Resting-state Graph Theory Metrics Predict Processing Speed and Correlate with Disease Burden in Relapsing-Remitting Multiple Sclerosis

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Abstract

Multiple Sclerosis is a common, neurodegenerative disorder characterized by the accumulation of gray and white matter lesions within the central nervous system and presenting with progressive disability within the physical, sensory, and cognitive domains. Understanding the relationship between cognitive dysfunction, which affects an estimated 70% of people with multiple sclerosis (PwMS), and overall disease burden (commonly measured by the Expanded Disability Status Scale; EDSS) is exceedingly important for informing research interventions to preserve patient quality of life. The current study utilized resting-state fMRI to derive connectome-based predictive models (CPM) of two cognitive domains affected in PwMS (information processing speed and working memory) and determine the contribution of model-derived metrics in explaining variance associated with EDSS. We were able to successfully derive a model of processing speed (r_s= .41, p= .03), but not working memory, perhaps due to processing speed deficits emerging earlier and more prominently in PwMS. fMRI-derived processing speed metrics uniquely accounted for 13.19% of the variance in explaining EDSS. In contrast, behavioral performance on processing speed measures, working memory measures, and calculated total lesion volume did not explain a significant amount of variance in EDSS, suggesting that functional connections associated with processing speed ability may importantly contribute to patient disease burden. This work therefore supports the hypothesis that models of MS disease burden may benefit from functional inputs in addition to structural and behavioral ones, though future work is needed to establish the mechanisms of MS-related disease processes on functional connections, cognition, and disease.

Keywords: functional connectivity, multiple sclerosis, cognition

Dedication

To my parents, Peanut, and Michael

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Fields of Study

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Chapter 1. Introduction

1.1 Multiple Sclerosis: Prevalence, Etiology and Diagnostic Criteria

Multiple Sclerosis (MS) is the most common inflammatory disease of the central nervous system (CNS) in young and middle-aged adults, affecting an estimated 1,000,000 people in the United States alone (Reich et al., 2018; Wallin et al., 2019). Notably, estimates of disease prevalence since 2010 appeared to increase at a rate of 2.3% per year, largely believed to be the result of increased longevity in people with MS (PwMS) due to advances in pharmacological treatments (Wallin et al., 2019). However, despite these treatment advances, MS is a neurological disease that continues to be significantly disruptive to both individuals with MS and society as a whole, due to the disease's early presentation and wide range of sensory, physical and cognitive deficits. The median age-of-onset of MS is 30 years old, when individuals are set to contribute substantially to both the workforce and their families (Dendrou et al., 2015; Wallin et al., 2012). The resulting economic burden is estimated to be approximately $10 billion dollars annually, due to a combination of healthcare costs and the eventual loss of these people to the job pool as physical and cognitive disability progresses, reducing patient independence and quality of life (Reich et al., 2018).

Similar to other complex auto-immune and neurodegenerative disorders, MS is believed to be caused by an interaction between genetic susceptibility and environmental factors, with the genetic components explaining approximately 30% of the variance in disease risk, making it a disease difficult to predict and detect (Dendrou et al., 2015). Many environmental factors have been associated with increased prevalence of MS - most compellingly, a history of exposure to the Epstein-Barr virus, history of infectious mononucleosis, history of smoking- and to a lesser degree- biomarkers associated with low vitamin D (Belbasis et al., 2015). Additionally, there exists a female to male ratio of 2.8:1 that is poorly understood, as well as a decreasing, but currently preserved, geographic gradient whereby MS prevalence decreases from north to south in the United States (Wallin et al., 2019) and worldwide (Simpson et al., 2011). Taken together, these correlations suggest a neuro-inflammatory basis of disease but give little hope of identifying a single catalyst on which to target preventative interventions.

Clinically, MS can be conceptualized as consisting of three disease stages, usually following a progressive presentation: 1) a first incidence in which lesions are present but clinical symptoms may or may not be; 2) a relapsing form of the disease in which unpredictable episodes of clinical symptoms occur, on average, once every 2 years, and are followed by varying levels of recovery; and finally, 3) a progressive form in which clinical symptoms slowly but steadily worsen without remissive periods (Ransohoff et al., 2015). Due to its slow progression, the diagnosis of MS is often delayed (Brownlee et al., 2017) and consideration of MS as a diagnosis usually occurs after a sudden, first episode of neurological symptoms, accompanied by the confirmed presence of MRI-visible white matter lesions (Thompson et al., 2018). A single presentation of clinical symptoms, even

accompanied by a single lesion on MRI, is not sufficient to meet diagnostic criteria of MS. Instead, diagnosis of MS firstly requires the elimination of other possible diagnoses, given that there is significant risk of MS pharmacological treatments exacerbating the course of other, similarly presenting diseases (Brownlee et al., 2017). Following this, diagnosis requires evidence of dissemination of lesions in space as well as in time, requiring both more than one lifetime episode as well as the presence of lesions in more than one location in the CNS (Thompson et al., 2018).

Further complicating diagnosis, some people with pathology and symptoms consistent with an MS episode do not progress to a relapsing or progressive form of disease. In these cases, the presence of a single, lifetime episode is referred to as Clinically Isolated Syndrome (CIS). Onset of CIS as well as conversion to clinically definite MS is considered idiopathic, however, several factors influence the likelihood of later conversion to MS. Of these, the number of white matter lesions on MRI at first attack is most predictive of conversion to full MS, followed by the degree of temporary neurological disability observed in initial episodes (Tintore et al., 2015). In terms of conversion prevention, receiving prompt diagnosis of CIS, and initializing treatment before a second attack is more associated with reduced risk of conversion to full MS than either lesion location or demographic factors (Tintore et al., 2015). Together, these findings suggest that early and accurate detection and understanding of MS disease pathology and associated symptoms, especially when these symptoms are mild, is a key clinical endeavor that is hindered by the disease's heterogenous presentation.

However, even with appropriate care, the majority of those initially diagnosed with CIS do experience a progression of the disease to clinically definite MS, with disease

progression most often following the prototypical relapsing-remitting multiple sclerosis (RRMS) subtype (Gaetani et al., 2018). RRMS is characterized by periods of active disease, in which demyelination and neurologic dysfunction progress rapidly for a time-limited period and are then followed by a remissive state of remyelination and varying degrees symptom remission. The ability of the CNS to fully repair and recover function after multiple, compounding relapses is limited. Therefore, over time these two alternating processes of relapse and repair are associated with progressive disconnection of structural and functional information pathways in the brain and body, altering communication within and between networks that support many physiological and cognitive processes.

Between 5 and 25 years after diagnosis with RRMS, PwMS usually convert to the third stage of the disease (Inojosa et al., 2019), known as secondary progressive multiple sclerosis (SPMS). Rather than aggressive and rapid disease progression followed by full-to-partial recovery, SPMS is characterized by a slow and steady advancement of neurodegeneration and lesion spread, coupled with a linear and unrelenting loss of function. This disease stage, more so than RRMS, is associated with increased levels of disability and decline in quality of life (Huijbregts et al., 2004; Patti et al., 2007). Approximately 10-15% of people with MS present immediately with this progressive form, which is known as primary progressive multiple sclerosis (PPMS), and as such experience a rapid decline over a short time course with little hope of recovery (Ransohoff et al., 2015; Reich et al., 2018). Regardless of time course and initial presentation, over the MS disease course disability compounds and disease burden increases in several domains of functioning that varies between patients, but can include cognitive, physical, and sensory deficits.

Due to heterogeneity of symptoms and variance in disease time course, progression of MS has been quantified in a variety of ways, including: the number, severity, and frequency of relapses in the early disease stages; structural measures of neurodegeneration such as total lesion volume; and self-reported or clinician-assessed disease burden. In particular, the Extended Disability Status Scale (EDSS) is widely used clinically as a measure that quantifies disease burden of individuals in any stage of disease (Kurtzke, 1983). The EDSS is scored on a scale of 0 – 10 in half point increments, with higher scores corresponding to higher levels of disability. The scale assesses eight key 'functional systems' often affected by MS: pyramidal function (limb motility and muscle weakness); cerebellar function (balance, tremors, coordination, ataxia); brainstem function (speech, swallowing, nystagmus); sensory function (numbness, loss of sensation); bowel and bladder function; visual function; cerebral functions (memory, problem solving, attention, processing speed); and other, a dichotomous indicator of whether other symptoms are present and believed to be attributable to MS. Given the heterogenous presentation of MS across these functional systems and the lack of a reliable biomarker of disease progression, the EDSS is currently considered the gold standard for MS disease assessment and monitoring, and commonly serves as a clinical endpoint for pharmacological and behavioral interventions (Meyer-Moock et al., 2014).

Notably, total lesion volume, defined as the percentage of white matter lesions observed on a T2 FLAIR image, has also been used commonly as a marker of disease progression in this population (Sormani & Bruzzi, 2013). In contrast to EDSS, which may be thought of as representing the cumulative, experienced symptoms of MS, total lesion volume has been used as a marker of disease pathology itself.

1.2 Multiple Sclerosis: Proposed Pathophysiological Mechanisms of Decline

It is currently unknown whether events internal or external to the CNS initially trigger MS disease pathology; the auto-immune response and infiltration of lymphocytes may be the response to an unknown process or infection within the CNS which triggers neurodegeneration as well as a peripheral autoimmune response (Dendrou et al., 2015). Alternatively, the peripheral autoimmune response and infiltration of lymphocytes into the CNS may itself be the triggering event that drives focal lesions and neurodegeneration (Dendrou et al., 2015). Regardless of etiology, what has been discovered about the pathophysiology of MS has resulted in a lesion-centric approach to conceptualizations of MS and has shaped not only MS diagnostic decision-making, but many aspects of disease monitoring, treatment, and goals of pharmaceutical interventions.

The clinical stages of MS are differentiated not only by their presentation of symptoms, but by the progression and characteristics of their lesions and the activity of the body's autoimmune response. Kuhlmann et al. proposed a lesion classification system that differentiates between active, mixed active/inactive, and inactive lesions in order to better distinguish between disease stages (Kuhlmann et al., 2017). By their definition, active lesions contain macrophages in the majority or entirety of the lesion, mixed lesions are characterized by macrophages only in the border of the lesion and cell loss in the center of the lesion and inactive lesions lack macrophages nearly completely (Kuhlmann et al., 2017).

Systematic dissection of 182 MS brains from the Netherlands Brain Bank led to the investigation of 7562 MS lesions and demonstrated that there is significant inflammatory

lesion activity in progressive MS: 78% had at least one mixed active lesion present, and 57% of all lesions were either active or mixed (Luchetti et al., 2018). Furthermore, there was a correlation between disease burden and higher proportion of active/mixed to inactive lesions as well as total lesion load (Luchetti et al., 2018). People with progressive MS compared to RRMS had a higher lesion load and a lower incidence of remyelinated lesions. Of these, men had a higher proportion of active/mixed to inactive lesions compared to females across all disease stages (Luchetti et al., 2018).

In addition to the heterogeneity found in lesion presentation and characteristics across disease stages, lesions are also disseminated unpredictably in space. Areas of cortical demyelination may be guided by regions with low flow of CSF, such as the brain's sulci, while other studies have found that focal white matter (WM) lesions occurred more commonly in areas with high venous density, as well as the watershed regions of the brain characterized by low arterial blood flow (Haider et al., 2016). This proclivity may explain some of the shared presentation of symptoms across individuals, however, in general there is great variability regarding the location and progression of symptoms, resulting in an unpredictable disease course that is difficult to diagnose, predict, and treat.

