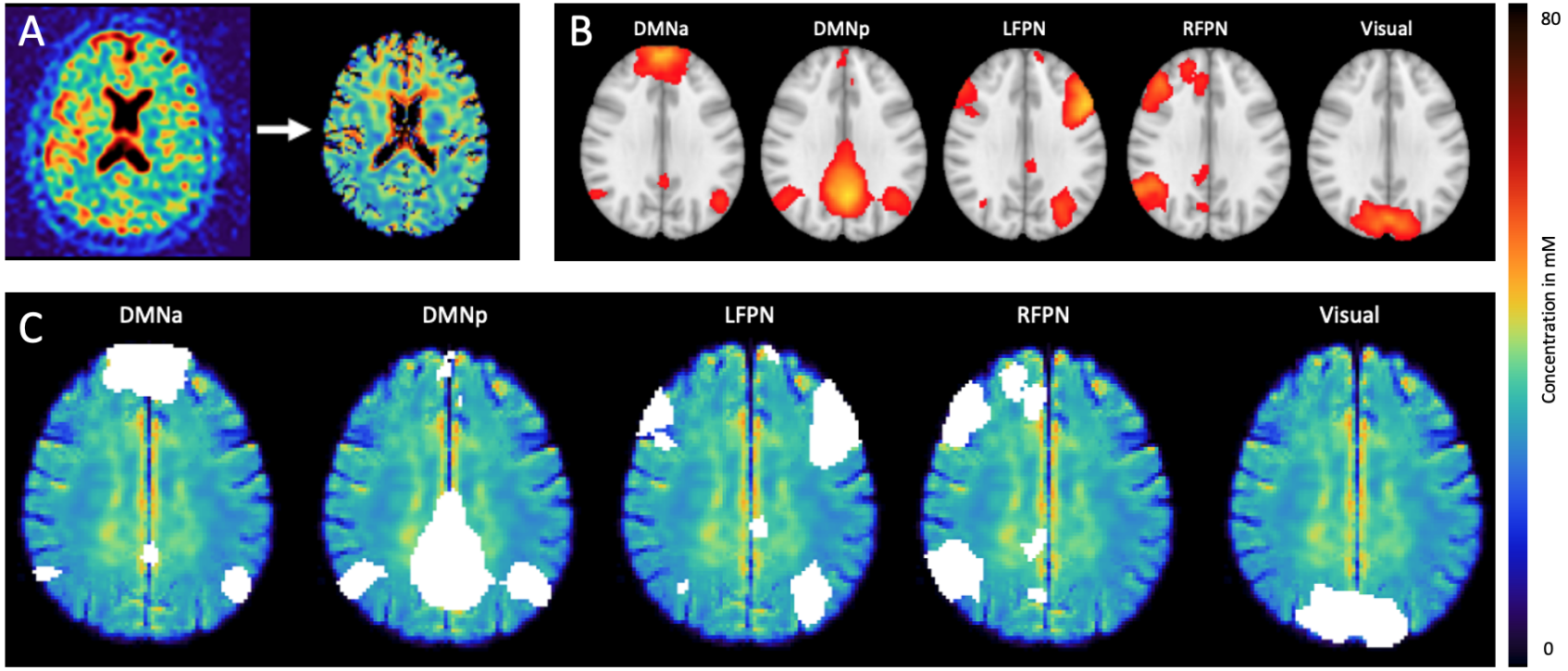

Figure 5.2: Extraction of total sodium concentration values from resting state networks
CSF was masked out of TSC maps before they were registered to MNI space (A). Four cognitively relevant RSNs and one control network (visual) were identified through independent component analysis (B). Binary masks of each network were applied to TSC masks in MNI space and median values were extracted for statistical analyses within and outside each RSN. TSC maps in section A are from a single control participation. RSNs in section B are overlaid on an MNI standard template. TSC map in section C is the average TSC map for all RRMS patients. Colour bar applies to sections A and C. Abbreviations: CSF = Cerebrospinal Fluid, DMNa = Anterior Default Mode Network, DMNp = Posterior Default Mode Network, LFPN = Left Frontoparietal Network, MNI = Montreal Neurological Institute, RFPN = Right Frontoparietal Network, RSN = Resting State Network, TSC = Total Sodium Concentration

5.2.8 Statistical analyses

Statistical analyses of demographic, clinical and MRI measures were performed in SPSS version 23.0 (IBM Corp., 2015). Distributions of all variables were checked through visual inspection of histograms in conjunction with application of Kolmogorov-Smirnov tests. Variables deviating from normality were analysed with non-parametric tests.

A longitudinal assessment of cognitive test scores of the 23 patients who attended both screening and MRI was assessed with a paired sample t-test and Wilcoxon signed-ranks test. We then carried out the following analyses:

1. To assess whether RSN regions are more susceptible to MS pathology than other regions in the brain, paired-samples t-tests and Wilcoxon signed-rank tests were used to compare TSC in each RSN and the rest of the brain. This comparison was performed in both MS patients and controls.
2. We then compared whether RRMS patients and controls differed in TSC within RSNs using independent samples t-tests and Mann-Whitney U-tests.
3. Finally, in the RRMS group, we examined the correlations between TSC values within RSNs and cognitive tests scores to understand the relationship between the energy state of the brain's 'hub' regions and cognitive function.

Due to the preliminary nature of the findings, no multiple comparisons correction was applied.

5.2.9 Data availability

Data are available from the corresponding authors upon reasonable request.

5.3 Results

5.3.1 Demographic, clinical, neuropsychological and conventional MRI characteristics

The demographic, clinical and conventional MRI characteristics of all participants are presented in **Table 1**.

At baseline (i.e. first cognitive test), 11 of the RRMS patients (24%) met the definition of cognitive impairment of $\geq$1.5 SD below normative values on $\geq$2 tests. Of the 23 patients who were retested two years later, four (17%) met the criterion for cognitive impairment at baseline. At follow up, a mean of 2.23 years (SD 0.36) from screening, one additional patient met the criterion for impairment (five patients, 22%). These rates however mask some key differences in cognitive performance in individual patients. Three patients who met the criterion for cognitive

impairment at baseline did not at follow up. Four patients classed as cognitively intact at baseline demonstrated cognitive impairment at follow-up.

At the group level there was no significant cognitive decline on any test in the battery or on a mean global cognitive Z-score calculated from normative values from baseline to follow up (**Table 2**). In contrast, we found some improvement on the serial recall test, long term storage condition ($Z = -2.91$, $p = .004$), and on the world list generation test ($Z = -2.36$, $p = .018$) from baseline to follow up.

Similarly, patients did not show worsening in physical functioning across the two time points in either EDSS bands, disease steps or on the 9 hole peg test. However, some worsening was seen on the 25 foot walking test ($Z = -3.24$, $p = .001$) (**Table 1**).

The RRMS subsample that returned for an MRI showed no significant difference in age or sex ratio compared to controls. However, patients had lower brain volume ($t(12.68) = 2.31$, $p = .039$), and white matter volume ($t(3.64) = 13.40$, $p = .003$), but showed no significant differences in grey matter volume (**Table 1**).

Table 5.1: Demographic and clinical characteristics

			RRMS sub-sample with follow-up		
	Controls (n=10)	**RRMS (n=45)**	**At screening (n=23)**	**At MRI (n=23)**	***Inferential statistics***
Demographic characteristics					
Mean age, years (SD)	59.7 (22.37)	41.3 (10.23)	41.13 (10.09)	43.52 (9.91)	$t(10.57) = 2.20$, $p = .051$[b]
Sex ratio, F/M	4/6	33/12	16/7	16/7	$p = .139$[b]
Education level, high/low†	*	30/15	13/10	13/10	
Median disease duration, years (range)	N/A	2.58 (0.08-6.00)	3.00 (0.17-6.00)	5.67 (2.00-10.42)	
On DMT, yes/no	N/A	38/7	18/5	19/4	$p = 1.00$[a]
Physical functioning					
EDSS band 0-4.0/4.5-6.5/7.0-7.5/7.5-9.5	*	41/4/0/0	22/1/0/0	21/2/0/0	$p = 1.00$[a]
Median disease steps (range)	*	1.00 (0.00-4.00)	1.00 (0.00-3.00)	1.00 (0.00-3.00)	$Z = -0.38$, $p = .705$[a]
Median MSFC 25FWT, sec (range)	*	5.47 (3.82-24.84)	5.35 (3.82-8.55)	6.78 (2.82-12.93)	$Z = -3.24$, $p = .001$[a]
Median MSFC 9HPT dominant hand, sec (range)	*	18.53 (14.64-41.59)	18.51 (14.64-34.83)	18.78 (14.48-38.28)	$Z = -0.37$, $p = .715$[a]
MRI measurements					
Median T2 lesion volume, mL (range)	N/A	N/A	N/A	0.74 (0.00-28.81)	
Mean normalised brain volume, L (SD)	1.45 (0.08)	N/A	N/A	1.39 (0.05)	$t(12.68) = 2.31$, $p = .039$[b]
Mean normalised grey matter volume, L (SD)	0.72 (0.05)	N/A	N/A	0.72 (0.03)	$t(12.52) = -0.22$, $p = .829$[b]
Mean normalised white matter volume, L (SD)	0.73 (0.05)	N/A	N/A	0.66 (0.04)	$t(3.64) = 13.40$, $p = .003$[b]

† Education below university considered low, Bachelor's degree and above considered high
* Data not available for control group.
[a]Comparison of RRMS subsample across two timepoints.
[b]Comparison of RRMS sub-sample at MRI timepoint and controls.
T-tests were used for comparisons of continuous variables with a normal distribution. Variables which were not normally distributed were assessed with Wilcoxon signed-ranks tests. Categorical variables were tested with the chi-squared test and McNemar test, for independent and repeated samples, respectively. Abbreviations: 25FWT = 25 Foot Walk Test, 9HPT = 9 Hole Peg Test, DMT = Disease Modifying Treatment, EDSS = Expanded Disability Status Scale, F = female, M = male, MSFC = Multiple Sclerosis Functional Composite, RRMS = relapsing remitting multiple sclerosis, SD = Standard Deviation

Table 5.2: Longitudinal neuropsychological test performance

	Sub-sample with follow-up		
	At screening (n=23)	**At follow-up (n=23)**	***Inferential statistics***
Median SRT LTS sum (range)	53.00 (8.00-66.00)	58.00 (35.00-70.00)	Z = -2.91, p = .004
Mean SRT CLTR sum (SD)	39.61 (15.45)	44.91 (15.70)	t(22) = -1.82, p = .083
Median SRT delayed sum (range)	10.00 (5.00-12.00)	10.00 (7.00-12.00)	Z = -1.11, p = .267
Mean SPART total over 3 trials (SD)	18.04 (3.72)	20.35 (6.55)	t(22)= -2.03, p = .055
Median SPART delayed (range)	7.00 (4.00-10.00)	8.00 (0.00-10.00)	Z = -0.65, p = .516
Mean SDMT (SD)	55.44 (11.28)	56.13 (10.27)	t(22) = -0.45, p = .657
Median PASAT2 (range)	33.00 (0.00-51.00)	35.00 (0.00-51.00)	Z = -1.09, p = .276
Median PASAT3 (range)	50.00 (0.00-57.00)	49.00 (0.00-60.00)	Z = -0.49, p = .626
Median WLG (range)	29.00 (16.00-37.00)	31.00 (17.00-37.00)	Z = -2.36, p = .018
Mean global cognitive Z-score (SD)	-0.28 (0.67)	-0.05 (83)	t(22) = -2.05, p = .052

Paired samples t-test used for variables with a normal distribution. Those variables which were not normally distributed were assessed with Wilcoxon signed rang test. Abbreviations: PASAT2 = Paced Auditory Serial Addition Test 2 second version, PASAT3 = Paced Auditory Serial Addition Test 3 second version, SD = Standard Deviation, SDMT = Symbol Digit Modalities Test, SPART = Spatial Recall Test, SRT = Selective Reminding Test, SRT LTS = Selective Reminding Test Long Term Storage, SRT CLTR = Selective Reminding Test Consistent Long-Term Storage, WLG = World List Generation test

5.3.2 Total sodium concentrations in resting state network regions

In both patients and controls TSC was higher within network regions than in the rest of the brain (**Table 3**). In the RRMS patients this finding was significant in the DMNa (t(22)= 3.95, p = .001), DMNp (t(22)= -9.23, p < .001), LFPN (Z = -3.47, p = .001) and RFPN (t(22)= 4.69, p < .001), and in control participants in the DMNa (t(9) = 3.34, p = .009), DMNp (t(9) = 6.00, p < .001) and RFPN (t(9) = 4.96, p = .001), but not the LFPN (Z = -0.36, p = .721).

5.3.3 Group differences in total sodium concentrations in resting state network regions

A comparison of TSC in RSN between RRMS patients and controls found TSC to be lower in patients in the DMNa (t(20.19) = 2.14, p = .045), RFPN (t(24.23) = 2.09, p = .047), and LFPN (t(25.45) = 2.27, p = .032). TSC did not differ significantly between groups in the DMNp (t(28.21) = 1.20, p = .241) (**Fig.3**). **Fig.4** shows the average TSC concentrations in controls and RRMS patients, respectively.

Table 5.3: Total sodium concentration inside and outside resting state network regions

	Controls (n =10)			RRMS (n=23)		
	Inside RSN	**Outside RSN**	***Inferential statistics***	**Inside RSN**	**Outside RSN**	***Inferential statistics***
DMNa TSC, mean (SD)	36.43 (5.46)	33.38 (3.76)	$t(9) = 3.34$, $p = .009$	31.74 (6.45)	30.34 (6.35)	$t(22) = 3.95$, $p = .001$
DMNp TSC, mean (SD)	35.56 (3.44)	33.38 (3.83)	$t(9) = 6.00$, $p < .001$	33.58 (5.97)	30.21 (6.38)	$t(22) = -9.23$, $p < .001$
LFPN TSC, median (range)	32.66 (29.52-41.45)	32.25 (28.74-40.94)	$Z = -0.36$, $p = .721$	30.17 (16.93-39.54)	30.84 (17.37-43.19)	$Z = -3.47$, $p = .001$
RFPN TSC, mean (SD)	34.68 (3.85)	33.41 (3.80)	$t(9) = 4.96$, $p = .001$	31.19 (5.49)	30.34 (6.41)	$t(22) = 4.69$, $p < .001$

Paired samples t-test used for variables with a normal distribution. Those variables which were not normally distributed were assessed with Wilcoxon signed rang test. Abbreviations: DMNa = anterior Default Mode Network, DMNp = Default Mode Network, LFPN = Left Frontoparietal Network, RFPN = Right Frontoparietal Network, RRMS = Relapsing Remitting Multiple Sclerosis, RSN = Resting State Network, SD = Standard Deviation, TSC = Total Sodium Concentration

5.3.4 Relationship between sodium concentrations in network regions and cognitive function

In RRMS patients, performance on the PASAT3 correlated positively with TSC values within the LFPN ($rs(21) = 0.437$, $p = .037$), RFPN ($rs(21) = 0.425$, $p = .043$) and visual networks ($rs(21) = 0.468$, $p = .024$) (**Fig.5**). No other associations between RSN TSC and cognitive test performance were statistically significant.