Additional data from autopsy studies and the examination of active lesions has resulted in two conclusions. First, data strongly suggests that that tissue injury is likely mediated by lymphocytes, and second, inflammation continues to drive the process of demyelination in all disease phases (Lassmann, 2018). However, these disease processes do differ between disease stages; RRMS is thought to be particularly characterized by the influx of immune cells into the CNS from the peripheral immune system, resulting in neuroinflammation as well as the formation of new lesion damage which may or may not be

repaired (Baecher-Allan et al., 2018). Contrastingly, in the progressive stage of the disease this influx is reduced and instead there are pathological processes within the CNS that primarily continue to drive inflammation (Baecher-Allan et al., 2018). Therefore, progressive MS is characterized by a marked reduction or absence of the formation of new focal white matter lesions and inflammatory blood brain barrier penetration, and instead the slow growth of pre-existing lesions (Lassmann & Bradl, 2017). Unique to this stage, mixed lesions form that slowly expand in size, and in the absence of the attempts at cellular repair found in early disease, become areas of complete cell death (Frischer et al., 2015). Notably, these "smoldering" plaques are more common in men than women for unknowns reasons, and men also have a smaller proportion of inactive plaques in older age, though an equal proportion of successfully-remyelinating lesions (Frischer et al., 2015).

This complex but striking disease course of inflammatory white matter lesions has become hallmark of MS, despite evidence that in addition to lesion pathology, the disease is characterized by other, significant but subtle changes that have gone largely unaddressed in clinical disease monitoring and treatment (Sormani et al., 2009). Despite this, treatment of MS has made significant advances in the past 25 years even by focusing primarily on limiting the accumulation of white matter lesions. Currently, there are approximately one dozen FDA-approved therapeutic drugs that target immune response and the inflammatory component of the disease in its relapsing-remitting stage. These pharmacological treatments are likely responsible for the observed delayed time to disability and lower relapse rate in current MS cohorts compared to previous ones (Baecher-Allan et al., 2018). However, these treatments work by reducing the burden of the inflammatory lesions typical of RRMS and have little effect on the secondary mechanisms that seem to drive the progressive stage of

disease (Ontaneda et al., 2017). Thus, even with accurate and early detection and treatment that may slow the accumulation of new lesions, MS still progresses through a set of secondary mechanisms as well as slowly expanding plaques from unprevented attacks. In addition, despite the success of pharmacological methods at delaying disease-related physical (Claflin et al., 2019) and cognitive (Landmeyer et al., 2020) changes in RRMS, these treatments extend the already dominating amount of time PwMS spend in the relapsing-remitting stage of disease (Ransohoff et al., 2015; Reich et al., 2018) and do not fully prevent the presentation of these symptoms nor the accumulation of disability in these domains. Due to these two gaps in treatment efficacy, a need still exists for a biomarker of disease progression, as well as methodological investigation into the ways in which disease pathology results in the accumulation of symptoms and relationship of those symptoms to overall disease burden.

Given that lesion accumulation and volume are considered the primary pathological features in MS, MRI-based biomarkers that utilize structural images have been the subject of much interest, with the hope that they would allow for monitoring of disease progression and further understanding of the mechanisms of symptom progression. However, given the complexity of disease pathology in MS - which may include focal demyelination, neuro-inflammation, widespread neurodegeneration, as well as the body's attempt to re-myelinate and compensate for these insults - there is also interest in functional MRI-based biomarkers which may be able to reflect the sum impact of these interacting processes. This is especially true as researchers move beyond the historical focus on MS-related physical decline and attempt to understand the relationship between MS-related disease processes and the decline of complex cognitive functions, such as working memory and information processing.

1.3 Cognitive Dysfunction in People with Multiple Sclerosis

Along with gray and white matter lesions, dysfunction of normal-appearing brain tissue, neurodegeneration, and inflammation, people with RRMS present most notably with decline in the sensory/motor, cognitive, and mood domains (Prakash et al., 2008). Although cognitive deficits have been associated with the progression of MS since Charcot first described the disease in 1877, motor deficits and physical disability have historically served as the primary measure of disease progression (Charcot, 1877; Sumowski et al., 2018a), until Rao and colleagues brought renewed interested to the prevalence of cognitive dysfunction (Stephen M. Rao, Leo, Bernardin, et al., 1991; Stephen M. Rao, Leo, Ellington, et al., 1991). Current estimates indicate cognitive impairment affects up to 70% of PwMS, with approximately 40-50% experiencing mild to moderate deficits, and 10-20% experiencing severe deficits (Hämäläinen, 2016). As such, cognition has received increased attention due to the predictive nature of the relationship between cognitive dysfunction and current quality of life (Benedict et al., 2005), as well as future disease progression (Moccia et al., 2016; Zipoli et al., 2010) and relationship to disease subtype (Sumowski et al., 2018). In particular, MS-research suggests that processing speed and working memory may be two domains that are particularly important for their contribution to more complex cognition-dependent behaviors (Berrigan et al., 2013), playing a foundational or supportive role in many behaviors and activities that are essential for preserved quality of life. For example, Rao and colleagues suggested that processing speed and working memory are important for the maintenance of social relationships, self-sufficiency, and mental well-being (Stephen M. Rao, Leo, Ellington,

et al., 1991), and other have shown their importance for retaining employment, and therefore financial independence (Macaron et al., 2020).

Information processing speed, measured as reaction time in simple matching or identification tasks and often conceptualized as the speed with which information is able to be processed by the brain, is perhaps the most notable cognitive domain affected in PwMS (Brochet & Ruet, 2019; Costa et al., 2017). Rao and colleagues first described slowed processing speed in PwMS in 1989, demonstrating that compared to healthy controls, PwMS had slower reaction times on tasks such as the Sternberg Paradigm, in which participants are presented with and asked to memorize a set of digits and respond yes/no to whether each number in a series of digits presented on a screen is member of that initial set (S. M. Rao et al., 1989). Since then, the study of information processing speed in MS has grown considerably, and importantly, researchers have increasingly utilized tasks that attempt to isolate processing speed from related cognitive domains, such as working memory and episodic memory. These tasks, such as the Symbol Digit Modalities Test, and the Symbol Search and Coding tasks as found in the Weschler Processing Speed Index, require participants to match symbols and provide an oral or written response (Costa et al., 2017; Wechsler, 1981).

Working memory deficits, while less commonly observed than processing speed deficits, are also widely reported in MS (DeLuca et al., 2004). Working memory, defined as the system used for temporary storage and manipulation of information subserving more complex cognitive tasks (Baddeley & Hitch, 1974; Baddeley, 2000), and the tasks most commonly used to measure it, can be decomposed into the following core components: the maintenance process of sensory information; the process of actively manipulating that

information; and the speed with which these processes can be performed. The most valid measures of working memory, such as the N-back task, the Paced Auditory Serial Addition Task (PASAT), and the Weschler Working Memory Index (WMI) address all three processes in some way. As a component of the Weschler Adult Intelligence Scale, the WMI is a neuropsychological test of digit span and item manipulation within a time limit (Wechsler, 1981). During the N-back, participants must maintain and continuously update their memory for constantly changing visual stimuli while making a match/no match judgment under time pressure – though it is important to note that the N-back relies on recognition memory and not recall (Kirchner, 1958). The PASAT, and its visual counterpart, the PVSAT, consists of the presentation of a series of single digit numbers and requires addition of each new number to the previous, requiring maintenance, manipulation and quick information processing (Gronwall, 1977; Fos et al., 2000). While the N-back has most commonly been used with healthy populations (Van Essen et al., 2012), the PASAT/PVSAT has been demonstrated to be particularly sensitive to disease-related changes to working memory function in patients with multiple sclerosis (D'Esposito et al., 1996; DeLuca et al., 2004; Chiaravalloti & DeLuca, 2008; Genova et al., 2009).

1.4 Attempts at a Structural Imaging-based Biomarker of Cognitive Impairment and Disease Burden

Due to the important role that processing speed and working memory play in quality of life for PwMS, and due to the integral role MRI already plays in the diagnosis and

monitoring of MS, neuroimaging work in PwMS has consistently sought an imaging-based biomarker with which to detect, monitor and predict cognitive symptoms of the disease, and to understand the relationship between cognitive symptoms and disease progression.

Several studies have linked features of structural MRI – white matter lesion volume, volumetric measures of atrophy and degeneration, and microstructural damage – to risk of future cognitive decline (Calabrese et al., 2012; Deloire, 2012; Filippi, Preziosa, et al., 2013). Much of the focus on associating MRI-detectable markers with disease progression have focused on T2-weighted hyperintense lesions, likely due to diagnostic criteria of clinically definite MS that requires this imaging sequence to evaluate the presence of these lesions disseminated in space and time (Louapre, 2018). Supporting this line of thought, white matter lesions appear to be an important predictor of conversion from CIS to clinically definite MS. For instance, in a large cohort study including 1058 CIS patients followed on average for 17 years, the presence of greater than 10 lesions at baseline MRI had a hazard ratio of 11.3 compared to 5.1 for those with fewer than 10 lesions (Tintore et al., 2015). In addition, the development of multiple white matter lesions within the first year of CIS increased the risk of not only developing RRMS, but also of developing SPMS (Brownlee et al., 2017). In RRMS, two meta-analyses of 23 and 31 randomized, double-blind, controlled clinical trials indicated that a relapsing episode in the following 12-24-month period from diagnosis is most strongly predicted by increasing lesion count and lesion volume accumulation over a six-month period (N. De Stefano et al., 2010; Sormani et al., 2009; Sormani & Bruzzi, 2013).

Many large studies have also sought to investigate the relationship between white matter lesions, disability, and cognitive impairment. Notably, in a five-year longitudinal study of 312 PwMS, white matter lesion volume at initial measurement predicted change in

overall disease burden as measured by the Expanded Disability Status Scale (EDSS), and also predicted cognitive status at the 5-year follow-up (Calabrese et al., 2012). In 721 PwMS, T2 lesion volume was a significant predictor of processing speed, and processing speed was in turn a significant predictor of employment status, independent of age and physical disability (Macaron et al., 2020). In another study of 997 PwMS, T2-measured lesion volume was strongly and negatively correlated with processing speed (Spearman's rank correlation of -.44) and outperformed all other evaluated MRI metrics, including whole brain fraction, thalamic volume, and cervical spinal cord cross sectional area volume (Nicola De Stefano et al., 2015). Similar results correlating T2 lesion volume with EDSS and processing speed have also been found in smaller samples of 20-40 PwMS (Mike et al., 2011; Randolph et al., 2005; Stankiewicz et al., 2011).

Attempts to correlate lesion volume with measures of working memory specifically have not been as promising, and some studies that have investigated both processing speed and working memory have corroborated the first relationship and either failed to find the second completely (Fulton et al., 1999) or, while indicative of a possible relationship, failed to reach statistical significance (Papadopoulou et al., 2013). However, other larger studies of 597 PwMS have found that lesion volume predicted impaired processing speed and working memory at a nine-year follow-up (Patti et al., 2015), perhaps because working memory deficits emerge later in the disease course (Brochet & Ruet, 2019), or perhaps because the weaker relationship requires larger sample sizes.

Despite this, lesion volume does not fully account for either disease progression or cognitive impairment. There are also several studies that have shown no meaningful relationship between lesion volume and either disability or cognitive decline (Filippi, Agosta,

et al., 2013; Healy et al., 2017) termed the ‘clinicoradiological paradox’ (Louapre, 2018). As such, more recent methods have focused both on more advanced structural methods requiring specialized MRI sequences that highlight cortical lesions, as well as utilizing high-resolution imaging of 7T scanners that can identify pathological tissue that appears normal on low resolution images, so-called “normal-appearing” gray and white matter. However, the potential improved clinical utility of these methods is limited by their availability and ease of acquisition. More importantly, even with these specialized sequences and increased resolution, these methods are still currently not sensitive enough for detection of cortical lesions in normal-appearing matter to be consistently reliable (Louapre, 2018). Recent investigations combining T1w and T2w MRI with diffusion tensor imaging have revealed that structural parameters are numerous and may be differentially important for prediction of cognition and disability dependent on the specific domain of interest (Tóth et al., 2019) and may differ by disease subtype (Eijlers et al., 2018), making the goal of realizing a structural MRI biomarker not an impossible task, but an arduous one - and one that is potentially cost- and time-prohibitive in a clinical setting.

Additionally, while structural measures of disease burden may reflect important information regarding disease progression and even remyelination, they may not fully account for individual variation in functional reserve. By contrast, functional neuroimaging could ideally provide a proxy measure of the functioning of the brain, reflecting both disease progression and the brain’s ability to ‘rewire’ in a compensatory way. There is evidence to suggest that functional connectivity is supported by underlying structural connections (Greicius, 2008; Hermundstad et al., 2013; Honey et al., 2009), and therefore structural damage may also be reflected in changes to brain function. The use of connectivity fMRI

may provide additional insight into disease-related cognitive risk over and above traditional methods quantifying lesion load and neurodegeneration, or even increases and decreases in localized activity, and instead may be able to reflect the sum impact of these multifactorial structural insults. In addition, functional MRI may allow for the prediction of cognitive domains that are not easily correlated with structural measures of disease burden, such as working memory. Given that cognitive dysfunction in MS is likely multifactorial (white matter lesions, but also widespread neurodegeneration, neuroinflammation, and damage to white and gray matter than appears normal on standard MRI), the study of functional brain imaging to assess the effective impact of this wide array of disease-related pathology has become of increased interest. In particular, recent work has attempted to use graph theory to model the relationship between disease progression and the functional organization of the brain.

1.5 A Graph Theoretical Approach to Functional Neuromarkers in MS

Functional connectivity can be defined as the co-activation of neuronal signals between two distinct brain regions. Typically, functional connectivity networks are defined as either spontaneous or task evoked. Whereas task-evoked networks were first described in terms of coordinated signal changes in response to a specific activity or stimulus (Kwong, 2012; Ogawa et al., 1998), spontaneous functional connectivity in the absence of a specific stimulus was first described by Biswal et. al in participants who were asked to rest and think of nothing in particular (1995). Rather than finding a lack of connectivity in the absence of a

directed behavior, Biswal and colleagues observed strong functional connections between an a-priori "seed" region in the motor cortex and other regions of the brain (Biswal et al., 1995). Initial reaction to the observation of this and other resting-state networks that followed was to suggest that this co-activation was the result of high frequency cardiac and respiratory noise aliasing back into the range of the bold response (van den Heuvel & Hulshoff Pol, 2010). However, in the two and a half decades since, the presence of multiple "resting state" networks have been widely replicated even when controlling for the influence of artifacts (Buckner et al., 2008; Fox & Raichle, 2007; van den Heuvel & Hulshoff Pol, 2010). Evidence that these resting-state functional connectivity patterns are reflective of neuronal signal is threefold: first, these signals are observed between regions with overlapping function and neuroanatomy; second, the frequency component of these resting-state signals appear to be dominated by low, not high, frequencies; and third, studies have shown associations between resting-state connectivity signals and direct electrophysiological neuronal recordings (van den Heuvel & Hulshoff Pol, 2010).

To this day, the fact that rs-fMRI does not evoke cognition-relevant signal in the same way that task-fMRI, in which participants engage in a behavior of interest during fMRI acquisition, does appear to suggest weaker brain-behavior correlations (Greene et al., 2018). However, several advantages to the use of resting-state fMRI, especially in clinical applications, exist (Fox & Greicius, 2010). Resting-state fMRI allows for the measurement of more than one network simultaneously - and these networks are reliably observed - most notably, the default mode network, the somatosensory network, the frontoparietal control network, an auditory network, a language network, and the dorsal and ventral attention networks (Lee et al., 2013). Additionally, evidence suggests that these resting-state networks

are inherently tied to the structural organization and connections of the brain (Greicius, 2008; Hermundstad et al., 2013; Honey et al., 2009). That resting-state networks have been associated with the surrounding structure of the brain's white matter is of key importance for the study of neurodegenerative diseases with white matter lesion pathology.

In addition, the ease with which resting-state fMRI can be collected has resulted in wider implementation in clinical research applications than task-fMRI (Smitha et al., 2017), with some large open access protocols collecting only resting-state fMRI. Even in endeavors that do collect task-fMRI, the expensive and time-consuming nature of MRI work necessitates limiting the collection of task-fMRI to a few, carefully chosen cognitive domains and measurements. There is, as of yet, no consensus on which cognitive task best measures each cognitive domain within the research community, nor which cognitive domains are most crucial to collect, given that this varies greatly depending on the question of interest. Resting-state fMRI, however, has applicability to a wide range of questions pertaining to multiple cognitive domains of interest, and is ubiquitously collected, allowing for investigations with larger, more varied datasets and populations.

In calculating functional connectivity at rest, several options exist to detect these networks. Most commonly: a "seed based approach" in which a region of interest, either informed by prior literature or the results of a localization task, is chosen and used to identify a network by isolating other regions that are functionally connected to that seed; an Independent Components Analysis (ICA) technique, which decomposes the time series data into groups of regions with similar signals across time (Fox et al., 2005); and graph theory methods (Rubinov & Sporns, 2010): a typically whole-brain approach which conceptualizes the brain as a network.

In this last case, the study of functional networks at rest offers the opportunity to study the inherent architecture of the brain using a mathematical approach in which the brain is conceptualized as a graph. The advantage of this approach, as compared with others, is that it allows for a more comprehensive characterization of the whole brain as a system of interconnected networks (Bassett & Sporns, 2017). In a graph theoretical framework, regions of the brain, typically voxels or larger regions of interest as defined by an atlas, are treated as vertices, or nodes, and the functional or structural connections between them are represented by either a binary or a weighted value, known as an edge. Using this method, all of the connections of the brain are represented by a functional connectivity matrix of these edge values, where each cell of the matrix represents the relationship between two nodes in the brain (Fornito et al., 2016). The values of these edges are themselves flexible, allowing for varying amounts of information. In the case of a binary matrix, values of 1 represent connected nodes, whereas values of 0 suggest nodes not connected functionally. In the case of a functional graph, this could reflect whether a functional connection meets a predefined threshold of significance; in the case of a weighted matrix, the values of the cell can represent the strength of that connection between nodes. Conceptualizing the connectivity of the brain in this way allows for the calculation of several metrics that further our understanding about how the brain is organized overall, but also how the sub-networks within it are organized. Commonly, these metrics have been used to examine both features shared by groups, but also to identify differences between individuals and groups as a function of individual differences, age, and disease.

Network measures, such as measures of network centrality, segregation, and integration, can characterize the functional organization of the brain and be used to both

reveal disease-related abnormal brain organization and be linked to disease status and cognition. Network centrality identifies the key nodes in a network based on some criteria of interest, and measures of segregation and integration are complementary metrics of the communication between nodes and groups of nodes, often referred to as modules (Sporns, 2018).

Graph theory studies in MS have to date been limited, but have described a pattern of local reorganization and long-range disconnection, and a resulting decrease in global efficiency of information transfer (Fleischer et al., 2019). Investigations into resting-state network changes have revealed decreases in local and global efficiency in people with relapsing-remitting MS (Rocca et al., 2016; Shu et al., 2016), with one study demonstrating that overall connectivity strength could be used to differentiate PwMS from people with CIS with an accuracy of 77% (Liu et al., 2017). Others have found increased network centrality in the DMN in cognitively impaired PwMS compared to both healthy controls and cognitively preserved PwMS (Eijlers et al., 2017). Particularly relevant to the current study, Gamboa et al. (2017) investigated working memory performance in people in the early stages of MS or CIS with low to no disability and found increased modularity compared to healthy controls, which correlated negatively with working memory; modularity was also used to classify PwMS from CIS with 75% accuracy. Impairment on a working memory task has also been associated with increased brain segregation, and processing speed associated with small worldedness and global efficiency (Welton et al., 2020). PwMS with cognitive impairment have also demonstrated a significant correlation between lower path length and better processing speed (Hawkins et al., 2020). In another study, a support vector machine built using graph theory metrics successfully capitalized on local efficiency and node strength

metrics to differentiate PwMS from healthy controls, and to differentiate those who were cognitively impaired from those who were cognitively preserved (Solana et al., 2019).

Together, these studies have resulted in a proposed model of cognitive dysfunction whereby the impact of disease-related structural changes to network efficiency are moderated by resilience or compensation of the network as measured by its retained modularity (Fleischer et al., 2019; Schoonheim et al., 2013, 2015). According to this model, at some point during disease progression with accumulating increased tissue damage, network resilience eventually reaches a breaking point, resulting in a collapse of information transfer efficiency and catalyzing steep increases in cognitive, and other, deficits (Fleischer et al., 2019; Schoonheim et al., 2015).

Thus, the association of graph theory metrics with clinical and cognitive variables has done much to advance our understanding of how metrics of brain organization correlate with both MS disease and cognitive changes. It has also led to theoretical models of disease-catalyzed network collapse and cognitive decline and has shown to have some utility in the classification of disease subtypes. However, the field still lacks research into the development of whole-brain functional neuromarkers that are specific to particular cognitive domains, rather than descriptive of general brain organization. The development of individual markers of processing speed and working memory, for example, would allow for investigations into how functional processes that support these domains are affected in MS, and how those functional processes relate to disability.

1.6 Connectome-based Predictive Modeling Techniques for Network Derivation

Machine learning, broadly defined, is a subset of artificial intelligence techniques that can be used in conjunction with large or highly dimensional datasets to identify and characterize signal within that data (unsupervised learning), or to make individual-level classifications or predictions by mapping data onto a target variable (supervised learning). Whereas the former category has long been used by fMRI researchers interested in defining the spatial or hierarchical organization of the brain (Khosla et al., 2019), the latter is of special interest to clinical researchers who may wish to make brain-behavior associations and predict clinically relevant variables in patient populations (Bzdok & Meyer-Lindenberg, 2018).

Despite variation in the purpose and implementation of these techniques and their increasing application in clinical research, supervised machine learning in the neuroimaging domain has been limited by the vast array of methods and a lack of understanding regarding their implementation. In general, these techniques require that a researcher make a series of choices, each with critical consequences. Typically, these steps and the associated choices are: 1) evaluation method, 2) feature selection and optimization, 3) algorithm implementation, and 4) performance metric.

Connectome-based predictive modeling (CPM; Xilin Shen et al., 2017) is one recent methodological framework that allows for the creation of a whole-brain, data-driven functional network of behaviorally-relevant and domain-specific edges, and generates a model of the relationship between graph theory metrics in these edges and the domain of interest, which is then tested on novel individuals. It is an implementation of a supervised

machine learning procedure that: 1) utilizing cross-validation, 2) selects edges that are significantly correlated with behavior across members of the training set, 3) takes the average connectivity strength from thresholded edges to predict behavior using linear regression, and 4) evaluates the model by correlating predicted and observed behavioral scores.

Although the generalizability of these models is generally unknown without repeated validation on separate, external datasets, the 'internal validation' procedure provides a first step toward the potential development of a biomarker of behavior and allows researchers to explore within-sample associations of domain-specific connectivity and clinical or behavioral variables. Thus, the network of significant edges resulting from the CPM method may have potential use in the monitoring and prediction of clinical outcomes.