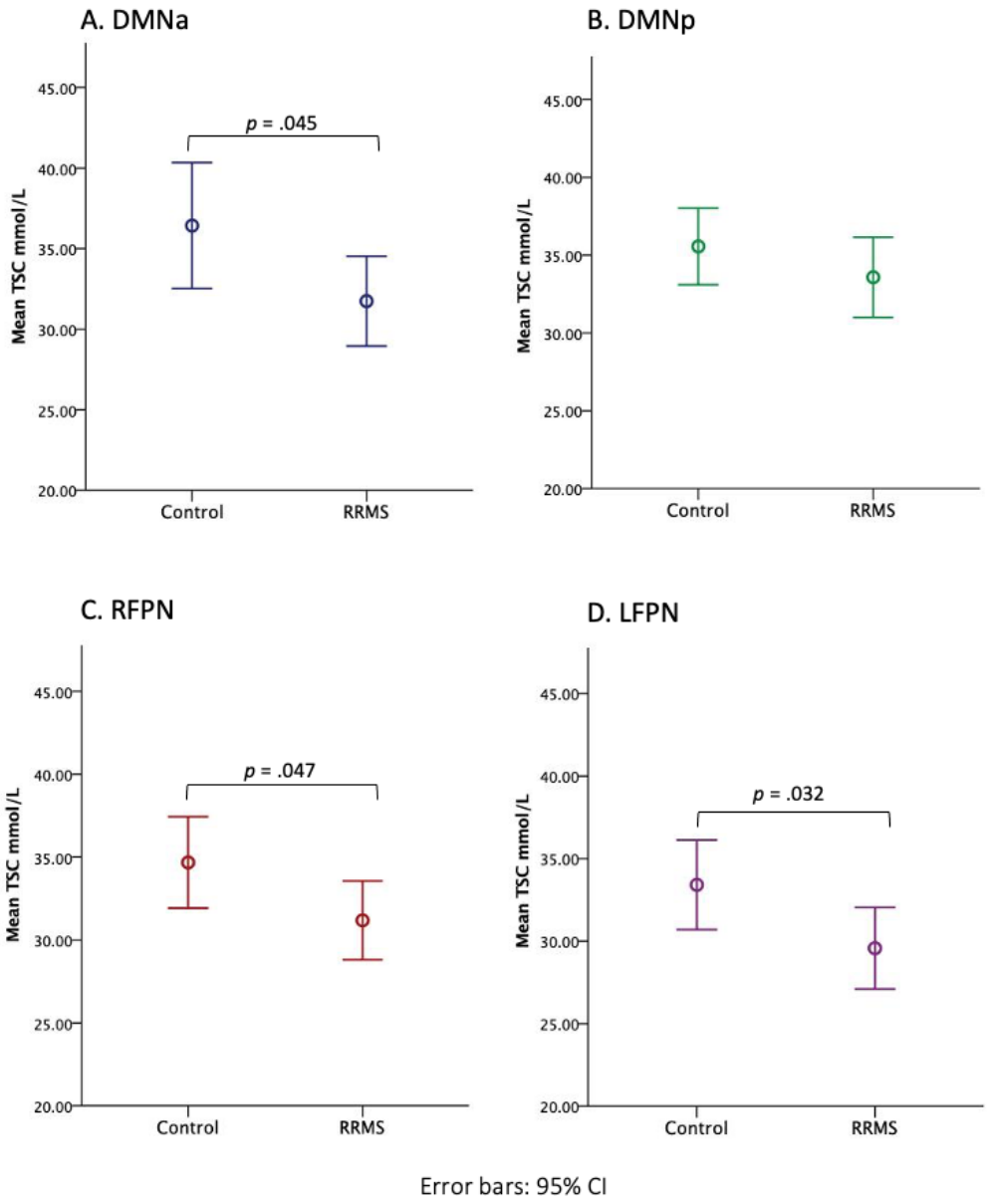

Figure 5.3: Mean total sodium concentrations in resting state network regions in RRMS patients compared to controls

Mean total sodium concentrations in controls and RRMS patients in the (A) anterior default mode network (DMNa), (B) posterior default mode network (DMNp), (C) right frontoparietal network (RFPN), and (D) left frontoparietal network (LFPN). Error bars show 95% confidence intervals. p values represent independent samples t-tests. Abbreviations: RRMS = relapsing remitting multiple sclerosis, TSC = total sodium concentration

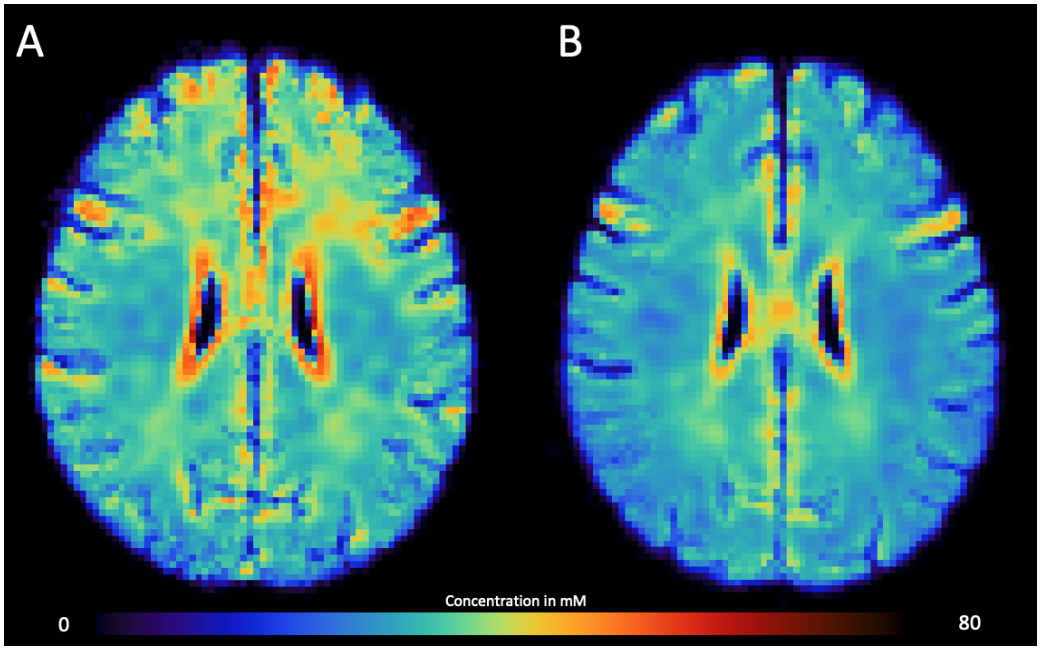

Figure 5.4: Average total sodium concentrations across the brain in controls (A) and RRMS patients (B)

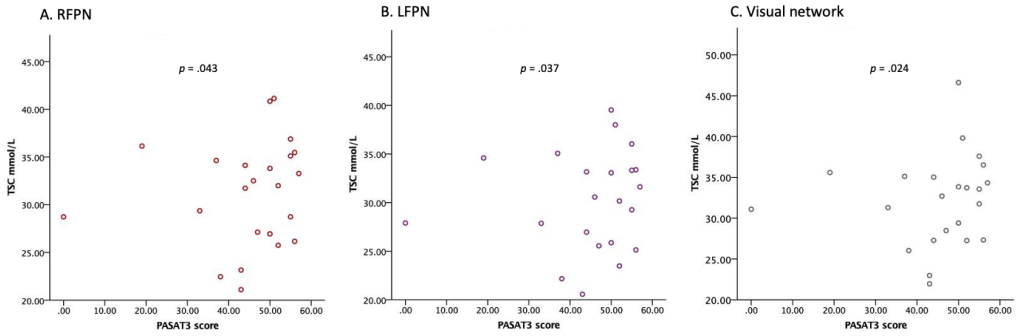

Figure 5.5: Associations between total sodium concentrations in resting state network regions and PASAT3 performance in RRMS patients
Significant positive correlations between PASAT3 score and TSC in the (A) right frontoparietal network (RFPN), (B), left frontoparietal network (LFPN) and (C) visual networks. p values represent Spearman correlation tests. Abbreviations: PASAT3 = Paced Auditory Serial Addition Test 3 second version, RRMS = relapsing remitting multiple sclerosis, TSC = total sodium concentration.

5.4 Discussion

In this study we examined whether resting state network hubs show differences in their concentrations of sodium levels compared to the rest of the brain, and whether this may be affected in MS. We provide the first evidence that sodium concentrations are generally greater in functional network hub regions than other brain regions, that they may be abnormal in people with early to mid-RRMS, and that this relates to deterioration in cognitive function. We consider what these findings tell us about the energy state of network regions, and how this can be further addressed in future research.

5.4.1 Rate of cognitive impairment

Rates of cognitive impairment were low in our sample of RRMS patients with a short disease duration. Within the first six years following diagnosis less than a quarter of the sample met criteria for impairment. This rate is substantially lower than the 40-70% often cited in research literature for MS overall (Chiaravalloti and DeLuca, 2008; Sumowski et al., 2018), as well as the rate of around 40-50% reported in early studies of newly diagnosed patients (Deloire et al., 2005; Patti et al., 2009; Reuter et al., 2011). However, more recent studies report lower rates of impairment in early MS, of around 20% at year five (Achiron et al., 2013) and 25% at year ten (Carotenuto et al., 2021), consistent with the rate found in the present study. A possible explanation for this large difference between studies could be availability of DMTs. Earlier studies did not report on whether patients were on any treatment, which likely reflects limited availability of DMTs at the time they were conducted, while in recent studies a substantial proportion of patients were on DMTs, as was also the case in the present study. While no currently available DMTs have proven efficacy for slowing cognitive decline from clinical phase 3 trials (DeLuca et al., 2020), they are known to slow disease progression and recent evidence suggests that DMT-treated patients experience lower rates of cognitive impairment, suggesting a protective effect on cognitive function (Harel et al., 2019; Landmeyer et al., 2020).

5.4.2 Stability of cognitive function

The rate of cognitive impairment in our sample remained largely unchanged over a two year delay. At the group level we saw no significant decline on any cognitive test; performance on two tests improved over time. This is in line with longitudinal and retrospective cross-sectional studies which have assessed progression over time and found it to increase slowly in the first years following diagnosis (Achiron et al., 2013; Carotenuto et al., 2021). This stability likely reflects well-controlled disease, as very little evidence of physical disability progression was also found in the current sample. It may also reflect psychological adjustment to being diagnosed with a neurological disorder. The improvement in function on two tests is difficult to interpret in such a small sample, and may reflect practice effects, but should be further explored in larger studies.

5.4.3 Sodium concentration in network hub regions

We found evidence of regional variation in total sodium concentration throughout the brain. Resting state network regions showed higher TSC within all four networks than in the rest of the brain, in both patients and controls (not significant in the LFPN in controls). The reasons why we see greater sodium concentrations in resting state network regions are currently unclear. We can however speculate that these regions tend to be more metabolically active (Zhou et al., 2012; Liang et al., 2013; Mann et al., 2020), and so more sensitive to energy changes than the rest of the brain, reflected by higher sodium levels.

TSC is a weighted average of intra- and extracellular sodium concentration and increased TSC could be a result of an increase in sodium concentrations in either or both spaces. One possibility is that because network hub regions are more metabolically active, there is increased neurovascular coupling, which increases the contribution of venous fluid to the TSC measure in these regions compared to rest of the brain. Venous blood has been shown to positively bias sodium concentration measurements in both grey and white matter (Driver et al., 2020). This would explain increased TSC in network regions relative to the rest of the brain in both RRMS patients and controls.

Another possibility is that network regions are more susceptible to atrophy (Chiang et al., 2019), and that loss of neurons in these regions creates a greater extracellular space and thus higher TSC. Given the vastly different implications about the relative health of network hub regions by these two proposed mechanisms, it will be important to investigate them in future studies, by combining Na-MRI with measures of cerebral blood flow or metabolism, or with histological studies, to help explain why functional connectivity network hubs may show increased sodium levels.

5.4.4 Mechanisms of reduced TSC in RRMS compared to controls

The finding of lower TSC in patients than controls differs from previous studies on sodium brain concentrations in MS, which all report sodium accumulation in MS relative to controls, albeit typically within white matter or whole brain grey matter (Inglese et al., 2010, 2013; Zaaraoui et al., 2012; Paling et al., 2013; Petracca et al., 2016; Maarouf et al., 2017; Brownlee et al., 2019; Collorone et al., 2021). These findings were borderline significant and may not have survived multiple comparison correction, so they should not be overinterpreted. Nevertheless, the potential mechanisms must be considered. One possibility, if TSC is influenced by venous flow (Driver et al., 2020) related to neurovascular coupling, is that there is on balance lower neural activity in MS than controls. This could reflect pathological changes in the early stages of the disease, when network regions are not under much stress. With increased pathology and increased metabolic stress over time it would be expected that sodium accumulation occurs in MS relative to controls, but this would need to be tested through longitudinal or larger cross-sectional

studies assessing different disease stages. Moreover, future studies should combine Na MRI with CBF measures to determine the influence of venous fluid of TSC in MS.

Another possibility has been proposed in a study of mild traumatic brain injury, which found lower TSC in patients than controls, and considered that this finding could reflect an increase in the intracellular space, due to cell swelling (Gerhalter et al., 2021).

To consider the mechanisms of TSC decreases in MS, it must be emphasised that the TSC measure is a weighted average of intra- and extracellular sodium concentration. Any change in TSC could therefore reflect mechanisms either in the intracellular space related to demyelination, or in the extra-cellular space, such as due to axonal loss. An upregulation of sodium-potassium ion channels occurs following demyelination and sodium influx into the cell occurs if the cell is not adequately supplied with energy to fuel the ion channel to pump the sodium out again (Foster et al., 1980; Craner et al., 2004; Madelin et al., 2015). However, other effects of this intracellular osmolarity can include water inflow through aquaporin channels, leading to cell swelling and an increase in intracellular volume (Liang et al., 2007; Stokum et al., 2016). If this occurs in MS, it would give greater weighting to the swollen intracellular space relative to the extracellular space in the calculation of the TSC measure, and even if sodium accumulation in the intracellular space is present, it may be masked by the increased ratio of the intracellular space, where TSC is substantially lower than the extracellular space. Given that sodium accumulation is more prominent in advanced and progressive stages of the disease (Paling et al., 2013), intracellular oedema may be an earlier pathological mechanism in the brain's network hubs. Of course, this requires suitable evidence from longitudinal or larger cross-sectional studies, or combined MRI-histological studies.

This potential mechanism of intracellular swelling showing as TSC decreases could also explain the positive correlation between PASAT3 scores and TSC in the LFPN, RFPN and visual networks. This result, showing that low, rather than high, TSC within functional connectivity network regions is related to poor cognitive test performance, differs from findings examining grey matter more generally in MS (Paling et al., 2013; Maarouf et al., 2017; Brownlee et al., 2019; Collorone et al., 2021). However, if oedema is present in the intracellular space it is feasible that it impairs normal network functioning, which in turn influences cognitive performance.

However, like in mild traumatic brain injury (Gerhalter et al., 2021), this explanation of intracellular swelling is speculative in MS and would need to be tested with more advanced methods which can separate intracellular signal, such as inversion recovery (IR) or multiple quantum filtering (MQF) (Ouwerkerk, 2011).

5.4.5 Limitations

This was a preliminary study and several limitations must be acknowledged. First, this was a small sample of RRMS patients with low rates of cognitive impairment. Future studies can involve a larger sample of patients with a greater range of deficits, providing increases in sensitivity to

detect associations, and the opportunity to examine separate groups of ‘cognitively intact’ and ‘cognitively impaired’ patients. Moreover, future studies would benefit from a larger control group, to better understand normal variation in sodium levels throughout the brain. Some of our control participants had a history of vestibular schwannoma. This sits outside the brain parenchyma and therefore should not affect sodium concentrations, however, this cannot be ruled out without formal comparison with an age-matched sample. Related to this, we did not have a control group for several of the key assessments in this study, including neuropsychological testing and rs-fMRI acquisition, and for that reason normative values for cognitive test scores were used and no assessment of functional connectivity abnormalities in RSN regions was performed. For more informative results, it will be important to have a matched control group through all stages of the study, to better understand the relationship between cognitive function and MR measures in RRMS relative to controls. We also did not use a sodium MR measure which can differentiate intra- and extracellular sodium concentrations. Use of more advanced methods, like IR or MQF, which can do this, will be essential for understanding the specifics of sodium abnormalities in MS. Finally, as this was a preliminary study, multiple comparison corrections were not applied, and the possibility of the presence of type I errors must be considered.

5.5 Conclusion

In this first study to combine sodium MRI and rs-fMRI in MS, we found sodium imbalances in the brain’s network hub regions in RRMS patients relative to controls. TSC in functional network hub regions was increased compared to the rest of the brain, but lower in RRMS patients than controls. These changes related to performance on cognitive testing. We speculate that these changes in sodium may reflect early pathological mechanisms following demyelination, such as intracellular oedema, before sodium accumulation occurs. Overall, the findings suggest that sodium abnormalities exist in RSN regions in RRMS. Future studies with larger, well-matched samples are needed to understand why sodium levels may be raised in functional connectivity network regions, and why these may be affected at early stages in people with MS.

5.6 Funding

This work was funded by a Medical Research Council Doctoral Training Partnership grant (MR/N013751/11). Funding for acquisition of control participant data was provided by CRUK and EPSRC Cancer Imaging Centre in Cambridge and Manchester (Award Number: C8742/A18097) and by the Manchester Academic Health Sciences centre (MAHSC).

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Chapter 6

A Tractometry Investigation of White Matter Tract Network Structure and Relationships with Cognitive Function in Relapsing-Remitting Multiple Sclerosis

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This is the Submitted Manuscript version (pre-peer review) of a paper submitted to *NeuroImage:Clinical*, with minor editing to position figures and tables where they should appear in the text.

Abstract

Understanding the brain changes underlying cognitive dysfunction is a key priority in multiple sclerosis to improve monitoring and treatment of this debilitating symptom. Functional connectivity network changes are associated with cognitive dysfunction, but it is less well understood how changes in normal appearing white matter relate to cognitive symptoms. If white matter tracts share a similar network structure it would be expected that tracts within a network are similarly affected by MS pathology. In the present study, we used a tractometry approach to explore patterns of variance in diffusion metrics across white matter (WM) tracts. We investigated whether separate networks, based on normal variation or pathology, appear, and how this relates to neuropsychological test performance across cognitive domains. A sample of 102 relapsing-remitting MS patients and 27 healthy controls underwent MRI and neuropsychological testing. Tractography was performed on diffusion MRI data to extract 40 WM tracts and microstructural measures were extracted from each tract. Principal component analysis (PCA) was used to decompose metrics from all tracts to assess the presence of any co-variance structure among the tracts. Similarly, PCA was applied to cognitive test scores to identify the main cognitive domains. Finally, we assessed the ability of tract components to predict test performance across cognitive domains. We found that a single component which captured pathology across all tracts explained the most variance and that there was little evidence for separate, smaller network patterns of pathology. WM tract components were weak, but significant, predictors of cognitive function in MS. These findings highlight the need to investigate the relationship between the normal appearing white matter and cognitive impairment further and on a more granular level, to improve the understanding of the network structure of the brain in MS.

6.1 Introduction

The main pathological feature in multiple sclerosis is the demyelinating lesion, yet magnetic resonance imaging (MRI) measures of lesions correlate poorly with clinical symptoms, a finding termed the 'clinico-radiological paradox' (Barkhof, 1999, 2002). This is particularly problematic for cognitive symptoms, which affect a large proportion of people with MS, are disabling and associated with poor outcomes, but are poorly understood in terms of pathology (Sumowski et al., 2018), making cognitive dysfunction a challenge in the management of MS. Identifying MRI correlates of cognitive impairment in MS to understand pathological mechanisms is therefore an important, but challenging, research priority in order to inform clinical decisions about diagnosis, monitoring and treatment of cognitive impairment.

Cognitive deficits in MS are often evaluated as a global impairment based on results of neuropsychological tests. However, cognitive impairment involves deficits in separate domains, including processing speed and memory (Charcot, 1877; Benedict et al., 2006; Sepulcre et al., 2006; Migliore et al., 2016; Matias-Guiu et al., 2017; De Meo et al., 2021). Understanding if and how different cognitive domains are susceptible to different underlying brain abnormalities can inform our understanding of the mechanisms of cognitive impairment in MS.

Cognitive functions have been shown to rely on brain networks, rather than individual brain regions (McIntosh, 2000; Bressler and Menon, 2010). In people with MS, cognitive symptoms have been associated with functional network connectivity abnormalities, assessed by resting state fMRI (rs-fMRI, reviewed in Chard et al., 2021; Jandric et al., 2021), but the mechanisms causing these functional connectivity changes are not known. There is evidence to suggest that white matter (WM) damage can influence functional connectivity (Schoonheim et al., 2015; Patel et al., 2018; Tewarie et al., 2018), possibly through alteration of anatomical connections between functionally connected regions (Catani and ffytche, 2005; Dineen et al., 2009). While WM lesions are a poor predictor of cognitive symptoms, tissue outside of lesions is also known to be affected by pathological processes, either due to secondary axonal loss from inflammatory activity in lesions, such as Wallerian degeneration, or to lesion-independent degeneration of axons following demyelination resulting from more diffuse inflammation (Trapp et al., 1998; Bitsch et al., 2000; Trapp and Stys, 2009). Such damage to normal appearing white matter (NAWM) on a clinical MRI scan could cause damage to WM tracts connecting spatially separate but functionally connected regions that support specific cognitive functions. Moreover, if lesions cause damage to a brain network, the functioning of the entire network may be affected.

A number of diffusion MRI studies (dMRI) have established associations between cognitive impairment and damage to NAWM in MS. These studies have largely used whole-brain analyses of the WM, such as tract-based spatial statistics (TBSS, Smith et al., 2006), to show that non-lesional damage in specific WM areas, such as the corpus callosum and cingulum, correlates with cognitive symptoms (e.g. Dineen et al., 2009; Sbardella et al., 2013; Meijer et al., 2016). More recently, there has been evidence of covarying patterns of pathology in white matter

tracts. In healthy participants, independent component analysis (ICA) based-approaches have demonstrated patterns of covariance between white matter tracts, thought to reflect shared phylogenetic and functional relationships (Wahl et al., 2010; Li et al., 2012). It can be expected that tracts that share characteristics and/or are part of the same networks are similarly susceptible to pathology. One study demonstrated this in a sample of secondary progressive MS patients (SPMS). Using ICA on a TBSS skeleton to identify patterns of covariance, possibly reflecting WM pathology, Meijer et al., (2016a) found eighteen components corresponding to WM tracts and visually grouped them into six different WM classes on the basis of anatomical features. FA values within some of these classes correlated with cognitive function, suggesting cognitively-relevant patterns of neurodegeneration (Meijer et al., 2016a).

Determining whether such patterns of pathology are also present in those with relapsing-remitting disease is necessary to identify at what stage in the disease such neurodegeneration occurs. While it is known that there is a greater degree of WM damage in SPMS (Kutzelnigg et al., 2005), people with RRMS also show both cognitive impairment and functional network changes, so it is plausible that they also show shared patterns of WM damage. It is also important to understand whether the patterns of WM damage appear when using an unbiased principal component analysis, which does not rely on manual grouping of component classes.

The standard for whole brain WM analysis has long been TBSS, which works by skeletonising the centre of each tract, based on high average FA values, to improve registration of non-homologous brains (Smith et al., 2006). As such TBSS does not reconstruct individual WM tracts, raising concerns about its anatomical accuracy (Bach et al., 2014). An alternative to TBSS for obtaining anatomically accurate WM tracts is tractography, which fits a diffusion tensor or alternative model at each voxel to trace the fibre orientation through the WM (Mori et al., 1999; Basser et al., 2000; Catani et al., 2002; Jeurissen et al., 2019). While challenging in its own right, technological developments have improved the ease and accuracy of individual, automated tratography (Warrington et al., 2020), and it has been shown that newer tracking algorithms can perform satisfactorily in the presence of MS lesions, and reconstruct even tracts with a high prevalence of lesions (Lipp et al., 2020). This makes tractography a feasible option for segmenting the brain into a large number of functionally meaningful WM units for investigating whether damage to non-lesioned parts of specific tracts can help understand cognitive symptoms in MS.

In the present study we conduct an exploratory analysis of WM microstructure diffusion metrics in a large sample of RRMS patients using a tractometry approach (Bells et al., 2011). We use automated individual tractography to reconstruct 40 WM tracts and extract four diffusion metrics from the non-lesioned parts of the tracts. By conducting principal component analysis (PCA) of extracted metrics we can test whether their grouping reflects the known network structure of the brain and covarying patterns of damage across tracts. Exploring this can help us understand the patterns of degeneration in normal appearing tissue in MS.

Thus, the present study aims to: 1) determine if WM tracts can be decomposed into components of shared covariance based on a network or pathology structure; 2) assess the cognitive domains structure present in common neuropsychological test data; 3) explore the relationship between WM tract components and cognitive domains in RRMS.

6.2 Material and Methods

6.2.1 Participants

Demographic, clinical and MRI data was collected in one study session from 102 RRMS patients and 27 healthy controls. This cohort has also been investigated and described in previous work (Jandric et al., 2021b) . All participants were between 18 and 60 years of age, right-handed and had no contraindications for MR scanning. Patients fulfilled additional eligibility criteria of having no relapses or change to treatment for 3 months prior to the MRI scan, and not having any comorbid neurological or psychiatric disease.

Patients were recruited through the Helen Durham Centre for Neuroinflammation at the University Hospital of Wales and controls from the community. The study was approved by the NHS South-West Ethics and the Cardiff and Vale University Health Board R&D committees. All participants provided written informed consent to participate in the study.

6.2.2 Cognitive Assessment

Participants were assessed with the Multiple Sclerosis Functional Composite (MSFC) (Cutter et al., 1999) and the Brief Repeatable Battery of Neuropsychological Tests (BRB-N) (Amato et al., 2006). The BRB-N consists of the following tests: the selective reminding test of verbal memory, which is scored as the sum of words in long term storage (SRT L sum), the sum of words consistently recalled (SRT C sum) and the words recalled after a delay (SRT delayed); the spatial recall test of visual memory, which is scored over three consecutive trials (Spatial1to3) and on a delayed trial (Spatial delayed); the symbol digit modalities test (SDMT) of attention and concentration; the paced auditorial serial addition test of processing speed, with a three second delay (PASAT3) and with a two second delay (PASAT2); and the word list generation test (WLG) of verbal fluency.

6.2.3 MRI acquisition

MRI data were acquired on a 3 T MR scanner (General Electric HDx MRI System, GE Medical Devices, Milwaukee, WI) using an eight channel receive-only head RF coil. A high-resolution 3D T1-weighted sequence was acquired for identification of T1-hypointense MS lesions, segmentation, registration and volumetric measurements (voxel size = 1 mm x 1 mm x 1 mm, TE = 3.0 ms, TR = 7.8 ms, matrix = 256x256x172, FOV = 256 mm x 256 mm, flip angle = 20°). A T2/proton-density

(PD)-weighted sequence (voxel size = 0.94 mm x 0.94 mm x 4.5 mm, TE = 9.0/80.6 ms, TR = 3000 ms, FOV = 240 mm x 240 mm, 36 slices, flip angle = 90°) and a fluid-attenuated inversion recovery (FLAIR) sequence (voxel size = 0.86 mm x 0.86 mm x 4.5 mm, TE = 122.3 ms, TR = 9502 ms, FOV = 220 mm x 220 mm, 36 slices, flip angle = 90°) were acquired for identification and segmentation of T2-hyperintense MS lesions. A twice refocused diffusion-weighted spin echo echo-planar (SE-EPI) sequence with 6 volumes with no diffusion weighting and 40 volumes with diffusion gradients applied in uniformly distributed directions was acquired for tractometrics analyses (diffusion directions: Camino 40, b = 1200 s/mm2, voxel size = 1.8 mm x 1.8 mm x 2.4 mm, TE = 94.5 ms, TR = 16000 ms, FOV = 230 mm x 230 mm, 57 slices, flip angle = 90°). In addition, a 3D MT sequence (voxel size = 0.94 mm x 0.94 mm x 1.9 mm, TE = 1.8 ms, TR = 26.7 ms, FOV = 240 mm x 240 mm, flip angle = 5°) and mcDESPOT sequence (voxel size = 1.7 mm x 1.7 mm x 1.7 mm, TE = SPGR: 2.1 ms, bSSFP: 1.6 ms, IR-SPGR: 2.1 ms, TR = SPGR: 4.7 ms, bSSFP: 3.2 ms, IR-SPGR: 4.7 ms, FOV = 220 mm x 220 mm, flip angle = SPGR: [3, 4, 5, 6, 7, 8, 9, 13, 18] degrees bSSFP: [10.6, 14.1, 18.5, 23.8, 29.1, 35.3, 45, 60] degrees IR-SPGR: 5 degrees) were acquired to obtain microstructure parameter maps as described in Lipp et al., (2019).

6.2.4 MRI processing

6.2.4.1 Structural image analysis and lesion marking

Structural 3D T1-weighted images from patients were lesion filled, as described in previous work (Lipp et al., 2019), to allow better segmentation and registration of brain tissue, then segmented into grey matter (GM), WM and cerebrospinal fluid (CSF) using FSL's Automated Segmentation Tool (FAST) (Zhang et al., 2001). Intracranial volume (ICV) was calculated with fslstats as the number of voxels in skull-stripped T1-weighted images. Volumetric measurements normalised for head size, including normalised brain volume (NBV), normalised GM volume (NGMV) and normalised WM volume (NAWM) were quantified from lesion-filled 3D T1-weighted images with FSL's SIENAX tool (Smith et al., 2002). Lesion volume was calculated from binary lesion masks created as part of lesion marking. The lesion-filled 3D T1-weighted images were non-linearly registered to the Montreal Neurological Institute (MNI) 152 template space using FSL's FNIRT tool and the warps saved for subsequent analyses.