In comparison to other techniques, the use of machine learning techniques introduces several advantages, such as the generation of more generalizable and reliable models (Scheinost et al., 2019; Sripada et al., 2019) and a less stringent approach to correcting for multiple comparisons during feature selection through the use of cross validation (Shen et al., 2017). Whereas univariate functional connectivity methods that examine the reliability of individual edges across multiple sessions has poor reliability, multivariate methods that examine the reliability of all edges simultaneously result in significant improvements to reliability (Noble et al., 2017, 2019). The test-retest reliability of resting state fMRi has direct relevance to the prediction of cognitive measures because reliability sets an upper limit on validity (Noble et al., 2017). Thus, an unreliable measure cannot be used for reliable prediction because too much of the signal varies from measurement to measurement.

CPM in particular has been shown to produce reliable and generalizable models across constructs, first demonstrated through the derivation of networks and models

predictive of fluid intelligence (Finn et al., 2015) and sustained attention (Rosenberg et al., 2015). The model built to predict sustained attention has been shown to generalize to an ADHD population (Rosenberg et al., 2015), as well as a separate sample of younger and older adults (Fountain-Zaragoza et al., 2019). Researchers have also successfully derived models of working memory in younger adults that generalized to older adults with and without Alzheimer's disease (Avery et al., 2019) and people with multiple sclerosis (Manglani et al., in prep). Additionally, the CPM technique has been used successfully in a broad array of disciplines to derive functional connectivity models of temperament (Jiang et al., 2018), stress (Goldfarb et al., 2020), processing speed in older adults (M. Gao et al., 2020), cognition in a sample of older adults with mild cognitive impairment and Alzheimer's disease (Lin et al., 2018), and cognitive decline in breast cancer patients (Henneghan et al., 2020), in a list that is quickly growing.

An additional advantage of CPM over many supervised and unsupervised learning techniques is the preservation of select features, in this case edges, that are important for prediction, and dichotomizing edges into those that are positively and negatively related to the target variable. This allows researchers to avoid worrisome 'black box' prediction, where the mapping of inputs to outputs is unintelligible and potentially driven by confounds. CPM additionally provides a binary or weighted mask of relevant edges that can be checked against previous knowledge but also treated as a data-driven whole-brain approach to explore the contribution of all edges, potentially revealing new information about the relationship between functional connectivity and behavior.

For example, CPM has revealed that connectivity of frontoparietal, medial frontal, DMN and motor nodes may mediate age-related differences in sustained attention (Fountain-

Zaragoza et al., 2019), that predicting processing speed in older adults may rely on within-network connectivity of the motor and visual networks (M. Gao et al., 2020), and that contributions of hippocampal and PFC connectivity may be larger than those of the basal ganglia, amygdala and cingulate gyrus for predicting temperament (Jiang et al., 2018). CPM itself can also be used in a hypothesis-driven way through the use of a specific atlas or the restriction of looking at a particular region that has historically been linked to the target variable, such as investigating the relationship between hippocampal connectivity and stress (Goldfarb et al., 2020).

Thus, CPM's transparency, interpretability, and ease of implementation make it an appealing technique when compared to methods that maximize predictive accuracy, often beyond what CPM is capable of (Dadi et al., 2019), but which inform the researcher little about how prediction is being achieved. As the neuroimaging community continues to strive toward translational applications of machine learning to clinical populations, potentially informing treatments and standard of care, interpretability is an important ethical consideration. Black box machine learning methods that maximize prediction without researcher oversight open themselves to the possibility of relying on information that is spuriously related to target variables, potentially introducing bias into the medical decision-making process.

One key advantage to the application of CPM to an MS population is the potential development of a domain-specific, fMRI-based predictor of disease progression and cognitive decline. Structural methods such as volumetric and lesion-based approaches lend themselves well to interpretable data-reduction to single metrics that can be used to track and predict disease progression and cognitive decline. Aforementioned studies indicate that

increases in lesion load volume and atrophy is predictive of future disease progression and cognitive status. Likewise, graph theory approaches to functional connectivity allow us to characterize the organization of the human brain and relate decreases in a modular structure to disease progression and cognitive status. However, a gap remains in identifying biomarkers specific to cognitive domains of interest, such as processing speed and working memory in PwMS. CPM allows researchers to identify networks to serve as clinical markers specific to these cognitive domains, and potentially aid in the prediction of behavioral change.

For this reason, the current study aimed to investigate whether CPM could be used to develop domain-specific functional connectivity neuromarkers of working memory and processing speed as measured by the Wechsler Adult Intelligence Scale, in which these domains are separable and non-overlapping. Currently, associations of disease and behavior in research with PwMS tend to rely on tasks that tax multiple cognitive domains simultaneously, such as the Paced Auditory Serial Addition Test, Symbol-Digit Modalities Test, and the N-back task. The prediction of multiple cognitive domains simultaneously could potentially increase predictive power, but they do not allow for the examination of differential onset of cognitive dysfunction in PwMS. Additionally, use of the WAIS-IV is common among healthy controls, allowing for contextualization of cognitive decline in PwMS within the larger population.

1.7 Specific Aims

The ***long-term goal*** of this work is to develop fMRI-based neuromarkers of cognitive functioning in people with MS that can be employed to understand progression of disease course as well as serve as surrogate endpoints in clinical trials targeting cognitive improvements. To achieve that goal, ***the overall objective*** of the current study was to build a whole-brain model of functional connectivity that is predictive of working memory functioning and processing speed in people with MS. Our main hypothesis is that these two resting-state models, derived to predict cognitive functioning in two key domains impacted in those with MS, will additionally predict disease status in this population. Using an existing dataset in our laboratory, we will build the WM-fc and PS-fc models to predict working memory and processing speed, respectively. The ***rationale*** for the proposed study is to employ functional MRI to develop neuromarkers that are non-invasive, accurate, and accessible to detect and monitor cognitive status and disease burden in those with MS. To accomplish our objective, the main hypothesis will be tested in accordance with the following two specific aims:

1. **Derive whole-brain based markers of functional connectivity using performance on validated measures of cognitive functioning (WM-fc and PS-fc).** Employing connectome-based predictive modeling that captures the co-activity of the entire brain during resting-state fMRI to build predictive models, we aimed to first derive whole-brain models of functional connectivity in order to determine in-sample fit for working memory abilities and processing speed in our sample of

individuals with MS. Our hypothesis was that the use of the multivariate predictive modeling approach, codifying interactions across the entire brain, would significantly predict working memory and processing speed in people with MS.

2. **Determine the validity of the WM-fc and PS-fc models in explaining variance in measures of disease status.** In order to assess the potential clinical utility of functional neuromarkers of cognition for predicting disease status compared to structural and cognitive measures, we aimed to build a multiple linear regression model with CPM-derived metrics from functional connectivity, lesion load, and cognitive performance on WMI and PSI to predict EDSS. Given the established associations between cognitive functioning and metrics of disease status, we hypothesized that our connectome models of working memory and processing speed functioning would be associated with total lesion volume and EDSS scores, but that metrics from the functional MRI markers of cognition would account for unique variance in EDSS compared to total lesion volume and cognitive performance scores.

Chapter 2. Method

The current analyses were completed using a dataset intended to test the association between physical activity and working memory performance in people with multiple sclerosis. The current analyses utilized resting-state functional magnetic resonance imaging (rs-fMRI) data, processing speed and working memory subtests of the Wechsler Adult Intelligence Scale (WAIS-IV; Wechsler, 1981), calculations of whole-brain lesion volume, and the Expanded Disability Status Scale (Kurtzke, 1983).

2.1 Sample Recruitment and Screening

Participants in this dataset were recruited from the Columbus, Ohio area through a combination of outreach through The National Multiple Sclerosis Society website, the established Clinical Neuroscience Lab database of people with MS, as well as flyers distributed throughout the community and in nearby medical and MS treatment centers. Inclusionary criteria required that eligible participants be 30-59 years of age, score greater than a 23 on the Mini-mental Status Exam (Folstein et al., 1975), have a corrected vision of at least 20/40, score less than or equal to 19 on the Beck Depression Inventory II (Beck, A. T., Steer, R. A., & Brown, 1996), be right-handed, have an Expanded Disability Status Scale score of between 0 and 5.5, be free of comorbid neurological or psychiatric disorders, be

relapse and corticosteroid use free for 30 days prior to enrollment, have a clinically definite diagnosis of relapsing-remitting MS, and have no contraindication to the MR environment. Eligible participants who consented to study participation completed neuropsychological assessment and a neuroimaging session. Additionally, they completed a 7-day physical activity monitoring in service of the parent study's original aims, which is of no direct relevance to the current thesis. This study was approved by the Ohio State University Institutional Review Board.

2.2 Neuropsychological Assessment

In the week prior to the neuroimaging visit, participants completed the Working Memory (WMI) and Processing Speed (PSI) indices from the WAIS-IV.

Processing Speed Index: The Processing Speed Index from WAIS-IV is composed of two subtests that measure an individual's ability to complete time-dependent graphomotor tasks by visually inspecting and categorizing symbols. In the symbol search subtest, individuals scan rows of symbols attempting to identify and mark those that match a set of target symbols. In the Coding subtest, individuals use a symbol-digit key to transcribe as many symbols with their corresponding digit as they can within a time limit. The dependent variable was the age-corrected Processing Speed Index score.

Working Memory Index: The Working Memory Index from WAIS-IV is likewise composed of two subtests that measure the ability to hold and manipulate numbers, testing, among other things, attention, encoding, and mental manipulation abilities. In the Digit Span

subtest, participants are asked to listen to an increasingly large set of numbers and repeat them, either in reverse or chronological order. In the Arithmetic subtest, individuals are orally administered mathematical word problems, requiring that they both attend to and retain the information in the problem and manipulate the information in their head. The dependent variable was the age-corrected Working Memory Index score.

2.3 Clinical Variables

Expanded Disability Status Scale

The EDSS is an MS-specific, self-report questionnaire intended to quantify the total disease burden of the disease and provide a method to measure disease progression over time (Kurtzke, 1983). The scale ranges from 0 to 10 in half point increments, with higher numbers representing higher levels of disease burden (Figure 2). Scores from 1 to 5.5 represent individuals who have retained the ability to walk without aid, and additionally assesses for dysfunction across eight systems: muscle weakness and/or impaired limb function; balance and coordination issues, tremors, ataxia; speech and swallowing function and nystagmus; numbness or sensory issues; bladder or bowel dysfunction; visual dysfunction; memory and cognitive dysfunction, and any other deficit observed. EDSS scores above 5.5 apply to individuals with impaired walking ability, and primarily scores the severity of this impairment.

2.4 Neuroimaging Data Acquisition

Each participant underwent a 75-minute scan, collecting both structural and functional MRI data on a Siemens 3 Tesla Trio scanner with a 32-channel head coil. For the structural scans, a high-resolution 1-mm voxel T1-weighted image (TR = 1950ms, TE = 4.44ms, flip angle = 12°, field of view = 256 x 232 x 176mm^3, slice thickness = 1.00mm^3, voxel size standard = 1.0-mm^3, 176 sagittal slices acquired interleaved), and a T2-weighted Fluid Attenuated Inversion Recovery (FLAIR) image (TR = 14000ms, TE = 7.3ms, flip angle = 120°, field of view = 350 x 263 x 350mm^3, slice thickness = 2.00mm, voxel size standard = 0.8 x 0.8 x 2.0-mm^3, 60 slices acquired interleaved). In addition to several task sequences outside the scope of the present study, a 15-minute, 450 volume resting-state scan was collected (TR = 2000 ms, TE = 30 ms, flip angle = 73°, field of view = 220 x 200 x 116mm^3, slice thickness = 3.4mm^3, voxel size = 3.4mm^3, 34 slices acquired interleaved), in which participants were asked to fixate on a white cross presented on a black screen while thinking of nothing in particular. All data was converted from DICOM to Nifti format using the Brain Imaging Data Structure (BIDS; Gorgolewski et al., 2016), and image quality and subject in-scanner motion was assessed using a validated quality control pipeline (MRIQC; Esteban et al., 2017).