6.2.4.2 dMRI analysis: quantification of FA and RD maps

Preprocessing of dMRI data in ExploreDTI (v 4.8.3 (Leemans et al., 2009)) included motion correction and corrections for eddy current and EPI-induced geometrical distortions. The latter was achieved by registering each diffusion image to its respective (skull-stripped and downsampled to 1.5 mm) 3D T1 image (Irfanoglu et al., 2012) using Elastix (Klein et al., 2010), with appropriate reorientation of the diffusion-encoding vectors (Leemans and Jones, 2009). dMRI images were further processed in FSL's FDT tool to fit diffusion tensors and fit the probabilistic diffusion model

using the Bedpostx tool (Behrens et al., 2003, 2007). Fractional anisotropy (FA) and radial diffusivity (RD) maps were normalised to MNI space through the application of the previously obtained warps. FA and RD maps were available for all participants.

6.2.5 MTR and MWF maps

MTR and MWF maps were calculated as described in Lipp et al., (2019), which included co-registration with participants' T1-weighted images. T1-weighted to MNI warps were applied for registration to MNI space. MTR maps were obtained for all HC and 101 MS patients, and MWF for 25 HC and 95 MS patients. MTR and MWF maps could not be obtained for some participants due to specific absorption rate (SAR) constraints of the mcDESPOT sequence, or due to logistical reasons.

6.2.6 Tractography and tractometry

Bedpostx outputs and T1-weighted to MNI registration warps were fed into FSL's Xtract tool which uses standardised protocol seeding, exclusion, waypoint and termination masks to perform automated individual tractography to reconstruct 42 WM tracts, then uses the warps to register the outputs to MNI space (Warrington et al., 2020). All tracts were visually inspected to ensure that they had reconstructed well. In a large proportion of participants, both MS and HC, the fornix failed to reconstruct or was missing portions of the tract. As such, it was not considered for any analyses. The remaining 40 tracts yielded reconstructions in line with their anatomical descriptions and were retained.

Because the protocol masks are based on probability atlases of tracts, they are not strictly limited to the WM. To ensure that tract masks used for our analyses were limited to the WM to be suitable for tractometry analyses, we first thresholded the masked probabilistic tractography outputs at 0.001 and then masked the output further with the respective WM mask from the segmented T1 weighted scan. These tracts were binarised and all voxels marked as lesions were removed to get a mask of only the non-lesioned part of the tract. The proportion of each tract affected by lesions in each participant was calculated by counting the lesion voxels in each tract relative to the voxels of the whole tract, averaged over all 102 participants for each tract.

To obtain FA, RD, MTR and MWF metrics from each reconstructed tract, each metric was averaged across all voxels in the non-lesioned tract masks. Distributions of each metric in each tract were assessed through histogram inspection in MATLAB (v R2020a). The majority of the FA, RD, MTR and MWF tract maps had distributions deviating from normality so median values were extracted.

6.2.6.1 Metrics dimensionality reduction

The four WM microstructure metrics were extracted from each tract and decomposed into one metric using principal component analysis. This dimensionality reduction analysis was performed on the FA, RD, MWF and MTR metrics in RStudio v 1.4.1103 using the principal function (RStudio Team, 2020). Mean values were imputed for the missing MTR and MWF values and a dataset comprising of 4 WM metrics x 40 WM tracts x 27 or 102 participants (for HC and MS, respectively) was created. The four metrics were reduced to a lower dimensionality that explains the maximum amount of variance in the data through a PCA performed across participants and tracts, as described by Chamberland et al., (2019). First, a correlation matrix of Pearson's r was calculated to determine feasibility of a PCA based on high correlations and tested with Bartlett's test of sphericity to ensure a significant difference from an identity matrix. The metric principal component for further analyses was chosen on the basis of an inspection of the scree plot (Cattell, 1966) and eigenvalues >1. A metric component score for the first extracted principal component, explaining most variance, was calculated for each tract and participant.

6.2.6.2 Principal component analysis of WM tract covariance

To assess whether patterns of shared covariance exist across the WM, an additional PCA, following the same process, was performed in HC and MS, respectively. For this PCA, the metric component score of the first extracted component was used as the WM microstructure metric for each tract.

6.2.6.3 Regression of sources of heterogeneity in data

To identify the sources of variance in a tract component (TC) resulting from this PCA, its component scores were correlated with a number of demographic and anatomical variables: age, sex, years of education, ICV, lesion volume, NBV, NGMV and NWMV. Multivariate regressions were performed to identify which of these variables explained most variance of the TC. First, all demographic and anatomical variables were inputted into a correlation matrix to assess the degree of multicollinearity. As there was high correlation between NBV, NGMV and NWMV, only NBV was included in the model, along with age, sex, education ICV and lesion volume. The demographic and anatomical variables that came out as the strongest predictors in the regression analysis were regressed out of the raw data and the metric dimensionality reduction and PCA of WM tract covariance steps were performed again. The aim of this was to explore whether any potential heterogeneity in the sample could have influenced the ability of the PCA to identify different components reliably. A Varimax rotation was applied to the first four principal components, based on eigenvalues >1 and proportion variance explained, to improve interpretability.

6.2.6.4 Cognitive test principal component analysis

Finally, we aimed to find the cognitive domain structure in this dataset. As for the metric and tract PCAs, a correlation matrix was constructed based on the scores on each of the BRB-N tests, and on the basis of confirmed correlations between tests and a significant Bartlett's test, a PCA was performed to decompose the battery tests into cognitive domains. Principal components were extracted on the basis of scree plots, eigenvalues and variance explained. A Varimax rotation was applied for interpretability. To understand what influences cognitive function, the resulting rotated cognitive components (CCs) were correlated with the tract components and all demographic and anatomical variables, after checking multicollinearity among predictors. NBV, NGMV and NWMV correlated highly so the variables included were age, sex, education, ICV, lesion volume and NBV. Multivariate regression analyses were performed to determine the relationship between WM tract microstructure and cognitive domains.

6.2.7 Statistical analyses

All analyses were performed in RStudio v 1.4.1103 (RStudio Team, 2020) with the exception of analyses of demographic and clinical variables, which were analysed in SPSS version 23.0 (IBM Corp., 2015). All variables were tested for normality through visual inspection of histograms and Q-Q plots and application of Kolmogorov-Smirnov tests. Variables which did not have a normal distribution were analysed with non-parametric tests.

6.3 Results

6.3.1 Participant characteristics

Demographic and clinical characteristics of the sample are presented in Table 1. Overall, patients were older and less educated than healthy controls, had lower NBV and NGMV, poorer upper and lower limb function, and performed worse on all cognitive tests except the word list generation test assessing verbal fluency.

Table 6.1: Demographic, clinical and neuropsychological characteristics

	HC (n=27)	RRMS (n=102)	*Inferential test results*
Age, yr (median, range)	37.00 (23-59)	45.00 (18-60)	$U = 958.00$, $p = .015$
Male/female, n	12/15	33/69	$\chi^2(1) = 1.37$, $p = .241$
Education years (median, range)	19.00 (12-30)	15.00 (10-30)	$U = 613.50$, $p < .001$
Mean disease duration, yr (median, range)	N/A	12.24 (1-39)	N/A
Timed 25 Foot Walk Test (median, range)	4.35 (3.2-5.4)	5.25 (3.6-26.8)	$U = 572.50$, $p < .001$
9-Hole Peg Test (median, range)	18.65 (15.35-23.00)	21.75 (16.35-59.50)	$U = 537.50$, $p < .001$
SRT L sum (median, range)	0.00 (-1.26-1.37)	-0.54 (-4.72-1.47)	$U = 914.00$, $p = .009$
SRT C sum (mean, SD)	0.00 (1.00)	-0.88 (1.22)	$t(49.06) = 3.86$, $p < .001$
SRT delayed (median, range)	0.06 (-2.13-1.16)	-0.49 (-4.31-1.15)	$U = 881.00$, $p = .004$
Spatial1to3	0.00 (1.00)	-0.74 (1.20)	$t(47.76) = 3.29$, $p = .002$
Spatial delayed	0.11 (-2.45-1.14)	-0.91 (-2.96-1.14)	$U = 794.00$, $p = .001$
SDMT	0.00 (1.00)	-0.88 (0.98)	$t(40.14) = 4.09$, $p < .001$
PASAT3 (median, range)	-0.03 (-2.61-1.26)	-1.32 (-8.26-1.42)	$U = 692.00$, $p < .001$
PASAT2	0.17 (-1.71-2.29)	-0.77 (-4.45-2.41)	$U = 768.00$, $p < .001$
WLG	0.00 (1.00)	-0.24 (0.88)	$t(37.48) = 1.14$, $p = .263$
Normalised brain volume, L (mean, SD)	1.56 (0.07)	1.51 (0.08)	$t(41.94) = 3.33$, $p = .002$
Normalised grey matter volume, L (median, range)	0.81 (0.72-0.89)	0.77 (0.61-0.89)	$U = 755.00$, $p < .001$
Normalised white matter volume, L (median, range)	0.76 (0.68- 0.81)	0.74 (0.66- 0.83)	$t(40.43) = 1.56$, $p = .127$

Independent samples t-tests were used for comparisons of variables with a normal distribution. Mann-Whitney U tests were used for variables which were not normally distributed. Sex, being a categorial variable, was tested with the chi-squared test. Cognitive test scores are reported as Z-scores.

Abbreviations: HC = healthy controls; PASAT2 = paced auditory serial addition test 2 second delay; PASAT3 = paced auditory serial addition test 3 second delay; RRMS = relapsing remitting multiple sclerosis; SD = standard deviation; SDMT = symbol digit modalities test; Spatial1to3 = spatial recall test average score over three trials; Spatial delayed = spatial recall test score at

the delayed trial; SRT delayed = serial recall test scores at the delayed trial; SRT L sum = serial recall test long term storage sum of scores; SRT L sum = serial recall test consistent recall sum of scores; WLG = word list generation test.

6.3.2 Metric dimensionality reduction

The following results are for the MS group unless otherwise indicated. Detailed results for healthy controls are presented in Appendix 1.

Bartlett's test of sphericity was significant (2(6) = 155.85, p <0.001) indicating the suitability of performing a PCA. Based on scree plot inspection and eigenvalues >1, only the first principal component, which explained 60% of variance, was extracted. The component loadings were 0.92 for FA, -0.87 for RD, 0.88 for MWF and 0.15 for MTR, indicating that the main contributors to the component were FA, RD and MWF.

6.3.3 Principal components of WM tract covariance

In MS patients, a correlation matrix of all WM tracts was shown to be significantly different from an identity matrix using Bartlett's test of sphericity (2(780) = 5803.14, p <0.001), indicating the suitability of performing a PCA to assess the covariance structure of WM tracts (see Figure 1A for the metric and tract correlation matrices and scree plots). The scree plot showed one strong principal component (65% variance explained), but three additional components had eigenvalues >1 (7%, 4% and 3% variance explained, respectively). A Varimax rotation was therefore applied to these first four principal components to improve interpretability. After rotation all tracts still loaded positively on tract component 1 (TC1), demonstrating a great degree of shared variance between white matter tracts.

In MS patients TC1 correlated most strongly with lesion volume (r=-0.73), NGMV (r=0.41), and NBV (r=0.31) (see Figure 1B). A multiple linear regression model showed that the variance of TC1 was best explained by lesion volume (ß = -0.74, p < 0.001) in a model explaining 54% of variance (R2 = 0.54, F(6, 95) = 20.58, p < 0.001). See Table 2 for full model statistics.

After regressing out lesion volume, correlations matrices for WM metrics and tracts, respectively, showed somewhat weaker correlations but still passed Bartlett's test of sphericity (2(6) = 90.01, p < 0.001 for metrics, 2(780) = 4347.86, p < 0.001 for tracts), and yielded the same PCA structure (see Figure 1C), indicating that most tracts still load positively onto a single component. After a component rotation of the four tracts that explained most of the variance (after rotation:79% cumulative variance; 30%, 25%, 21%, 0.03% for TCs 1-4, respectively) most tracts still loaded positively on the first tract component, especially large WM tracts like the optic radiations, middle longitudinal fasciuli, forceps major, inferior fronto-occipital fasciculi, vertical occipital fasciculi and acoustic radiations. Similarly, the tracts which loaded most highly on TC2 were large tracts connecting distal areas of the brain, including the superior thalamic radiations,

corticospinal tracts, frontal aslants, superior longitudinal fasiculi and the arcuate fasciculi. TC3 in contrast consisted mainly of shorter tracts, including sub-sections of the cingulum, the anterior commissure, forceps minor and uncinate fasciuli. Only the middle cerebellar penduncle loaded highly on TC4. The principal component analysis screeplot showing a single dominant component and the high tract loadings of all tracts onto the first of the four rotated components demonstrates a high covariance between all tracts investigated. Please see Table 3 for full details of tract loadings on the four components.

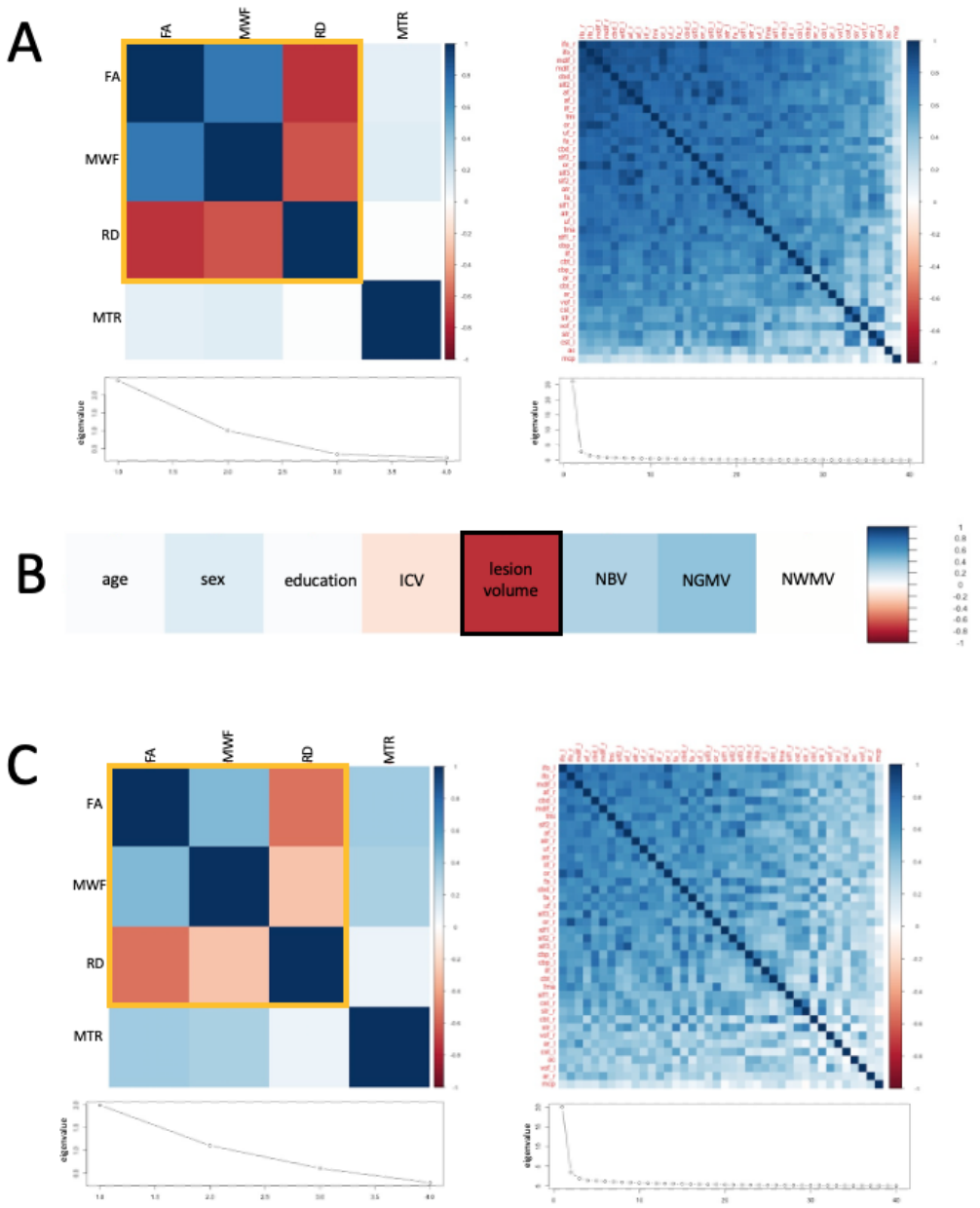

Figure 6.1: Metric and tract principal component analysis in multiple sclerosis patients
Figure 1A shows the correlation matrices and scree plots for the PCA ran on the four white matter microstructural metrics (left) and the white matter tracts based on the first component from the metric PCA (right). Those metrics marked with a yellow line load most on principal component 1. All tracts loaded positively on tract principal component 1. Figure 1B shows correlations between rotated tract principal component 1 (TC1) and demographic and anatomical variables. Variable marked with a black line, lesion volume, was a significant predictor of the principal tract component from Figure 1A in multiple linear regression models. Figure 1C shows the correlation matrices and scree plots for metric and tract PCAs after lesion volume was regressed out.
Abbreviations: FA = fractional anisotropy; RD = radial diffusivity; MWF = myelin water fraction; MTR = magnetisation transfer ratio; ICV = intracranial volume; NBV = normalised brain volume; NGMV = normalised grey matter volume; NWMV = normalised white matter volume.

Table 6.2: Predictors of WM tract covariance and cognitive domains

Dependent variable	Predictors	Model statistics
Unrotated tract component 1	Age: ß = 0.03, *p* = 0.727 Sex: ß = 0.13, *p* = 0.186 Education: ß = -0.03, *p* = 0.690 ICV: ß = -0.03, *p* = 0.721 *Lesion volume: ß = -0.74, p < 0.001* NBV: ß = 0.01, *p* = 0.451989	R^2 = 0.54, *F*(6, 95) = 20.58 , *p* < 0.001
CC1: Verbal cognition	*TC1: ß = 0.30, p = 0.009* TC2: ß = -0.07, *p* = 0.444 TC3: ß = 0.14, *p* = 0.120 TC4: ß = 0.08, *p* = 0.379 Age: ß = -0.01, *p* = 0.957 *Sex: ß = 0.35, p = 0.010* Education: ß = 0.06, *p* = 0.558 ICV: ß = 0.12, *p* = 0.365 *Lesion volume: ß = -0.22, p = 0.049* *NBV: ß = -0.32, p = 0.028*	R^2 = 0.17, *F*(10, 91) = 3.04, *p* < 0.001
CC2: Visuospatial cognition	TC1: ß = -0.01, p = 0.938 TC2: ß = -0.13, p = 0.191 TC3: ß = 0.08, p = 0.442 TC4: ß = -0.11, p = 0.290 *Age: ß = -0.32, p = 0.009* Sex: ß = 0.10, p = 0.482 Education: ß = 0.09, p = 0.358 ICV: ß = 0.01, p = 0.963 Lesion volume: ß = -0.04, p = 0.708 NBV: ß = -0.04, p = 0.800	R^2 = 0.05, *F*(10, 91) = 1.50, *p* = 0.153
CC3: Information processing	TC1: ß = 0.06, p = 0.628 TC2: ß = 0.12, p = 0.236 *TC3: ß = 0.24, p = 0.017* TC4: ß = -0.16, p = 0.101 Age: ß = 0.18, p = 0.127 Sex: ß = -0.14, p = 0.318 Education: ß = -0.02, p = 0.839 ICV: ß = -0.10, p = 0.493 Lesion volume: ß = 0.04, p = 0.739 NBV: ß = 0.20, p = 0.197	R^2 = 0.06, *F*(10, 91) = 1.65, *p* = 0.106
CC4: Executive function	TC1: ß = -0.02, p = 0.870 TC2: ß = 0.05, p = 0.604 TC3: ß = 0.15, p = 0.101 TC4: ß = 0.03, p = 0.747 *Age: ß = -0.27, p = 0.016* Sex: ß = -0.03, p = 0.851 Education: ß = 0.07, p = 0.443 ICV: ß = -0.02, p = 0.872 *Lesion volume: ß = -0.23, p = 0.035* NBV: ß = 0.13, p = 0.352	R^2 = 0.18, *F*(10, 91) = 3.19, *p* = 0.002

Significant predictors are presented in italics. Significance threshold p < 0.05 applied unless otherwise indicated. Abbreviations: CC = cognitive component , NBV = normalised brain volume, NWMV = normalised white matter volume, TC = tract component, WM = white matter

Table 6.3: Tract loadings on each component derived from the tract PCA, after regressing out significant predictors of tract variance and applying Varimax rotation

TC1		TC2		TC3		TC4	
Tract	Loading	Tract	Loading	Tract	Loading	Tract	Loading
or_l	0.77	str_l	0.89	cbp_r	0.77	mcp	0.84
mdlf_l	0.75	cst_l	0.86	ac	0.73	vof_r	0.26
or_r	0.75	str_r	0.82	cbd_r	0.72	ac	0.25
fma	0.75	cst_r	0.78	cbt_r	0.70	fma	0.23
mdlf_r	0.74	fa_l	0.73	cbt_l	0.67	ar_r	0.23
ifo_r	0.72	fa_r	0.68	cbp_l	0.67	or_r	0.23
vof_l	0.70	af_l	0.66	fmi	0.65	cst_r	0.23
ar_l	0.70	slf1_l	0.65	uf_l	0.64	atr_r	0.21
vof_r	0.69	slf3_l	0.65	cbd_l	0.63	atr_l	0.18
ar_r	0.68	af_r	0.63	uf_r	0.60	mdlf_r	0.16
ifo_l	0.67	slf2_r	0.62	atr_l	0.59	ifo_r	0.14
ilf_r	0.67	slf2_l	0.59	atr_r	0.58	ilf_l	0.12
slf2_l	0.62	atr_l	0.59	ilf_l	0.55	fmi	0.12
af_r	0.62	slf3_r	0.59	ifo_l	0.50	af_r	0.09
slf1_r	0.61	atr_r	0.56	ilf_r	0.46	or_l	0.09
slf3_r	0.60	mdlf_r	0.47	mdlf_l	0.44	slf3_r	0.08
ilf_l	0.58	slf1_r	0.44	ifo_r	0.43	cbt_r	0.08
slf2_r	0.58	ifo_l	0.44	fa_l	0.39	ilf_r	0.07
af_l	0.56	ifo_r	0.43	slf1_l	0.39	str_r	0.06
cbd_l	0.56	cbd_l	0.42	fma	0.38	uf_r	0.04
uf_r	0.55	fmi	0.41	slf2_l	0.37	slf2_r	0.03
cbt_l	0.54	mdlf_l	0.40	or_l	0.37	ifo_l	0.02
slf3_l	0.53	or_r	0.40	ar_l	0.37	vof_l	0.02
fa_r	0.52	ilf_r	0.39	af_l	0.34	str_l	0.02
fmi	0.49	uf_r	0.37	mdlf_r	0.33	cbd_r	0.02
cbd_r	0.47	uf_l	0.37	fa_r	0.33	cbd_l	0.01
uf_l	0.45	cbd_r	0.35	vof_l	0.33	cbt_l	0.00
slf1_l	0.44	or_l	0.35	slf1_r	0.32	mdlf_l	0.00
cbt_r	0.44	ar_r	0.32	slf3_l	0.32	slf3_l	-0.03
cbp_l	0.42	cbp_l	0.31	af_r	0.31	cst_l	-0.03
fa_l	0.38	cbp_r	0.27	slf3_r	0.30	slf1_r	-0.03
atr_r	0.31	ilf_l	0.21	or_r	0.29	af_l	-0.04
atr_l	0.31	fma	0.21	slf2_r	0.28	cbp_l	-0.04
cbp_r	0.31	vof_r	0.15	vof_r	0.21	fa_r	-0.04
cst_r	0.24	ar_l	0.14	ar_r	0.17	slf2_l	-0.05
mcp	0.22	cbt_l	0.13	str_r	0.16	cbp_r	-0.05
str_r	0.19	vof_l	0.11	cst_r	0.14	slf1_l	-0.06
str_l	0.14	cbt_r	0.11	mcp	0.13	uf_l	-0.07
cst_l	0.08	ac	0.04	cst_l	0.09	fa_l	-0.12

Abbreviations: ac = anterior commissure; af = arcuate fasciculus; ar = acoustic radiation; atr = anterior thalamic radiation; cbd = cingulum subsection, dorsal; cbp = cingulum subsection, peri-genual; cbt = cingulum subsection, temporal; cst = corticospinal tracr; fa = frontal aslant; fma = forceps major; fmi = forceps minor; ifo = inferior fronto-occipital fasciculus; ilf = inferior longitudinal fasciculus; mcp = middle cerebellar peduncle; mdlf = middle longitudinal fasciculus; or = optic radiation; slf1-3 = superior longitudinal fasciculus 1-3; str = superior thalamic radiation; uf = uncinate fasciculus; vof = vertical occipital fasciulus. Left and right hemisphere tracts are denoted with _l and _r, respectively.

6.3.4 Cognitive domains

A correlation matrix of cognitive test scores showed a large number of moderate to high correlations and was significantly different from an identity matrix as assessed by Bartlett’s test

of sphericity (2(36) = 558.62, p <0.001), indicating a likely domain structure of cognition and confirming suitability for a PCA. Based on eigenvalues of at or near 1 and proportion variance explained, four components explaining 85% of variance were extracted. After a Varimax rotation a clear component structure emerged whereby cognitive component (CC) 1 reflects verbal cognition and CC2 visuospatial cognition, while CCs 3 and 4 reflect information processing speed and executive function, respectively. The component weights for rotated cognitive components (CCs) were as follows: Serial Recall Test Consistent recall (0.87), Serial Recall Test Long term storage recall (0.82), Word List Generation Test (0.80) and Serial Recall Test delayed recall (0.73) for CC1; Spatial Recall Test over three trials (0.90) and Spatial Recall Test delayed recall (0.93) for CC2; Paced Auditory Serial Addition Test 3 second delay (0.88) and Paced Auditory Serial Addition Test 2 second delay (0.89) for CC3; and Symbol Digit Modalities Test (0.84) for CC4, see Table 4.

6.3.4.1 Tract components are weak predictors of cognition

In MS, tract components were modest to weak predictors of cognitive components, as were demographic and MRI variables (see Table 2). The first cognitive component, CC1, was best predicted by TC1 (ß = 0.30, p = 0.009), sex (ß = 0.35, p = 0.010), lesion volume (ß = -0.22, p = 0.049) and NBV (ß = -0.32, p = 0.028), in a model explaining 17% of variance (R2 = 0.17, F(10, 91) = 3.04, p < 0.001). The final cognitive component, CC4, was best predicted by age (ß = -0.27, p = 0.016) and lesion volume (ß = -0.23, p = 0.035), in a model explaining 18% of the variance (R2 = 0.18, F(10, 91) = 3.19, p = 0.002). For cognitive components 2 and 3, the regression models were not significant. Given the low predictive values of tract components on cognitive components, WM variance patterns are weakly linked to cognitive domains. Please see Table 2 for full statistical results.

Table 6.4: Cognitive component weights in MS patients

	Cognitive RC1	Cognitive RC2	Cognitive RC3	Cognitive RC4
SRT L sum	**0.82**	0.16	0.10	0.36
SRT C sum	**0.87**	0.18	0.21	0.24
SRT delayed	**0.73**	0.25	0.29	0.37
Spatial1to3	0.15	**0.90**	0.23	0.06
Spatial delayed	0.10	**0.93**	0.01	0.15
SDMT	0.20	0.17	0.27	**0.84**
PASAT3	0.17	0.18	**0.88**	0.20
PASAT2	0.25	-0.06	**0.89**	0.11
WLG	**0.80**	-0.05	0.17	-0.29

Abbreviations: PASAT2 = paced auditory serial addition test 2 second delay; PASAT3 = paced auditory serial addition test 3 second delay; SDMT = symbol digit modalities test; Spatial1to3 = spatial recall test average score over three trials; Spatial delayed = spatial recall test score at the delayed trial; SRT delayed = serial recall test scores at the delayed trial; SRT L sum = serial recall test long term storage sum of scores; SRT C sum = serial recall test consistent recall sum of scores; WLG = word list generation test.

6.4 Discussion

In this study we combined PCA with tractometry to determine whether cognitive performance in people with MS relates to one or many patterns of white matter tract pathology. A decomposition approach of microstructure metrics from WM tracts showed a high degree of covariance across most tracts, indicating a global WM structure rather than a network-specific structure. This global WM microstructure component was largely explained by lesion volume, but retained largely a single covariance pattern even after this factor was regressed out. Cognitive domains were only weakly explained by WM microstructure components. This demonstrates that changes in white matter microstructure in people with MS is dominated by a single pattern of pathology, which is weakly associated with impaired cognition.

6.4.1 Metric dimensionality reduction

Tract decomposition was based on several diffusion metrics combined into one, consisting of FA, RD and MWF. Traditionally FA is used in MS studies of cognition, but FA has been shown to be susceptible to many factors, including myelination, axonal density and orientational dispersion of fibre populations in a voxel (Beaulieu, 2014; De Santis et al., 2014; Lazari and Lipp, 2021). A multimodal approach is a useful alternative for obtaining more comprehensive information about WM microstructure, and has been shown to produce a sensitive component measure of WM microstructure when several metrics are combined in a tractometry approach (Chamberland et al., 2019; Geeraert et al., 2020; Bosticardo et al., 2021).