2.5 Neuroimaging Data Preprocessing

All preprocessing of structural and functional images was completed using the fMRIPrep pipeline version 1.4.1 (Esteban et al., 2019) which uses Nipype 1.2.0 (K.

Gorgolewski et al., 2011). fMRIPrep completes minimal preprocessing utilizing an array of tools from various analysis software packages, including brain extraction, motion correction, field unwarping, normalization, and bias field correction (Esteban et al., 2019).

Total Lesion Volume

Due to the fact that structural MRI images from PwMS have T2w, and sometimes T1w, visible lesions that may interfere with standard preprocessing steps such as segmentation and normalization (Battaglini et al., 2012), we included in the analysis pipeline a high-resolution lesion map which fMRIprep utilized to fill lesion locations with signal from non-lesion matter. To create this lesion map, T2 images were first brain extracted using FSL's BET tool, and then imported to the Xinapse Systems Jim 6.0 software (Xinapse Systems, 2011), where two independent raters (ICC = .968, $p < .01$) manually identified white matter lesions by marking lesion voxels in each slice of the T2 images. This manual lesion map was then combined with an automated lesion finding tool segmentations in the Jim software that uses prior knowledge of MS lesions from 300 expert manual lesion to create an additional lesion probability map (Xinapse Systems, 2011). These two lesion maps, the manual and the automated, were algorithmically combined giving equal weight to each, to create a final, semi-automated lesion map. Total lesion volume measure in cubed centimeters was calculated in the Jim software, and utilized in the regression analysis predicting EDSS. Using FSL, the lesion map was converted to high-resolution space in order to be used for lesion filling of the T1w image in fMRIprep, a procedure which improves segmentation and registration of the T1w image to standard space by refilling lesion spaces with the intensity values from normal-appearing, non-lesion white matter.

Preprocessing of Structural Data

The T1 image was first corrected for intensity non-uniformity with the ANTs *N4BiasFieldCorrection* function, and then skull-stripped with the ANTs *antsBrainExtraction* tool (ANTs 2.2.0; Avants et al., 2011). The lesions in the T1w image were identified and filled with surrounding voxel intensities in order to improve subsequent registration and segmentation. Spatial normalization was performed using ANTs' *antsRegistration* tool, using nonlinear registration for alignment of the T1w image to standard space (MNI152NLin2009cAsym). Brain tissue in the T1 was segmented into cerebrospinal fluid (CSF), white-matter (WM), and gray-matter (GM) using FSL's (FSL 5.0.9; Jenkinson et al., 2012) *fast* tool (Zhang et al., 2001), and then Freesurfer (FreeSurfer 6.0.1;Fischl, 2012) was used to refine these segmentations using both the T1w and FLAIR image.

Preprocessing of rs-fMRI

For the single BOLD resting-state run, a single reference image and a skull-stripped version of this image was generated, and the susceptibility distortion was estimated from the fieldmap image using a custom methodology from fMRIPrep. The sequence was corrected for susceptibility distortion using the fieldmap image. The BOLD reference image was co-registered to the T1w reference using Freesurfer's *bbregiste*r tool (FreeSurfer 6.0.1;Greve & Fischl, 2009), using nine degrees of freedom. Head motion was estimated in relation to the BOLD reference and corrected for using FSL's *mcflirt* (FSL 5.0.9;(Jenkinson et al., 2002), and then slice-timing corrected using AFNI's *3dTshift* (Cox, 1996, 2012). Volumes that

exceeded a .5 mm FD cutoff were considered high motion outliers and included as regressors in the following step.

After data preprocessing, the first 5 volumes of the functional data were excluded to allow for the stabilization of the BOLD signal. A final regression step was implemented using the *signal.clean* function in the Nilearn Python package (Abraham et al., 2014) to implement high pass temporal filtering at .01 Hz, as well as the regression of 36 motion parameters (WM/CSF/global timecourses, 6 rigid body motion parameters, their temporal derivatives, and their quadratic term), high motion confound volumes, and mean signal from the WM, CSF, and global signal. No spatial smoothing was performed, given recent work suggesting smoothing may reduce between subject differences in graph theory metrics (Alakörkkö et al., 2017).

2.6 Prediction of Processing Speed Index and Working Memory Index

Connectivity Matrices

Connectome-based predictive modeling was used to examine the explained variance in processing speed and working memory from resting-state functional connectivity. After nuisance regression of the time-series from each node of the Shen atlas (X. Shen et al., 2013), each time-series was correlated with every other time-series to create a 268 by 268 functional connectivity matrix for each participant, with each edge representing the degree of correlation between two nodal time-series. Given the small sample size of this study, nodes

were removed from every individual's connectivity matrix if they were missing in any participant.

One potential confound in functional connectivity analyses is the influence of in-scanner head motion, which can create the spurious appearance of functional connections between nodes. In addition to motion correction at the preprocessing stage, to examine the potential influence of residual motion during rs-fMRI on CPM models, a three-step procedure was followed.

First, for each subject's processed rs-fMRI data, a mean framewise displacement measure was calculated by taking the average absolute temporal derivative of 6 motion parameters (3 translational and 3 rotational) from volume to volume in the motion-corrected data (Power et al., 2012). This post-processed mean FD therefore represented the remaining motion in each participant's resting-state timeseries. Processed mean FD was Pearson correlated with PSI and WMI, respectively, to determine the overlap between behavioral data and subject motion. Second, participants who averaged a processed FD of above .15 were determined to have excessive motion and excluded from analyses. This cutoff has been widely used in CPM analyses and was selected *a priori* in order to balance the tradeoff between sample size and clean data. Third, a CPM was built specifically to predict motion. This allowed us to: a) determine whether exclusion of high motion subjects reduced the influence of residual motion on CPM models, and b) identify the sample-specific mean FD cutoff required to reduce motion artifact such that it is no longer sufficient to predict subject-level motion from functional connectivity and compare it to our *a priori* cutoff of .15.

Network Derivation and Model-Building

Networks and models were built separately for PSI and WMI utilizing a leave-one-out cross validation approach (Figure 1). For PSI and WMI respectively, participants were divided into a training set, consisting of n-1 participants, and a testing set, consisting of the left out individual. Within each cross-validation iteration, each edge in the functional connectivity matrix across all individuals was Spearman correlated with the behavioral input while statistically controlling for processed motion scores, and thresholded at a significance level of $p < .01$, creating a set of edges significantly correlated with behavior. These edges were separated into two distinct networks: a high network, consisting of all edges positively correlated with behavior, and a low network, consisting of all edges negatively correlated with behavior.

Within each of these networks, connectivity values in all selected edges were averaged to form a summary statistic. The validity of averaging the edge connectivity values into a single score was evaluated by comparing the correlation of the most predictive edge in the entire network with behavior to the correlation of the summary strength scores with behavior. Three regression models were formed by regressing behavioral scores onto the high and low summary statistics for the n-1 individuals. Using the training-set network, summary strength scores were then calculated for the left out individual and inserted into the linear model in order to produce an estimated behavioral score from the unseen individual's data.

This procedure was repeated for every individual such that every individual's data was left out of model training once, and thus received an estimated behavioral score. Estimated scores from the high and low models were correlated with observed behavioral

scores to assess model fit. To assess the robustness of any significant models, and to calculate a final *p*-value for each correlation between observed and estimated scores, permutation testing was performed by shuffling the behavioral scores such that they were no longer matched with the brain matrix from the same individual. CPM was shuffled and run 1000 times, creating a null distribution of rho values representing the accuracy of the CPM models observable by chance. Our true model's *rho* value was placed into this distribution to determine the likelihood that it occurred due to chance.

After model derivation, network edges in significant models were evaluated by examining region location and participation 10 canonical networks: cerebellar; medial frontal; frontoparietal; default mode; motor; visual I; visual II; association; salience; subcortical. Using the following formula, the contribution of within and between network connections was calculated as the ratio of the proportion of model edges from network A to B, to the proportion of total edges in the brain from network A to B. This allowed us to examine the relative contribution of networks in our final models by controlling for overall network size.

$$Contribution_{A,B} = \frac{m_{A,B}/m_{total}}{E_{A,B}/E_{total}}$$

Examination of Model Assumptions

As CPM relies on a linear regression of behavioral scores onto functional connectivity summary strength scores, the relationship between network connectivity and PSI and WMI, respectively, was evaluated post-derivation for linearity, normality, homoscedasticity by examining the residuals and estimated Y values derived for each subject

during cross-validation. These assumptions may be categorized into primary, which when violated affects the meaning of the statistics applied, and secondary, which when violated does not change the interpretation of the statistics but may not accurately reflect population inference (Hayes, 2005). Linearity, the only primary assumption of linear regression, was evaluated visually, by constructing a detrended scatterplot of the residuals of estimated Y (model-estimated PSI and WMI scores) against summary strength scores. In addition, variance accounted for by the simple linear model was compared to variance accounted for by the introduction of a second degree and third-degree polynomial to determine whether the relationship between connectivity and behavior was better explained by a curvilinear model.

The secondary assumption of conditional normality of the estimation errors assumes that the model's errors in estimation are normally distributed at every value of estimated Y. This was assessed by visual inspection for normality by plotting a histogram of the frequencies of the residuals, however, the possibility of non-normal estimation errors was proactively accounted for by the use of permutation testing to determine the final p-values, given that permutation testing makes no assumption about the shape of the error distribution and is therefore valid regardless of its shape.

Homoscedasticity is the secondary assumption that the conditional estimation errors are equally variable for all estimated Y values in the population. This was examined by visually inspecting a plot of the residuals against the estimated Y values, as well as non-visually by computing the Pearson correlation between estimated Y and the absolute value of the residuals.

2.7 Association of Functional Connectivity with Disease Course

Functional connectivity models determined to have successfully identified a brain-behavior relationship were evaluated for overlap with clinical disease measures by correlating summary strength scores with EDSS. Additionally, the explanatory power of functional connectivity and total lesion volume in predicting EDSS were examined by creating a multiple linear regression model that utilized summary strength scores from WMI and PSI models and lesion load as predictor variables in the prediction of EDSS. The unique contribution of each of these variables was assessed by completing a semi-partial correlation for each of the three predictor variables.

Chapter 3. Results

3.1 Sample Demographics, Neuropsychological and Clinical Variables

Of the 44 individuals who participated in the study, 39 completed the neuroimaging portion of the study and were included in these analyses. The demographic characteristics of this sample can be found in Table 1, which consisted of 31 females and 8 males with an average age of 45.95 years (*SD* = 7.62), an average of 16.37 years of education (*SD* = 2.37). With regards to race and ethnicity, 33 participants identified as 'White', 3 identified as 'Black or African American', 2 identified as 'Other', and 1 responded 'Prefer not to Answer'; no participants identified as 'Hispanic'. The average duration of disease was 11.39 years (*SD* = 8.42), with a mean EDSS score of 3.74 (*SD* = 1.13). Mean WAIS-IV PSI percentile score was 98.92 (*SD* = 12.81) and mean WMI percentile score was 104.10 (*SD* = 15.62), indicating a cognitively normal sample.

Although MS study samples vary in clinical and demographic factors, making comparison between samples difficult, compared to similar neuroimaging studies with relapsing-remitting MS samples, our study had a comparable patient sample size (Bonavita et al., 2015; Faivre et al., 2016; Finke et al., 2015; Gamboa et al., 2014; Leonardi et al., 2013; Sbardella et al., 2015; Shu et al., 2016), and was similar in average age (Bonavita et al., 2015; Castellazzi et al., 2018; Finke et al., 2015), and disease duration (Finke et al., 2015;

Sbardella et al., 2015). Our sample had approximately 3 more years of education, a larger female to male ratio (Cruz-Gómez et al., 2014; Faivre et al., 2016; Finke et al., 2015; Leonardi et al., 2013; Sbardella et al., 2015) and had a higher average EDSS score (Castellazzi et al., 2018; Cruz-Gómez et al., 2014; Finke et al., 2015; Leonardi et al., 2013) than many similar neuroimaging samples. Race and ethnicity in our sample could not be adequately compared to similar studies due to infrequent reporting of these variables.

After excluding subjects with an average mean FD head motion estimate of $> .15$, an exploratory analysis in the final sample of 33 was performed to examine the relationship between clinical, demographic, and neuropsychological variables by correlating PSI scores, WMI scores, disease burden (EDSS), disease duration, education, total lesion volume (TLV), and motion estimates during rs-fMRI measured as mean framewise displacement (mean FD) (Table 2). A significant negative correlation was found between disease duration and PSI scores, r (33) = -.35, $p < .05$, such that longer disease duration was associated with worse PSI scores. In addition, a positive relationship was observed between motion and disease burden, r (33) = -.36, $p = .04$, such that higher average motion was associated with higher self-reported disease burden. Several notable relationships were examined but did not reach significance in this sample: total lesion volume was inversely correlated with both PSI scores, r (33) = -.34, $p = .06$, and WMI scores, r (33) = -.34, $p = .05$, with higher total lesion volume associated with lower PSI and WMI scores. In addition, there was no significant association between WMI scores and education level, r (33) = .30, $p = .09$, or WMI scores and in-scanner motion, r (33) = -.30, $p = .09$.

3.2 Neuroimaging Results

Network Derivation and Model Performance

Twenty-one nodes were excluded from the analyses prior to network derivation due to insufficient brain coverage in at least one individual. These nodes consisted of 15 cerebellar nodes, 2 brainstem nodes, 2 prefrontal nodes, and 2 temporal nodes, resulting in a 247 x 247 symmetrical connectivity matrix for inclusion in the main analysis. Within each iteration of the separate PSI and WMI cross-validation loops, edges were correlated with assessment scores, forming 33 iterations of behavioral networks and 33 iteration of models. During PSI derivation, the number of edges in the model-building iterations ranged from 168-227 in the high network, and 133-179 in the low network. The low-PSI model was found to successfully predict processing speed (correlation between predicted and estimated scores: r_s (33) =.41, p = .02), however the high network was not significantly able to predict processing speed performance, r_s (33) =.03, p = .86 (Figure 2). During WMI derivation, the number of edges in the model-building iterations ranged from 111-157 in the high network, and 148-214 in the low network. Neither the high nor the low networks were able to successfully predict working memory scores (high model: r_s (33) =-.15, p = .40; low model: r_s (33) =.13, p = .48) (Figure 2). The only significant model, the low PSI CPM, survived permutation testing suggesting low likelihood of this model occurring due to chance, p = .03 (Figure 2).

Regression Model Assumptions

The high and low models within each round of cross validation were assessed for PSI and WMI individually in order to examine model assumptions of the regression between

summary strength scores and behavior, including linearity, normality, and homoscedasticity (Figure 5). For PSI high and low models, visual inspection of the residuals showed no discernable trend, nor visible homoscedasticity, suggesting a linear model was appropriate (Figure 5 b). The absolute value of the residuals and the predicted value of Y were correlated and demonstrated no significant relationship for any of the networks. A histogram plot of the residuals showed a normal distribution, satisfying the requirement for normality (Figure 5 a). Testing whether the relationship between summary scores and PSI would be better modeled by a curvilinear relationship using 2nd and 3rd degree polynomial terms, we found no change in nonsignificant models. For the significant low network, a 2nd degree polynomial term improved the captured variance in PSI scores but decreased statistical significance due to the added degree of freedom, r_s (33) =.48, $p < .05$. For the WMI high network, visual examination of the residuals indicated a slight negative trend (Figure 5 b), indicating a linear model may not be appropriate. A second order polynomial term increased the captured variance of WMI scores, but still failed to reach significance, r_s (33) =.32, $p = 0.20$ (Table 6). Upon visual inspection, normality of the residuals for the WMI high network was also questionable (Figure 5 a). The low WMI network appeared to be normal, linear and have appropriate homoscedasticity.

Network Topology Predictive of Worse Processing Speed

In order to characterize edges utilized during cross-validation, edges that were significantly correlated with behavior in every iteration of the cross-validation loop were identified for each of the processing speed and working memory network derivations. For PSI, we found 71 common edges in the high network, and 58 in the low network, accounting

for .23% and .19% of total available edges, respectively. For WMI, we found 46 edges in the high network, and 72 in the low network, .15% and .23% of available edges in the whole matrix.

The anatomical and network localization of the significant, low-PSI network was then assessed (Table 2). The 58 edges in the common low-PSI network showed slightly greater involvement of the right hemisphere than the left with the largest number of contributions originating from the left and right prefrontal regions (Figure 3). Contribution of the 10 canonical networks was assessed by dividing these edges into the networks they participate in and examining the within and between network connections relative to the expected contribution given the number of edges in those networks (Figure 4). This analysis revealed that prediction in the low-PSI CPM was driven by edges in several different networks. By raw count, the network consisted primarily of within network connections in the cerebellar, medial frontal, subcortical, and motor networks, as well as increased between-network connectivity between the cerebellar and subcortical networks, cerebellar and frontoparietal networks, subcortical and frontoparietal networks, salience and motor networks, visual I and motor networks, visual I and DMN, and frontoparietal and medial frontal networks. Relative to the number of edges located in each of these networks, the greatest relative contributions were from within network connections in the cerebellar, subcortical, and motor networks, in descending order. The greatest relative contributions from between network connections were from connections between cerebellar and subcortical, cerebellar and frontoparietal networks, visual I and DMN, motor and DMN, in descending order (Figure 6).

Processing Speed Model Metrics Correlate with Measures of Disease Burden

Summary scores estimated for each individual during model derivation for the significant low-PSI network were significantly correlated with EDSS, r (33) =.38, p = .03, suggesting that functional connectivity within edges negatively correlated with processing speed were also associated with increased disease burden. Neither PSI cognitive performance scores, r_s (33) = -.13, p = .48, WMI cognitive performance scores, r_s (33) = -.20, p = .27, nor total lesion volume, r_s (33) = .15, p = .40, were significantly associated with disease burden.

In order to determine the unique contribution of functional connectivity, lesion accumulation, and cognitive performance in the prediction of current disease burden, we built a multiple linear regression model using summary strength scores from the successful processing speed derivation, total lesion volume, and cognitive performance on the WAIS-IV PSI and WMI subtests (Table 4). Combined, TLV, WMI, PSI, and the PSI-CPM summary strength scores accounted for 18.2% of the variance in EDSS. Of this, PSI-CPM summary strength scores uniquely accounted for a significant amount, 13.19%, of variance in EDSS. Cognitive performance as measured by the PSI uniquely accounted for .58% of variance in EDSS, WMI uniquely accounted for 3.54% of variance in EDSS, and lesion volume uniquely accounted for a miniscule .002% of variance in EDSS. The shared variance accounted for by lesion volume, PSI, WMI, and PSI-CPM summary strength scores was only 1.02%. As a final analysis, a model was built to predict EDSS using WMI, PSI and TLV, but not CPM summary strength scores. This model accounted for only 5% of the variance in EDSS, meaning that including CPM summary strength scores resulted in an increase of 13.20%.

Motion Correction

In-scanner motion was Pearson correlated with WMI and PSI in the full sample, which failed to find a relationship between motion and neuropsychological assessment scores (correlation between mean FD and PSI, r (39) = -.30, p = .06; correlation between mean FD and WMI, R(39)= -.24, p= .14). Six participants were excluded *a priori* for having average motion estimates greater than .15mm, resulting in a final sample of 33, after which the correlation between motion and behavior was r (33) = .10, p =.55 for PSI, and r (33) = -.030, p = .09 for WMI (Table 2).

As an additional check for the potential confounding role of motion during model derivation in this low-motion sample of 33 participants, a CPM model was built specifically to predict motion estimates using rs-functional connectivity. We found that enough motion artifact remained in the data to result in a highly predictive model (correlation between predicted and observed mean FD scores: r (33) = .72, p < .001). As an exploratory analysis, models were iteratively built to remove high-motion subjects in descending order in an attempt to determine an ideal motion cutoff. At n = 27, corresponding to a processed mean FD cutoff of > .1097, CPM could no longer be used to predict motion. The ability to predict motion decreased steadily with the removal of high motion subjects, suggesting that removing high-motion individuals does reduce the chance of predictive models being influenced by motion artifact. Due to the residual motion in the sample indicated by the successful prediction of mean FD using CPM, motion was subsequently statistically controlled for during the feature selection stage of the processing speed and working memory CPM derivation by using a partial correlation to select edges that remained significantly

associated with cognitive scores even after removing the variance accounted for by mean FD scores.

Chapter 4. Discussion

Current conceptualization of multiple sclerosis is as a common, idiopathic and unrelenting inflammatory disease, afflicting young and middle-aged adults with progressive structural damage to the central nervous system (Baecher-Allan et al., 2018; Dendrou et al., 2015; Lassmann, 2018; Wallin et al., 2019). Pharmacological treatments fail to fully prevent disease progression (Amato et al., 2019; Baecher-Allan et al., 2018; Claflin et al., 2019; Ontaneda et al., 2017), and thus PwMS experience a complex disease course characterized by MRI-visible white matter lesions, dysfunction of "normal-appearing" gray and white matter, neuroinflammation, and the body's own attempts to repair this damage (Calabrese et al., 2012; Louapre, 2018; Tintore et al., 2015). Together, these factors result in a highly unpredictable and heterogenous disease that challenges the standard clinical goals of diagnosis, symptom monitoring, identification of mechanisms of decline, and evaluation of treatment efficacy. Previous research has attempted to use structural and functional MRI to quantify individual disease burden as a means to address these clinical goals, particularly focusing on lesion accumulation. These studies have revealed that although lesion accumulation is often correlated with impaired processing speed as well as increased EDSS, this relationship is not unequivocal (Calabrese et al., 2012; Filippi, Preziosa, et al., 2013; Healy et al., 2017; Louapre, 2018; Mike et al., 2011; Patti et al., 2015; Randolph et al., 2005; Stankiewicz et al., 2011). And whereas a graph theoretical approach to functional connectivity has led researchers to propose that structural damage, including but not limited

to lesion accumulation, negatively impacts network efficiency and interacts with compensatory mechanisms in a way that may theoretically explain the heterogenous and unpredictable nature of MS symptomatology (Fleischer et al., 2019; Schoonheim et al., 2015), to date, the development of functional neuromarkers is in its infancy and their usefulness above and beyond structural measures of disease burden have not been sufficiently investigated. The present study demonstrated that a successful model of processing speed, but not working memory, could be derived from resting-state fMRI and used to create a preliminary network of processing speed specific to this population. Additionally, we demonstrated that metrics summarized from this processing speed network explained significant variance in disease burden that was unique to, and in excess of, variance explained by either total lesion volume or neuropsychological assessment scores of processing speed and working memory.