Such dimensionality reduction has been shown to overcome the problem of multiple comparisons of data containing overlapping information while maintaining good sensitivity of WM microstructure (Chamberland et al., 2019; Geeraert et al., 2020; Bosticardo et al., 2021). Recently it has been shown to provide a more sensitive measure of MS pathology for connectomics approaches than the number of streamlines traditionally used (Bosticardo et al., 2021).

6.4.2 WM microstructure organisation

A number of studies have demonstrated functional network changes (Chard et al., 2021, Jandric et al., 2021a) and patterns of grey and white matter pathology associated with cognitive impairment in MS (Meijer et al., 2016a; Steenwijk et al., 2016; Colato et al., 2021). Such network changes are thought to be driven by the degradation of structural connections between network regions (Catani and ffytche, 2005; Dineen et al., 2009; Schoonheim et al., 2015). So far, only one study has shown covarying patterns of WM abnormalities in MS, but several other studies have shown that WM tracts share features which may make them similarly susceptible to pathology (Wahl et al., 2010; Li et al., 2012, Meijer et al., 2016a). Understanding the nature of WM degeneration holds the key to elucidating the relationship between functional and structural connectivity and is an important aim in mapping the pathology of cognitive impairment in MS. Thus, in this study we aimed to assess whether WM tracts can be decomposed based on shared pathological or other features, and whether the resulting components reflect known functional network structures.

Our results provide limited evidence of separate covariance structures of WM tracts in MS patients. A single dominant component consisting of all tracts was found in both people with MS and healthy controls, although with somewhat different tract loadings. In MS the main component was largely explained by lesion volume. Even though lesions were masked out of each tract and only non-lesioned tissue was included in the analyses, inflammatory activity in lesions is known to have an effect on surrounding tissue and Wallerian and retrograde degeneration is known to occur in remote areas from the lesions (Trapp et al., 1998; Bitsch et al., 2000; Trapp and Stys, 2009). After regressing out this predictor, the tract pattern covariance was still shared between all tracts (i.e. no separate patterns of pathology emerged).

A rotation of the first four components (explaining most of the variance in the data) revealed that some tracts loaded more strongly on these components than others. The first component consisted of all the tracts, but those which loaded most highly on this component were large associations tracts connecting distant regions of the brain like the inferior fronto-occipital fasciculus, middle longitudinal fasciculus, optic radiations and vertical occipital fasciculus. Similarly, the second component consisted of association and projection tracts connecting distant brain regions, including the superior thalamic radiation, corticospinal tract and superior longitudinal fasciculus. The third and fourth component consisted of smaller tracts, including commissural tracts, such as the forceps minor and middle cerebellar penduncle. While this

suggests differences between types of tracts which will be important to investigate further, these results lack the granularity to draw conclusions about brain networks supported by the tracts in each component. For instance, the first and second components consist of high loadings from tracts which together connect most of the brain. Moreover, most tracts loaded positively on most of the four extracted components, suggesting considerable overlap. This, coupled with the dominant first component prior to rotation, suggests that our results first and foremost show some global aspect of WM microstructure rather than distinct covariance patterns reflecting known functional networks.

There are a number of possible reasons for why pathology patterns associated with functional networks may not have emerged. First, white matter may not show a strong network structure or patterns of covarying pathology. We also found only one dominant tract component in healthy controls, and thus no evidence of a network structure in the white matter (see Appendix 1). Studies which do report patterns of WM pathology have grouped tracts into classes manually, rather than statistically based on shared features (Li et al., 2012, Meijer et al., 2016a). However, despite manual grouping, each class determined by Meijer et al., (2016a) did show that both FA values and component loadings within a class were associated with cognition, suggesting possible shared damage within a class. Second, patterns of WM pathology may only emerge at later stages of the disease. While network changes measured by rs-fMRI are common in RRMS and occur even in clinically isolated syndrome (CIS, reviewed in Jandric et al., 2021), they have been shown to be more pronounced in progressive MS (Meijer et al., 2018; Rocca et al., 2018). It is therefore feasible that if there is shared susceptibility to MS pathology in the WM, like in the grey matter, it comes more pronounced as the disease advances. This would need to be tested in longitudinal studies or large cross-sectional studies with both RRMS and SPMS samples. Third, patterns of pathology may only become apparent when looking at regions within tracts and not, as assessed in this study, across whole tracts. It is known that many major tracts support several separate functions, for example the interior fronto-occipital fasciculus is involved in cognition and sensorimotor functions as well as other behaviours (Sarubbo et al., 2013). Indeed, Li et al., (2012) found different FA covariance patterns for different segments of the corpus callosum. In support of this point, a recent study found that WM tract metrics of volume and microstructural integrity from specific section of specific tracts, including subsections of the corpus callosum, superior longitudinal fasciculus and the striato-prefrontal and striato-parietal pathways, better predict cognitive test performance than global tractography and lesion measures, and also better than whole tract measures (Winter et al., 2021). This lack of granularity in our data may therefore account for the weak component structure that emerged after rotation. Further studies comparing regional ICA and PCA approaches can help to determine the extent to which each of these factors is at play.

6.4.3 Relationships between WM microstructure and cognition

We found shared variance in WM microstructure across all tracts – this was present in people with MS, despite the heterogeneity of the disease, and determined links to cognitive performance. A large body of literature has now demonstrated associations between WM microstructure metrics and cognitive test performance in MS (Dineen et al., 2009; Inglese and Bester, 2010; Hulst et al., 2013; Sbardella et al., 2013; Llufriu et al., 2014, Meijer et al., 2016b). We identified four cognitive domains: verbal cognition, visuospatial cognition, information processing speed and executive function, consistent with the known domain structure of the BRB-N (Sepulcre et al., 2006; De Meo et al., 2021).

We found that the first and main tract component was related to specific cognitive domains, but overall these associations were weak. This component, together with sex, lesion volume and normalised brain volume, explained less than 20% of the variance of the verbal cognitive domain. This tract component is made up of most of the tracts investigated, but those which load most highly are long association tracts which connect most of the brain. Interestingly, tracts which connect the occipital cortex to the rest of the brain load highly onto this tract component. It may seem as an unexpected finding that tracts associated with visual function predict a cognitive domain without a visual element, but it's important to consider that we found all tracts to correlate highly with each other, so this correlation between the tract component and cognition is not specific to visual tracts. Nevertheless, damage to the occipital cortex, including atrophy and functional connectivity abnormalities, (in line with known pathology within the optic nerve, i.e. optic neuritis) is commonly reported in MS (Pagani et al., 2005; Calabrese et al., 2007; Tona et al., 2014), so the present finding may reflect a non-cognitively specific marker of MS pathology, albeit weakly, as the tract component only explained a small proportion of this cognitive domain. Lesion volume and atrophy measures were also weak predictors of cognitive domain variance, confirming the clinical-radiological paradox and the need for more advanced brain pathology measures in the study of cognitive impairment in MS.

The weak relationship between test performance on the different cognitive domains and WM tract components contrasts with previous evidence linking WM microstructure in MS to cognitive function (Dineen et al., 2009; Inglese and Bester, 2010; Hulst et al., 2013; Sbardella et al., 2013; Llufriu et al., 2014, Meijer et al., 2016b). This may be due to our use of whole tract measures. There is evidence to suggest that spatial topography is important for cognitive deficits and that some tracts in particular are involved in supporting cognitive function. Most of the early diffusion studies of cognition in MS report correlations between cognitive performance and diffusion metrics in specific regions of tracts, despite analyses being conducted over a whole brain WM skeleton. Those which are commonly reported across the literature are the corpus callosum, cingulum and forceps major and minor (Dineen et al., 2009; Sbardella et al., 2013; Llufriu et al., 2014). In addition, in another study of the sample investigated in the present study, WM metric differences between cognitively impaired and non-impaired patients were found mainly

in the corpus callosum and cingulum (Jandric et al., 2021b). This possibility of spatial specificity has not been formally established through a meta-analysis to date and is therefore speculative. However, it is supported by recent graph theory studies which have found associations between structural connectome metrics in certain networks and cognitive function rather than across the whole connectome (Llufriu et al., 2017, 2019; Koubiyr et al., 2019; Has Silemek et al., 2020). The third tract component identified in this study had had loadings from sections of the cingulum and forceps minor, yet did not explain a great deal of variance of cognitive domains. However, this tract component also consisted of a large number of other tracts with high loadings, so is non-specific to the cingulum and corpus callosum. Future work should focus on establishing if certain WM tracts in particular, e.g. those connecting key hub regions of the brain, are more important for cognitive function and more susceptible to pathology.

6.4.4 Limitations

Our study is not without limitations. Few previous studies have used data decomposition approaches to WM metrics, and those that have used independent component analysis (ICA), which aims to separate sources of signals (Li et al., 2012, Meijer et al., 2016a). In both studies the WM skeleton fed into the ICA returned components which reflected individual tracts or sub-sections of tracts. Grouping of tracts was in both cases done manually, introducing the risk of bias. In contrast, we used PCA to identify shared variance within orthogonal components. This minimises the risk of bias and may also better reflect normal variation in white matter structure. By comparing dominant components between control and patients we were able to evaluate whether such structures are to be expected. However, the possibility should be considered that tract components are not actually orthogonal, perhaps due to the known multifunctional nature of WM tracts, and independent component analysis would in this case have been a more suitable approach. We could also have used factor analytic techniques, but PCA has been shown to produce very similar results to factor analysis when the communalities of variables investigated are greater than 0.7, which was the case in this study (Guadagnoli and Velicer, 1988). This nascent research area requires further detailed work to determine the optimal analysis strategy to identify patterns of white matter pathology. In doing so they can help to understand whether there are networks that are susceptible to MS disease and how these might change over time.

6.4.5 Conclusions and future directions

In this study we have demonstrated that a single dominant component explains the variation in microstructure of white matter in people with MS and in healthy controls. We demonstrate that in people with MS this may relate to the distal effects of WM lesions. These covarying patterns of WM tract variance showed a weak relationship with cognitive function. The study raises several questions about whether or not there is a structure to the pathological changes underlying cognitive impairment in MS. Future research should therefore consider whether the

effects of lesions spread between tracts and at what stage this may start to impact upon cognitive function. By doing so we can develop a greater understanding of why spatially heterogeneous damage may lead to similar impairments to affect the lives of people with MS.

6.5 Funding

This work was funded by a research grant of the MS Society UK and a Medical Research Council Doctoral Training Partnership grant (MR/N013751/11).

6.6 Potential Conflicts of Interest

The authors report no potential conflicts of interests.

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6.8 Appendix 1: Results for healthy controls

A PCA of the four microstructural metrics was conducted based on a significant Bartlett's test of sphericity 2(6) = 40.72, p <0.001). Based on scree plot inspection and eigenvalues >1, only the first principal component, which explained 61% of variance, was extracted. The component loadings were 0.92 for FA, -0.91 for RD, 0.86 for MWF and 0.24 for MTR.

Component scores of this principal component (PC) were calculated for each healthy control and each tract and correlated to determine the suitability of a PCA. However, the correlation matrix was not positive definite, suggesting the presence of linear dependencies, due to the small sample size relative to the number of variables. Smoothing was applied to the matrix, and a PCA was still run, but results must be interpreted with caution. The four components before the bend in the screeplot, with an eigenvalue >1, explained 79% of variance. After a Varimax rotation all tracts still loaded positively on rotated tract component (TC) 1. See Figure 1A for the metric and tract correlation matrices and scree plots and Table 2 for the rotated component tract loadings.

Age (r=-0.30), sex (r=-0.28) and intracranial volume (r=0.46) correlated most highly with RC1 weightings (see Figure 1B). In a multiple linear regression model no demographic or anatomical variables predicted TC1 (R2 = 0.16, F(5, 21) = 2.02, p = 0.12). Full statistics for this model are presented in Table 1.

Because no variables explained variance in TC1, for HC the PCA assessing WM tract covariance pattern was not performed again.

Cognitive test scores correlated moderately to highly and a Bartlett's test of sphericity (2(36) = 95.64, p <0.001) confirmed the suitability of a PCA. The first four principal components explained 82% of variance and were subjected to a Varimax rotation, after which the component weights for cognitive components (CCs) were as follows: SRT C sum (0.93), SRT L sum (0.91) and SRT delayed (0.88) for CC1; WLG (0.88), SDMT (0.71) and PASAT3 (0.60) for CC2; Spatial1to3 (0.90) and Spatial delayed (0.88) for CC3; and PASAT2 (0.96) for CC4, see Table 3. None of the cognitive components was significantly predicted by regression models containing the four WM tract components, age, sex, education, ICV or NVB, see Table 1.

Table 6.5: Predictors of WM tract covariance and cognitive domains

Model	Predictors	Model statistics
Unrotated tract component 1	Age: ß = -0.19 *p* = 0.465 Sex: ß = 0.09, *p* = 0.789 Education: ß = 0.01, *p* = 0.937 ICV: ß = 0.56, *p* = 0.101 NBV: ß = 0.14, *p* = 0.605	R^2 = 0.16, *F*(5, 21) = 2.02, *p* = 0.12
CC1: Verbal cognition	TC1: ß = 1.25, p = 0.258 TC2: ß = -0.10, p = 0.914 TC3: ß = 0.69, p = 0.286 TC4: ß = -2.03, p = 0.056 *Age: ß = -0.68, p = 0.021* Sex: ß = -0.74, p = 0.055 Education: ß = -0.08, p = 0.623 ICV: ß = -0.77, p = 0.054 NBV: ß = -0.35, p = 0.239	R^2 = 0.34, *F*(9, 17) = 2.50, *p* = 0.050
CC2: Visuospatial cognition	TC1: ß = -0.32, p = 0.807 TC2: ß = -2.10, p = 0.077 TC3: ß = 0.32, p = 0.682 TC4: ß = 2.05, p = 0.101 Age: ß = -0.03, p = 0.936 Sex: ß = -0.18, p = 0.676 Education: ß = -0.24, p = 0.248 ICV: ß = -0.51, p = 0.266 NBV: ß = 0.36, p = 0.317	R^2 = 0.05, *F*(9, 17) = 1.17, *p* = 0.375
CC3: Information processing	TC1: ß = -0.63, p = 0.641 TC2: ß = 0.75, p = 0.526 TC3: ß = -1.05, p = 0.198 TC4: ß = 0.86, p = 0.498 Age: ß = -0.19, p = 0.581 Sex: ß = 0.10, p = 0.833 Education: ß = -0.32, p = 0.142 ICV: ß = 0.15, p = 0.746 NBV: ß = 0.11, p = 0.771	R^2 = -0.03, *F*(9, 17) = 0.92, *p* = 0.535
CC4: Executive function	TC1: ß = -0.93, p = 0.451 TC2: ß = -0.20, p = 0.849 TC3: ß = 0.48, p = 0.503 TC4: ß = 0.38, p = 0.740 Age: ß = -0.16, p = 0.604 Sex: ß = -0.84, p = 0.053 Education: ß = 0.31, p = 0.119 ICV: ß = -0.35, p = 0.422 NBV: ß = 0.19, p = 0.563	R^2 = 0.16, *F*(9, 17) = 1.57, *p* = 0.204