4.1 Success and Composition of PSI and WMI CPM Networks

Connectome-based predictive modeling is a technique that builds upon traditional whole-brain graph theory approaches by allowing for the derivation of domain-specific networks of cognition. Previous work has used CPM in healthy adults to derive unique networks for domains such as fluid intelligence (Finn et al., 2015), attention (Rosenberg et al., 2015), processing speed (M. Gao et al., 2020), and working memory (Avery et al., 2019). Especially relevant to the current study, recent work by Gao et al. has used resting state functional connectivity and CPM to predict processing speed in 99 healthy older adults, successfully deriving two distinct networks of fast and slow processing speed,

respectively (M. Gao et al., 2020). A neuromarker of working memory has also successfully been derived from resting-state functional connectivity to predict n-back performance in a large sample of 502 healthy young adults, though this neuromarker was outperformed by a task-based model (Avery et al., 2019). These and other findings have suggested that CPM neuromarkers built on task data may be more predictive and have better generalizability to external data (Greene et al., 2018). Despite this, resting state functional connectivity has long been considered better-suited for use in clinical populations, given its high signal-to-noise ratio, the ability to apply it flexibly to multiple constructs, and its ease of collection in a wide array of populations (Fox & Greicius, 2010). However, the application of CPM to neurological disease has thus far been limited when compared to its use in healthy adults, though it has demonstrated some success in predicting cognitive impairment in cancer patients and older adults with MCI and Alzheimer's disease (Henneghan et al., 2020; Lin et al., 2018; Prakash et al., in prep) and psychological disorders such as anxiety, depression and disordered sleep (Giancardo et al., 2018; Ju et al., 2020; Ren et al., 2021). Encouragingly, however, recent work has demonstrated networks derived during CPM may have potential utility as clinical markers of treatment response in depression (Ju et al., 2020), opening a new field of domain-specific neuromarkers in patient populations.

Given the success of predictive model derivation in healthy adults, and the advantages of the method's whole-brain approach to functional connectivity, we hypothesized that we would be able to successfully derive processing speed and working memory networks in people with relapsing-remitting multiple sclerosis. In our current study, we were successfully able to derive a low-processing speed network and use that network to build a model to infer WAIS-IV processing speed scores in PwMS using resting-

state fMRI data, and a leave-one-out cross validation procedure to assess model fit. Our successful processing speed model was one that relied on edges negatively correlated with processing speed scores, and thus the resulting network is one representing a set of edges where increased connectivity in this sample of PwMS is indicative of slower processing speed. Our observed correlation between predicted and observed scores was moderately strong and survived permutation testing. Similar to the findings of Gao et al., we observed that our low-PSI network outperformed our high-PS network in the internal validation procedure, and that our correlation between observed and predicted processing speed scores was comparable (MS low-PSI network $r_s = .41$; older adult slow-PS network $r_s = .42$) (M. Gao et al., 2020).

When correcting for network size, this slow-PS network contained primarily within-network connections in cerebellar, subcortical, motor, and medial frontal networks, and between-network connections between the cerebellar and subcortical networks, cerebellar and frontoparietal networks, visual I and DMN, as well as motor and DMN. Within-network cerebellar connections were especially overrepresented, followed by between-network connections from cerebellar-to-frontoparietal networks, and within-network connections in the sub-cortical network. Other overrepresented network connections included Visual I-to-DMN, motor-to-DMN, frontoparietal-to-subcortical, and cerebellar-to-visual II (Figure 6). In comparison, the slow-PS CPM network identified by Gao et al. in healthy older adults demonstrated a strong contribution of within-network connections in the motor cortex, and between-network connections from motor-to-visual II and motor-to-frontoparietal networks (M. Gao et al., 2020). Differences in these two

networks may be due in part to edges associated with slowed processing speed in MS being related to MS pathology.

If this is the case, then high-probability lesion sites that are common to many PwMS, such as the cerebellum (Calabrese et al., 2010), should be highly represented, as they were in our sample. Notably, cerebellar lesions and degeneration have also previously been tied to deficits in reaction time tasks (Molinari et al., 1997) and processing speed in aging adults (Eckert et al., 2010) and PwMS (Moroso et al., 2017; Savini et al., 2019) . This may in part explain the high contribution of within-cerebellar connections contributing to our network and would suggest that lesion accumulation is in fact an important metric of disease burden, but one that may not be adequately or ideally measured by whole brain total volume.

Despite the successful derivation of a low-PSI network, we were unable to derive a high-PS network in this sample. In addition, our second hypothesis was that we would be able to successfully derive a resting-state working memory network in PwMS, which we were also unable to do. Deriving a network of working memory in PwMS would similarly have allowed us to examine the contribution of working memory to disease burden and compare this to processing speed. Research has suggested that processing speed deficits appear earlier in the MS disease course than working memory deficits (Brochet & Ruet, 2019), and research into these cognitive domains has led others to propose that working memory is supported by processing speed (Fry & Hale, 1996) and therefore working memory deficits may emerge directly as a result of processing speed deficits. Given that we were unable to derive either a high or low working memory model, these relationships could not be examined.

4.2 Assessing Predictors of Disease Burden

Given that processing speed is a core deficit observed in PwMS (DeLuca et al., 2004), identifying a population-specific network of processing speed allowed us to examine the role of this cognitive domain in overall disease burden. The second aim of this study attempted to establish whether a neuromarker of processing speed might have clinical utility, which we examined by associating metrics derived from this marker with disease burden as measured by the EDSS. We hypothesized that metrics derived from our successful predictive models would account for unique variance compared to total lesion volume and neuropsychological assessment scores, given that changes in functional connectivity may theoretically reflect the impact of lesions on networks supporting cognitive functions. In line with our hypothesis, we found that indeed functional connectivity metrics accounted for 13.19% of the variance in EDSS using linear regression.

Notably, and unexpectedly, total lesion volume did not account for a significant amount of variance in predicting EDSS, despite marginal associations of total lesion volume with PSI and WMI scores in our sample. Our failure to find a relationship between EDSS and total lesion volume fits into a literature of mixed findings, with several large studies previously finding evidence of associations between lesion volume and EDSS (Calabrese Mike 2011, Randolph 2005, Stankiewicz 2011) and several studies failing to do so (Fillipi, Agosta 2013, Healy 2017). This paradox can likely be explained due to issues already discussed, particularly that total lesion volume is likely an imperfect measure of pathology,

exacerbated by diversity in lesion location, the compensatory factors of functional networks, and heterogeneity of disease course (Louapre, 2018).

Importantly, we also included performance on the WAIS-IV PSI and WMI as covariates in our model to explain variance in EDSS, given that neuropsychological assessment is clinically used to monitor disease progression in MS and other disorders and has some practical advantages compared to fMRI (Ruet & Brochet, 2020). We did not observe that performance on either of these tests explained variance in disease burden. This finding was also unexpected, but important, given that the National MS Society has suggested the use of repeated cognitive testing in PwMS to monitor disease progression and evaluate treatment (Kalb et al., 2018). Our findings here suggest instead that although structural damage begets functional changes, these functional changes reflect the degree of damage to cognitive systems resulting from MS pathology, and that these functional changes may be more relevant to predicting disease burden than either structural damage or cognitive test performance.

4.3 Limitations and Future Directions

Together, these findings might suggest that for cognitive outcomes and overall disease burden, total lesion volume may be less important than a nuanced understanding of the relationship between lesion location and the resulting impact of lesions to the integrity of networks that support cognition. Previous work has demonstrated that functional networks are constrained by structural white matter connections (Honey, 2008), and therefore white matter integrity is likely to be directly related to the integrity of functional

networks. In PwMS, recent work has suggested that in individuals with structural damage to regions associated with the DMN, cognitive rehabilitation response is moderated by integrity of functional networks (Fuchs, 2020). This suggests first, that the impact of structural damage may be differential depending on the location of that damage, and second, that structural damage is moderated by functional reserve. Thus, as is supported by our results, functional connectivity is likely to contribute unique predictive variance to models of disease. However, our analysis did not investigate the impact of lesions dependent on their location. Future work would benefit from incorporating diffusion weighted imaging to examine this relationship, as well as the relationship between local structural damage and the impact on local and diffuse functional networks.

In addition, the current study proposed to examine the feasibility of implementing CPM to derive processing speed and working memory networks in a MS sample, and to determine whether metrics from those networks were associated with overall disease burden. Our findings are limited to this sample and cannot be generalized to the MS population as a whole without further work replicating this finding. In particular, two of our findings may limit the replicability of this experiment. First, our successful low-PSI network contained only 58 edges common across all individuals in the sample, whereas previous working memory, attention and processing speed CPM papers have reported that number to be in the hundreds (Avery et al., 2019; M. Gao et al., 2020; Rosenberg et al., 2015). Despite this, the high degree of correlation between the low-PSI network metrics and EDSS, and the disproportionate representation of certain canonical networks suggest that these edges were not selected by chance. One explanation for the low number of network edges is that the MS disease course introduces significant functional heterogeneity as a

result of the observed heterogenous and unpredictable structural damage. CPM is a method that seeks commonalities across individuals, and thus its implementation in PwMS might be hampered by processes that result in the need for functional reorganization. Future work would benefit from the use of longitudinal studies to determine the progression and nature of change in functional networks as the disease course progresses. We would hypothesize that processing speed and working memory networks would be intact and homogenous in pre-symptomatic individuals and become increasingly heterogenous with increased disease severity. An important future direction is to examine how focal white matter damage impacts whole-brain connectivity that supports cognitive functions. Creating joint structural and functional models and modeling the interaction between structure and function could account for inter-individual differences in disease presentation.

Another limiting factor in functional connectivity analyses is the potential confounding role of head motion, which is known to be a problem in both traditional functional connectivity techniques (Power et al., 2012) and CPM (Horien et al., 2018). It is possible that the failed derivations and low number of common edges was due to excessive noise introduced by participant motion. PwMS may be more prone to in-scanner motion than healthy adults given that tremors are a feature of the disease (Koch et al., 2007), and to address this we implemented rigorous motion controls, including: the regression of 36 motion parameters and high motion volumes during nuisance regression; exclusion of participants with an average framewise displacement greater than .15; and, controlling for motion during the edge selection step of CPM. Despite controlling extensively for motion artifact in this study, an important future direction would be to replicate these analyses in a larger sample with even stricter motion exclusion criteria. However, it should be noted that

translational models that utilize functional connectivity will not be useful to clinicians of MS and other clinical populations if they are only able to be derived from, and applied to, low-motion patients. Therefore, another key future direction is the development of additional methodology that accounts for motion in the preprocessing and model building stages.

Additionally, although the sustained attention CPM network originally derived in Rosenberg et al. (2015) consisted of a sample of 25 healthy adults and has been validated in predicting ADHD symptoms in a sample of young adults (Rosenberg et al., 2015) as well as Stroop task performance in older adults (Fountain-Zaragoza et al., 2019), most recent connectome-based predictive models in the literature have been derived on larger samples of closer to 100+ individuals. Models derived on smaller samples are likely to be less generalizable (Poldrack et al., 2020), though they are still useful for investigating relationships between variables within a single sample, as we do here. Future work would benefit from examining the variance accounted for by lesion volume and functional metrics in larger samples of PwMS to determine if these relationships hold. Relatedly, given the low prevalence of MS in the general population, recruitment for MS studies is a challenge for researchers, and thus data sharing and collaboration will be a necessary direction in this field, given the need for using large, open access datasets in these advanced methods.

A final limitation in this study exists within the network derivation method itself. CPM aims to account for multiple comparisons in the edge selection step by utilizing cross-validation (Finn, 2015). However, recent work, not yet peer reviewed, has experimented with the CPM method, and proposed several methods for improving feature selection and generalizability (O'Connor et al., 2020). Additionally, recent work published after the aims

of the current study were completed have utilized ridge and lasso regression in order to remedy this and account for the collinearity of edges (S. Gao et al., 2019). Future work in CPM would benefit from examining how different feature selection methods may be more or less suitable for different populations and samples, however, these methods may be less successful in small sample sizes such as those commonly found in pilot studies such as this one. In our current study, the low number of edges but the high relevance of those edges to disease burden suggests that our network itself may be sample-specific, but the relationship demonstrated between whole-brain functional connectivity, lesion volume, processing speed, and disease burden in PwMS may itself be highly replicable and of imminent importance to the MS literature.