Significant predictors are presented in italics. Significance threshold $p < 0.05$ applied unless otherwise indicated. Abbreviations: CC = cognitive component , NBV = normalised brain volumeNWMV = normalised white matter volume TC = tract component, WM = white matter

Table 6.6: Tract loadings on each component derived from the tract PCA, after regressing out significant predictors of tract variance and applying Varimax rotation

TC1		TC2		TC3		TC4	
Tract	**Loading**	**Tract**	**Loading**	**Tract**	**Loading**	**Tract**	**Loading**
slf3_r	0.82	cbp_l	0.86	cbt_r	0.88	vof_l	0.79
slf2_r	0.80	cbd_l	0.77	cbt_l	0.82	vof_r	0.78
str_l	0.79	cbp_r	0.76	mcp	0.56	fma	0.74
af_r	0.78	atr_l	0.75	uf_r	0.54	or_l	0.70
str_r	0.77	ar_l	0.74	ac	0.54	ilf_l	0.68
cst_l	0.76	atr_r	0.71	uf_l	0.46	or_r	0.66
slf2_l	0.74	fmi	0.68	ar_r	0.46	mdlf_r	0.65
fa_l	0.71	cbd_r	0.68	cbd_r	0.43	mdlf_l	0.63
slf1_l	0.70	ifo_l	0.55	slf1_r	0.38	ifo_r	0.60
slf3_l	0.69	af_l	0.52	str_r	0.38	ilf_r	0.57
af_l	0.69	mdlf_l	0.46	cst_r	0.36	cst_r	0.54
fa_r	0.67	fa_r	0.46	vof_l	0.36	slf1_r	0.53
ifo_r	0.64	fa_l	0.46	cbp_r	0.34	ifo_l	0.50
or_r	0.62	slf3_l	0.45	ar_l	0.31	slf3_r	0.42
cst_r	0.57	slf2_l	0.43	slf1_l	0.31	uf_r	0.41
ifo_l	0.56	str_l	0.40	vof_r	0.31	slf3_l	0.41
slf1_r	0.56	slf1_l	0.40	mdlf_r	0.27	slf2_l	0.39
or_l	0.53	ar_r	0.36	fa_l	0.27	af_l	0.38
mdlf_l	0.51	ilf_r	0.35	slf3_l	0.23	atr_l	0.37
uf_l	0.51	or_l	0.35	cbd_l	0.22	slf2_r	0.37
uf_r	0.49	ifo_r	0.35	cst_l	0.20	af_r	0.36
fmi	0.49	uf_l	0.35	fa_r	0.19	atr_r	0.34
mdlf_r	0.48	mdlf_r	0.30	ifo_r	0.19	cst_l	0.32
ilf_r	0.46	af_r	0.28	af_r	0.18	uf_l	0.32
atr_r	0.44	vof_r	0.27	ilf_l	0.18	slf1_l	0.32
fma	0.42	cst_l	0.27	atr_l	0.18	ac	0.32
cbd_l	0.41	fma	0.26	ilf_r	0.18	fa_r	0.31
mcp	0.41	cst_r	0.26	slf2_l	0.17	fmi	0.31
atr_l	0.40	ilf_l	0.24	slf2_r	0.14	cbd_r	0.31
cbd_r	0.39	or_r	0.24	atr_r	0.12	ar_r	0.26
ar_r	0.31	slf3_r	0.23	mdlf_l	0.12	ar_l	0.22
cbp_r	0.24	cbt_l	0.20	slf3_r	0.12	cbt_r	0.19
vof_l	0.20	mcp	0.18	or_r	0.11	str_r	0.18
vof_r	0.18	uf_r	0.16	af_l	0.10	cbd_l	0.16
ilf_l	0.16	cbt_r	0.15	str_l	0.09	cbp_l	0.16
ar_l	0.16	slf1_r	0.14	cbp_l	0.07	cbt_l	0.11
cbt_r	0.11	ac	0.10	or_l	0.04	fa_l	0.10
cbt_l	0.10	slf2_r	0.10	ifo_l	0.04	str_l	0.09
ac	0.08	str_r	0.09	fmi	-0.01	cbp_r	0.07
cbp_l	0.07	vof_l	0.02	fma	-0.15	mcp	-0.22

Abbreviations: ac = anterior commissure; af = arcuate fasciculus; ar = acoustic radiation; atr = anterior thalamic radiation; cbd = cingulum subsection, dorsal; cbp = cingulum subsection, peri-genual; cbt = cingulum subsection, temporal; cst = corticospinal tracr; fa = frontal aslant; fma = forceps major; fmi = forceps minor; ifo = inferior fronto-occipital fasciculus; ilf = inferior longitudinal fasciculus; mcp = middle cerebellar peduncle; mdlf = middle longitudinal fasciculus; or = optic radiation; slf1-3 = superior longitudinal fasciculus 1-3; str = superior thalamic radiation; uf = uncinate fasciculus; vof = vertical occipital fasciulus. Left and right hemisphere tracts are denoted with _l and _r, respectively.

Table 6.7: Cognitive component weights in healthy controls

	Cognitive RC1	Cognitive RC2	Cognitive RC3	Cognitive RC4
SRT L sum	**0.91**	0.08	0.10	0.02
SRT C sum	**0.93**	0.09	0.11	0.03
SRT delayed	**0.88**	-0.02	0.10	0.10
Spatial1to3	0.06	0.16	**0.90**	0.02
Spatial delayed	0.21	0.09	**0.88**	-0.10
SDMT	0.15	**0.71**	0.32	0.18
PASAT3	0.08	**0.60**	-0.32	0.53
PASAT2	0.09	0.11	0.01	**0.96**
WLG	-0.02	**0.88**	0.12	-0.00

Abbreviations: PASAT2 = paced auditory serial addition test 2 second delay; PASAT3 = paced auditory serial addition test 3 second delay; SDMT = symbol digit modalities test; Spatial1to3 = spatial recall test average score over three trials; Spatial delayed = spatial recall test score at the delayed trial; SRT delayed = serial recall test scores at the delayed trial; SRT L sum = serial recall test long term storage sum of scores; SRT L sum = serial recall test consistent recall sum of scores; WLG = word list generation test.

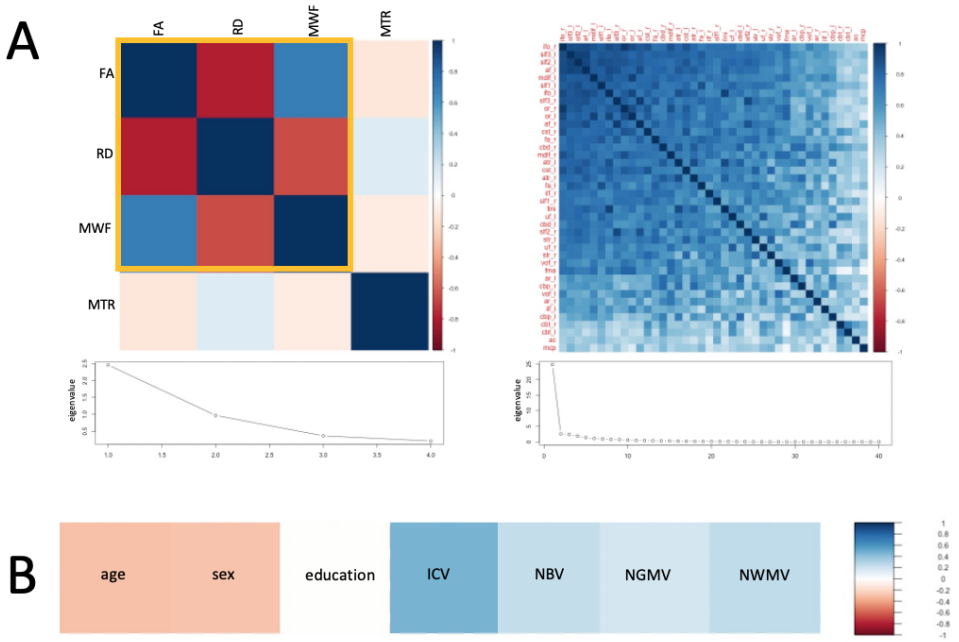

Figure 6.2: Metric and tract principal component analysis in healthy controls
FFigure 1A shows the correlation matrices and scree plots for the PCA ran on the four white matter microstructural metrics (left) and the white matter tracts based on the first component from the metric PCA (right). Those metrics marked with a yellow line load most on principal component 1. All tracts loaded positively on tract principal component 1. Figure 1B shows correlations between rotated tract principal component 1 (TC1) and demographic and anatomical variables. No demographic or anatomical variables predicted TC1 significantly, and the PCA was therefore not repeated, unlike in the MS group.
Abbreviations: FA = fractional anisotropy; RD = radial diffusivity; MWF = myelin water fraction; MTR = magnetisation transfer ratio; ICV = intracranial volume; NBV = normalised brain volume; NGMV = normalised grey matter volume; NWMV = normalised white matter volume

Chapter 7

General Discussion

The aim of this thesis was to understand the brain changes associated with cognitive impairment in people living with multiple sclerosis (MS), with particular focus on brain networks. This aim was pursued with four individual, but related, studies. A systematic review of the literature on functional connectivity abnormalities (FC) associated with cognitive impairment was conducted to provide an overview of the state of the field and research findings to date. This was followed by a multimodal MRI study assessing anatomical connectivity and cerebral blood flow (CBF) abnormalities in and around functional network regions, to test the hypothesis that the high metabolic demand of network 'hubs' makes them susceptible to degeneration. A third study assessed the metabolic state of network hub regions, as a potential mechanism of FC abnormalities, in more depth with sodium MRI, by investigating if an accumulation of sodium, indicative of energy failure, occurs in network regions compared to the rest of the brain. Finally, the network structure of normal appearing white matter was investigated in an exploratory analysis, to gain understanding of whether white matter pathology follows similar patterns to what FC studies have shown in the grey matter.

These four studies are presented in this thesis in a format suitable for publication, and the findings of each have been discussed in their respective chapters. The aim of this general discussion is to consider how the combined findings of these studies address the central research question underpinning each study and the thesis itself. It also considers the implications of these findings for how we understand network changes in MS, and finally, what research questions still remain to be answered.

7.1 Overview of findings

Prior to this work, network changes in the brains of people with MS were increasingly being explored with advanced MRI methods. Resting state fMRI (rs-fMRI) in particular, provided promising findings, with studies often reporting associations between abnormal FC and poor cognitive test performance. However, such findings were difficult to interpret. First, the findings lacked consistency; both increased and reduced FC were associated with cognitive impairment (Bonavita et al., 2011, Faivre et al., 2012; Basile et al., 2014; Cruz-Gómez et al., 2014; Rocca et al., 2018). Second, the pathophysiological mechanisms of FC abnormalities were not known and it was speculated on what a change in connectivity meant. Common interpretations considered that alterations in FC reflected a functional re-organisation, which could be either adaptative (i.e. compensatory plasticity) or maladaptive, but little evidence was produced to support this (Schoonheim et al., 2015a, 2017). Related to this, few theories or models existed on which to

base predictions of expected FC abnormalities, and those which had been proposed were rarely formally investigated.

Thus, the knowledge gaps were around what patterns of FC abnormalities are commonly associated with cognitive impairment, what pathological mechanisms lead to altered FC, and how well the existing models of FC changes explain research findings.

The first study of this thesis (Chapter 3) was the first systematic review of the literature on FC and cognitive impairment, conducted to address the knowledge gap of what pattern of FC abnormalities is most strongly associated with cognitive impairment. The main finding in this review was that any abnormality in FC is associated with worse cognition, but no clear pattern emerged as to whether this is mainly increased or reduced FC. Methodological and demographic factors which could influence study findings, including the brain regions or network investigated, or MS subtype studied, were considered. Overall, the body of literature showed too much heterogeneity to draw firm conclusions about what influences the direction of FC changes. It was also considered how a commonly cited model of FC changes, the *network collapse* model, fits the research findings. The model predicts that FC changes are driven by structural damage, which can be compensated for in the early stages of the disease, causing high FC, but resulting in network collapse, and low FC, when structural damage becomes too great (Schoonheim et al., 2015a). Although there is some support for this model (Patel et al., 2018, Tewarie et al., 2018), none was found in this review. When studies were considered by the average disease duration of the sample, the predicted pattern of FC increased followed by decreases did not emerge. The complexities of comparing different studies were acknowledged, and a call to action made for future research to ensure more consistent methodology to enable more direct comparisons.

This review highlighted the urgency to address the second knowledge gap, of identifying the mechanisms causing altered FC. The second study of this thesis (Chapter 4) tested another model of FC abnormalities, the *nodal stress* hypothesis, which suggests that FC changes in a functional network found at the resting state reflect neurodegeneration to network 'hubs,' the regions in the network that integrate information across the network (Buckner et al., 2009; Zhou et al., 2012). This degeneration is thought to be a result of unmet high metabolic demands of network hubs, and reflect a sort of 'wear-and-tear' damage. By combining rs-fMRI FC with anatomical connectivity measures from diffusion MRI and CBF from arterial spin labelling (ASL), we hypothesised that 'wear-and-tear' damage to network hubs should also affect the anatomical connectivity and blood flow to network hubs, and found some evidence in support of this. Both anatomical connectivity and CBF measures were abnormal around functional network regions which showed abnormal FC in cognitively impaired relative to non-impaired MS patients. While these findings provide the first preliminary support for the *nodal stress* hypothesis is MS, and suggest that functional networks are susceptible to metabolic stress, it was acknowledged that the methods used are indirect measures of metabolic function in the brain, and other MR modalities can offer further information about the metabolic basis of FC.

Chapter 5 was therefore a continuation of this work. Sodium MRI had previously been used in MS to show that sodium, which is essential for the metabolic functioning of neuronal axons, accumulates in people with MS and points to energy failure as a mechanism of axonal dysfunction (Paling et al., 2013). By combining sodium MRI with rs-fMRI for the first time, we found total sodium concentration to be higher in functional network regions than the rest of the brain in both people with MS and controls, pointing to sodium accumulation in these regions. Importantly, this study was conducted in participants recently diagnosed with MS, to understand what mechanisms are present at an early stage of the disease. While this study was severely affected by the COVID-19 pandemic, impacting sample size, it nevertheless provides evidence that functional network regions may be susceptible to metabolic pathology, and supports the findings in Chapter 4.

Finally, the study in Chapter 6 was conducted to understand structural connectivity better. Most work on network changes in MS have focused on FC through rs-fMRI, but it is known that the non-lesioned white matter is also affected by MS pathology and it is thought that white matter damage might be a driver of functional network failure through damaged anatomical connections between functional network regions (Catani and ffytche, 2005; Dineen et al., 2009). If this is the case, it would be expected that some white matter tracts, which support functional networks, are more susceptible to MS pathology than others, an idea for which some support had been provided by previous research (Meijer et al., 2016). Our tractometry approach involved conducting a principal component analysis of diffusion metrics from 40 white matter tracts. We didn't find evidence of a network structure in the normal appearing white matter, but acknowledge limitations which may have influenced findings. This study highlighted the need to research non-lesioned white matter further to understand how it is affected by pathology and to understand the relationship between functional and structural connectivity better.

Overall, this research has confirmed FC abnormalities as a key brain correlate of cognitive impairment in MS, emphasising the need to understand this metric better, and begun exploring potential mechanisms underpinning FC abnormalities. To progress this field further, three questions need to be addressed: whether FC changes related to cognitive impairment are meaningful, whether they are reliable, and what causes them.

7.2 Are the changes meaningful?

It is well-established that the white matter lesions identified on MRI scans in the MS clinic are a poor predictor of cognitive symptoms (Barkhof, 1999, 2002), and the use of advanced MRI measures in MS research aims to find a more sensitive marker of cognitive decline. As rs-fMRI FC has been shown to be consistently associated with cognitive impairment it is a good candidate, but it must be considered if FC changes are meaningful enough for clinical translation.

FC is a statistical concept rather than a biological one. It reflects the correlations between low frequency blood oxygenation level dependent (BOLD) fluctuations across spatially separate

regions (Bijsterbosch et al., 2017). Despite this, the functional networks obtained at the resting state reflect those which can be induced by a task during a functional MRI scan (Smith et al., 2009). Thus, without even knowing the biological basis of FC there is evidence to suggest that it is based on neuronal activity. For FC changes to be a meaningful marker of disease, their biological basis does not necessarily need to be fully understood. Rather, the potential clinical utility of FC changes needs to be considered.

For FC to be a meaningful marker of cognitive decline, there are certain criteria that need to be met. First, it needs to be sensitive to cognitive impairment, in that FC abnormalities should be present in MS patients with reduced cognitive function, but not in those without cognitive difficulties. Second, it needs to be specific, in that those without cognitive impairment don't show FC abnormalities for other reasons. In its simplest form, a biomarker can have this binary characteristic, but is typically considered more helpful if the volume of measurement of the biomarker increases or decreases as the symptom does (Biomarkers Definitions Working Group, 2001; Simon, 2015).

There is some evidence supporting the sensitivity criterion. In Chapter 4 FC was found to differentiate cognitively impaired MS patients from non-impaired patients, in the absence of differences in volumetric brain measurements, including lesion volume, in these groups. This is consistent with a substantial number of additional studies that found differences in FC between cognitively impaired and non-impaired patients (Rocca et al., 2010, 2016; Bonavita et al., 2011; Cruz-Gómez et al., 2014; Leavitt et al., 2014; Louapre et al., 2014, Schoonheim et al., 2015b; d'Ambrosio et al., 2017, 2019; Eijlers et al., 2017, 2018, 2019, Meijer et al., 2018b, a; Karavasilis et al., 2019).

However, as was shown in Chapter 3, at present the direction of FC changes, increased or decreased relative to a comparison group, does not appear to be meaningful as both types of changes are associated with cognitive worsening. The *network collapse* model (Schoonheim et al., 2015a) and related explanations that suggest that the pattern of FC abnormalities moves from high to low as the disease progresses (Meijer et al., 2018a) are not supported by the existing literature (Jandric et al., 2021). It has been suggested that the pattern of FC abnormalities varies spatially across the brain, with cortical regions and deep grey matter showing different patterns of abnormalities (Meijer et al., 2018a). This idea is understudied at present, and it is reasonable to assume that the pattern of FC may be a combination of degree of MS pathology in the brain and differing sensitivity of different brain regions and networks to damage. An important aim for future research of rs-fMRI FC in cognitive impairment in MS is therefore to identify what role factors such as spatial topography, structural damage and disease severity have in the relationships between FC and cognitive impairment.

Another challenge for FC is the specificity to cognition. A network approach has been applied to other symptoms of MS as well, including fatigue, motor symptoms and visual symptoms, and associations have been reported between abnormal FC and the symptoms investigated (Gallo et al., 2012; Wu et al., 2015; Zhong et al., 2016; Bisecco et al., 2018). Studies typically focus on the

network thought to be involved in that function, such as motor networks for motor symptoms, making it difficult to assess specificity of findings. Some studies have assessed both cognitive and non-cognitive symptoms and reported findings which suggest varying degrees of specificity. For example, one study found FC alterations in the default mode network and attentional networks, but not in the motor network in cognitively impaired compared to non-impaired patients (Louapre et al., 2014). But others have found associations between FC of networks which are thought to be mainly involved in cognition and measures of physical disability such as the multiple sclerosis functional composite (Faivre et al., 2012) and the expanded disability status scale (Meijer et al., 2017). Similarly, associations have also been found between cognitive measures and non-cognitive networks including the sensorimotor network (Manca et al., 2019; Soares et al., 2020). However, no study has systematically assessed the specificity of FC abnormalities in cognitively relevant regions to cognitive symptoms. So, although the present literature does not suggest high specificity of FC to cognition, further work is needed to determine whether some regionally (or network) specific changes are unique to cognitive impairment over other MS symptoms.

7.3 Are the changes reliable?

Another criterion which needs to be met for network changes to be clinically translatable as a marker of cognitive impairment is reliability. The FC measure needs to be reliable over time and across samples. A limitation comes from the method itself, which has low signal to noise ratio and is susceptible to noise, reducing its test-retest reliability (Chen et al., 2016; Shah et al., 2016; Noble et al., 2019). Some methodological factors have been identified that improve reliability, including the amount of data collected, the test-retest interval length and the corrections applied (such as removing physiological noise) (Noble et al., 2019). Further work is ongoing (Teeuw et al., 2021) and the reliability of the method is likely to improve further over time.

Despite this challenge, a few longitudinal studies in MS show evidence of FC stability over one month (Welton et al., 2020) up to five years (Høgestøl et al., 2021) and moreover, changes in degree of FC abnormalities during disease progression (Koubiyr et al., 2019; Huiskamp et al., 2021). FC has also been shown to respond to initiation of disease modifying treatments, with decreased FC observed over six months after start of treatment (Petsas et al., 2019). While small, this body of literature is promising, showing a degree of reliability of the FC measurements over time, which should be confirmed by further research.

Reliability of FC metrics across samples has been shown in healthy volunteers (Damoiseaux et al., 2006; Bijsterbosch et al., 2017), but is harder to assess in disease, as it requires the same protocol and methods across samples and additionally samples that are well matched on the symptom of interest, in this case cognitive impairment. Some multi-centre studies in MS have used the same protocol across study centres, but pooled participants from different centres into one sample rather than comparing across samples (e.g. d'Ambrosio et al., 2019). Therefore, a key

aim for future research is to understand whether FC abnormalities are reliably detected across samples with similar cognitive impairments.

Related to this, FC abnormalities need to be reliably detected in individuals to be translatable into a marker of cognitive decline. This work is lacking in MS, but results from healthy volunteers show that functional networks at the resting state are consistent across individuals (Damoiseaux et al., 2006; Shehzad et al., 2009). Nevertheless, recent studies have shown individual differences in functional connections (Mueller et al., 2013; Finn et al., 2015; Gordon et al., 2017), which may influence how FC metrics can be used in individuals with neurological disease. This makes establishing patterns of FC associated with cognitive functioning at the group level all the more important, because by knowing what the average relationship between FC and cognition is in the healthy brain, it becomes possible to assess how individuals with cognitive impairment differ from that average.

7.4 What causes these changes?

Understanding the mechanisms of functional connectivity changes associated with cognitive impairment in MS will be essential for understanding the role of this measure in MS diagnosis and management. This is a major and open-ended question, which can be approached from a multitude of angles. In this thesis, Chapters 3, 4 and 5 all attempted to address this question by investigating some of the proposed candidate mechanisms, specifically metabolic deficits and structural disconnection.

In the introduction of this thesis, three models that aim to explain the mechanisms of FC abnormalities were outlined: the *network collapse* model, the *network degeneration* hypothesis and the *nodal stress* hypothesis. Chapter 3 tested the *network collapse* model, which suggests that FC changes are a result of accrual of structural damage (Schoonheim et al., 2015a) and found little support for it. But that is not to say that this model can be discredited. As was acknowledged in Chapter 3, the data reviewed was too heterogeneous for any firm conclusions. Indeed, individual studies which have modelled the effect of structural damage on FC measures support it (Patel et al., 2018, Tewarie et al., 2018). In Chapter 4 we found abnormalities in white matter metrics, including anatomical connectivity mapping and fractional anisotropy around the network regions which showed FC abnormalities in cognitively impaired relative to non-impaired patient, demonstrating that anatomical connectivity abnormalities co-occur with functional connectivity abnormalities. These findings are consistent with the *network collapse* model, but the study did not assess whether anatomical connectivity changes precede FC changes.

Chapter 6 also assessed the role of anatomical connectivity in cognitive impairment in MS, based on the idea that if degradation of anatomical connectivity underlies functional network abnormalities, microstructural damage to white matter tracts should appear in patterns that reflect the network structure of the brain. One other study has suggested that this is the case in MS (Meijer et al., 2016), but we found no evidence of a network structure in the white matter,

again calling into question the predictions of the *network collapse* model. At present the evidence for this model is mixed, and the relationship between functional and structural connectivity needs to be understood further to determine whether and to what degree damage to the tracts connecting network regions influences functional connectivity.

Related to this, structural damage to the network regions themselves needs to be understood. The *network degeneration* hypothesis suggests that functional network hubs are more susceptible to atrophy than other regions (Chiang et al., 2019), and this hypothesis has been supported by a validation study in relapsing remitting MS (Chiang et al., 2021). Two recent studies assessed covarying patterns of cortical thinning and grey matter atrophy, respectively, and found some regions to be more susceptible than others, and moreover that these patterns are associated with cognitive functioning (Steenwijk et al., 2016; Colato et al., 2021). Similarly, we know that cortical lesions occur in MS and are associated with cognitive impairment (Roosendaal et al., 2009; Calabrese et al., 2012; Geurts et al., 2012), again highlighting the sensitivity of grey matter regions to structural damage. A key research question is therefore whether such damage affects network hub regions in particular, and how this influences functional connectivity.

Understanding whether damage to network hub regions and the anatomical connections of these influences white matter is a research priority for understanding the MS pathology responsible for cognitive symptoms. A further step is to probe the mechanisms by which such damage causes FC changes. This would give a better understanding of the FC metric and its biological substrates, and lead to more accurate use of it as a potential marker of cognitive decline. In this thesis the idea that the high metabolic demands of network hubs which integrate information from across the brain are susceptible to damage when those demands are not met, formulated in the *nodal stress* hypothesis (Buckner et al., 2009; Zhou et al., 2012), was supported. Previous research has found evidence of metabolic changes in demyelinated axons (Foster et al., 1980; Craner et al., 2004), and work in a drosophila model showed that metabolic demands driven by neural activity create changes in a functional connectivity network (Mann et al., 2020). In humans, fluorodeoxyglucose positron emission tomography (FDG PET), which is based on neurometabolic coupling, has been used to measure resting state networks and shown that glucose metabolism in the visual cortex influences the FC of the visual network (Savio et al., 2017). Moreover, there is evidence of hypoperfusion in the MS brain and associations with worse cognitive function, which could point to decreased energy demand (Lapointe et al., 2018). On the basis of this work, the study in Chapter 4 showed that FC abnormalities co-occur with anatomical connectivity and cerebral blood flow abnormalities, which would be expected on the basis of previous work if metabolic dysfunction is a mechanism of FC changes. The fact that these multimodal abnormalities were not in exactly the same locations casts some doubt on this and highlights the need to investigate this hypothesis in MS further with more direct methods of metabolism, such as FDG-PET. Chapter 5 pursued the hypothesis of energy failure in network regions with sodium MRI, and found higher sodium concentrations in network hubs than the rest of the brain, highlighting the potential altered metabolic state of these regions.

Additional outstanding questions concern the specific mechanisms of FC increases and decreases, respectively. Decreases could be easily explained as reduced correlations in the BOLD timeseries that underpins the FC measure, which in turn could result from any of the potential mechanisms discussed, severed anatomical connections between regions, loss of tissue in network regions themselves, or inadequately met metabolic demands. FC increases are harder to explain, as they show increased correlations between regions. It has been proposed that such increases could reflect disinhibition or some element of plasticity such as adaptation or compensation for damage to other parts of the wider network (Chard et al., 2021). Evidence of plasticity has been found in stroke patients (Zemke et al., 2003; Hartwigsen and Saur, 2019) and it is feasible to expect that it can also occur in MS, but needs to be empirically demonstrated.

In summary, several potential mechanisms of FC have been investigated in this thesis and the results presented support findings from other research in the field. At present, the results do not offer support for the *network collapse* model, but provide preliminary evidence in support of the *nodal stress* hypothesis. While some way has thus been made to address the knowledge gaps outlined at the start of this chapter, several outstanding questions remain priorities for future work.

7.5 Limitations

The work in this thesis has been conducted to fill existing knowledge gaps in the field of network changes associated with cognitive impairment with MS. Limitations of each individual study have been discussed in the respective chapters. The main limitation of the overall thesis relates to how methods have been used in this work. In this thesis predictions are made about the mechanisms that influence brain connectivity, and to that end chapters 4, 5 and 6 have used advanced MRI methods to assess what abnormalities occur alongside functional connectivity abnormalities or in functional network regions. However, throughout the thesis the methods have focused on abnormalities that co-occur on different modalities rather than attempted to assess the relationships between modalities. Establishing whether they co-occur was an important first step to test existing models of network changes and identify directions for future research. Thus, the thesis has begun to fill this knowledge gap. However, to progress the field further and understand the mechanisms of network changes better, a more integrative approach will be necessary to understand whether abnormalities on different MR modalities are related to each other. This could be achieved by correlating metrics or applying a more advanced approach, such as linked independent component analysis (Groves et al., 2011) to understand common variance between metrics from different modalities and neuropsychological variables.

7.6 Future directions

A number of priorities for future work have been identified in this chapter and the most pressing are summarised below.

- **Standardisation of network research in MS**

  A large body of network changes associated with cognitive impairment in MS exists already, but substantial heterogeneity in the literature makes comparisons and drawing of conclusions challenging.

- **Sensitivity and specificity of network measures**

  Network measures, particularly FC, are promising markers of cognitive decline in MS, but more work is needed to establish the sensitivity and specificity required for clinical translation to a biomarker.

- **Model-led research**

  Several models of the network changes in the MS brain exist, but the evidence base for each is limited. By conducting studies with the explicit aim to test these models the field can progress to understand the pattern and mechanisms of network changes better.

7.7 Concluding remarks

The four studies of this thesis have advanced our understanding of network changes associated with cognitive impairment in MS by addressing some key knowledge gaps and identifying priorities for future research. Cognitive impairment remains a hugely debilitating symptom of MS and one that is still poorly understood. The present body of research adds to existing literature to show that network measures are associated with cognitive impairment and show promise for being MRI markers of cognitive decline. There are challenges that need to be addressed before network measures are suitable for clinical translation. Future research investigating the mechanisms that cause changes in brain networks and determining the sensitivity, specificity and reliability of network measures has the potential to create clinically meaningful prognostic and diagnostic markers of cognitive impairment in MS.

7.8 References

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