4.4 Conclusions

The current study aimed to use connectome-based predictive modeling - a methodology that has been used widely in healthy and various clinical populations to identify functional connections associated with behavioral, physiological, and cognitive constructs - to derive resting-state, whole-brain functional neuromarkers of processing speed and working memory performance in individuals with relapsing-remitting multiple sclerosis, and to assess the potential use of this population and cognitive domain specific neuromarker as a method to explain variance in overall MS disease burden. We were able to derive a network of processing speed in a sample of people with relapsing-remitting multiple sclerosis, but not a working memory network. Additionally, this processing speed network demonstrated some clinical utility by predicting significant unique variance in patient disease burden, while total

lesion volume and neuropsychological processing speed and working memory assessment scores did not. Although the small number of edges in this derived network likely limits its use as a true neuromarker, together, these results suggest that the use of functional neuromarkers in addition to structural measures may in fact be a promising line of study in certain clinical populations, potentially able to reflect not the amount of disease progression as measure by structural damage, but rather the net impact of that damage on disease burden given individual variation in functional reserve. Multiple sclerosis is a disease marked by a high prevalence rate, consequential and disruptive symptoms, and idiopathic onset, and the high likelihood of progression to significant disability. The current study suggests that whole-brain approaches that include cerebellar regions are vital to future investigations of the mechanisms of the MS-disease processes and their effects on physical, sensory and cognitive processing. Future research could address the limitations of the current study by replicating this analysis on a larger sample and incorporating multimodal imaging (diffusion, task-fMRI, etc.) to increase model accuracy and generalizability.

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Appendix A. Tables and Figures

Table 1. Demographic variables for the full MRI sample

	n	*M*	*SD*	Range
Demographics				
Age	39	45.95	7.62	30-58
Sex	39	-	-	-
Female		31	-	-
Male		8	-	-
Education	39	16.37	2.37	12-23
Race	39			
White		33	-	-
Black		3	-	-
Other		2	-	-
Prefer not to answer		1	-	-
Ethnicity	39			
Non-Hispanic		33	-	-
Hispanic		0	-	-
Prefer not to answer		6	-	-
Clinical Characteristics				
EDSS	39	3.74	1.13	0-5.5
Disease Duration	39	11.39	8.42	.25-35
Lesion Volume	39	21.83 cm^3	12.65 cm^3	2.91-44.17cm^3
Neuropsychological				
Processing Speed (PSI)	39	98.92	12.81	76-127
Working Memory (WMI)	39	104.10	15.62	69-136

Note. EDSS = Expanded Disability Status Scale (Kurtzke, 1983). WAIS-IV PSI = Processing Speed Index standardized score, WMI = Working Memory Index standardized score (Wechsler, 1981).

Table 2. Correlations for Study Variables in the Final Sample

Variable	*n*	1	2	3	4	5	6
1. PSI Scores	33	-	-	-	-	-	-
2.WMI Scores	33	*r* = .25 *p* = .17	-	-	-	-	-
3. EDSS	33	*r* = -0.13 *p* = 0.48	*r* = -.20 *p* = .27	-	-	-	-
4. Disease Duration	33	*r* = -.35* *p* < .05	*r* = .02 *p* = .91	*r* = .25 *p* = .16	-	-	-
5. Education	33	*r* = .03 *p*= .86	*r* = .30⊥ *p* = .09⊥	*r* = -.03 *p* = .85	*r* = .10 *p* = .56	-	-
6. TLV	33	*r* = -.34⊥ *p*= .056⊥	*r* = -.34⊥ *p* = .05⊥	*r* = .15 *p* = .40	*r* = .24 *p* = .19	*r* = -.05 *p* = .80	-
7. Mean FD	33	*r* = -.10 *p* = .55	*r* = -.30⊥ *p* = .09⊥	*r* = .36* *p* = .04	*r* = .14 *p* = .43	*r* = -.17 *p* = .34	*r* = -.28 *p* = .12

Note: Values listed are WAIS-IV PSI = Processing Speed Index standardized score, WMI = Working Memory Index standardized score (Wechsler, 1981). EDSS = Extended Disability Status Scale (Kurtzke, 1983). Mean FD = Mean Framewise Displacement (Power et al., 2011). * $p < .05$, ⊥ $p < .01$

Table 3. Edges common to all models in derivation of low-PSI network

Edge	Region	Network	connection	Region	Network
1	R prefrontal	SAL	-	R prefrontal	FP
2	R prefrontal	MF	-	R motor	Mot
3	R prefrontal	FP	-	R motor	Mot
4	R insula	Mo	-	R parietal	VI
5	R parietal	SAL	-	R parietal	Mot
6	R parietal	FP	-	R temporal	Vas
7	R prefrontal	DMN	-	R occipital	VI
8	R prefrontal	DMN	-	R occipital	VI
9	R temporal	Vas	-	R limbic	SAL
10	R prefrontal	FP	-	R cerebellum	CBL
11	R cerebellum	FP	-	R cerebellum	SAL
12	R subcortex	SC	-	R subcortex	SC
13	R parietal	VAs	-	R subcortex	SC
14	R parietal	FP	-	R subcortex	SC
15	R prefrontal	FP	-	R subcortex	SC
16	R prefrontal	FP	-	R subcortex	SC
17	R temporal	Mot	-	L prefrontal	DMN
18	R prefrontal	SAL	-	L motor	Mot
19	L prefrontal	MF	-	L motor	FP
20	R motor	Mot	-	L motor	Mot
21	R prefrontal	MF	-	L motor	Mot
22	L prefrontal	DMN	-	L insula	Mot
23	R insula	Mot	-	L parietal	VI
24	L prefrontal	MF	-	L temporal	MF
25	L prefrontal	SAL	-	L temporal	MF
26	L parietal	MF	-	L temporal	MF
27	R temporal	MF	-	L temporal	FP
28	R cerebellum	CBL	-	L temporal	FP
29	L temporal	MF	-	L temporal	FP
30	R cerebellum	CBL	-	L temporal	MF
31	L temporal	MF	-	L temporal	MF
32	R parietal	VAs	-	L temporal	Mot
33	R cerebellum	CBL	-	L temporal	FP
34	R temporal	Vas	-	L temporal	Mot
35	R limbic	SC	-	L temporal	Vas
36	R parietal	Mot	-	L temporal	Mot
37	R prefrontal	FP	-	L occipital	Vas
38	R prefrontal	DMN	-	L occipital	VI
39	R insula	Mot	-	L occipital	VI
40	L prefrontal	SAL	-	L limbic	SC
41	L temporal	MF	-	L limbic	SC
42	R limbic	Mot	-	L limbic	SAL
43	R prefrontal	'MF	-	L limbic	SC
44	R brainstem	CBL	-	L cerebellum	CBL
45	R brainstem	CBL	-	L cerebellum	VII
46	R brainstem	CBL	-	L cerebellum	VII
47	R occipital	VII	-	L cerebellum	SAL
48	R cerebellum	CBL	-	L cerebellum	SAL
49	R brainstem	CBL	-	L cerebellum	CBL
50	R brainstem	CBL	-	L cerebellum	CBL
51	R cerebellum	CBL	-	L subcortex	SC
52	L cerebellum	VII	-	L subcortex	SC
53	R cerebellum	CBL	-	L subcortex	SC
54	R occipital	VII	-	L brainstem	SC
55	R cerebellum	CBL	-	L brainstem	SC
56	L subcortex	SC	-	L brainstem	SC
57	L cerebellum	CBL	-	L brainstem	CBL
58	L prefrontal	SAL	-	L brainstem	CBL

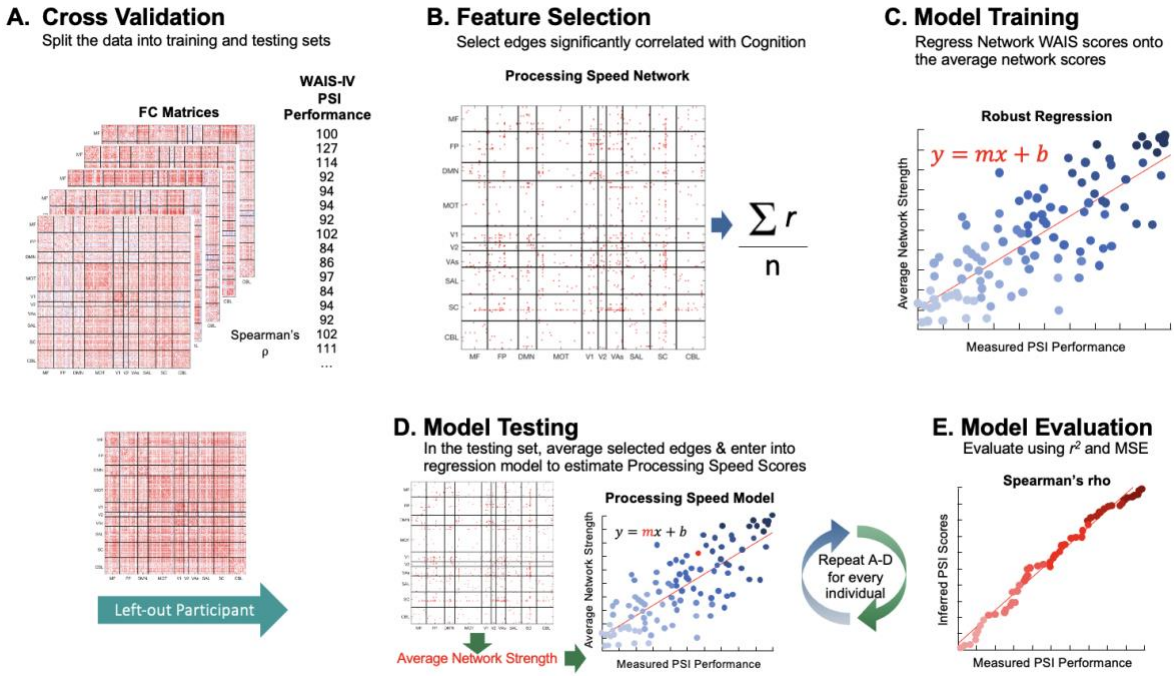
Note: ‘CBL’ = Cerebellar. ‘MF’ = Medial Frontal. ‘FP’ = Frontoparietal. ‘DMN’ = Default Mode Network. ‘Mot’ = Motor. ‘VI’ = Visual 1. ‘VII’ = Visual 2. ‘VAs’ = Visual Association. ‘SAL’ = Salience. ‘SC’ = Subcortical.

Table 4. Predicting EDSS from Cognitive Scores, Total Lesion Volume, and low-PSI Metrics

	Model 1			Model 2		
Variable	*B*	95% CI	*p*	*B*	95% CI	*p*
Constant	5.33	[0.56 10.10]	.03	3.97	[-0.73 8.67]	.09
PSI	-.01	[-0.04 0.03]	.75	.01	[-0.28 0.04]	.66
WMI	-.01	[-0.04 0.02]	.43	-.02	[-0.04 0.01]	.28
TLV	.01	[-0.06 0.09]	.69	-.00	[-0.08 0.07]	.98
Low-PSI CPM	-	-	-	22.89*	[0.71 45.06]	.04*
R^2	.05			.18		
F	.52			1.55		
Delta R^2	-			.13		

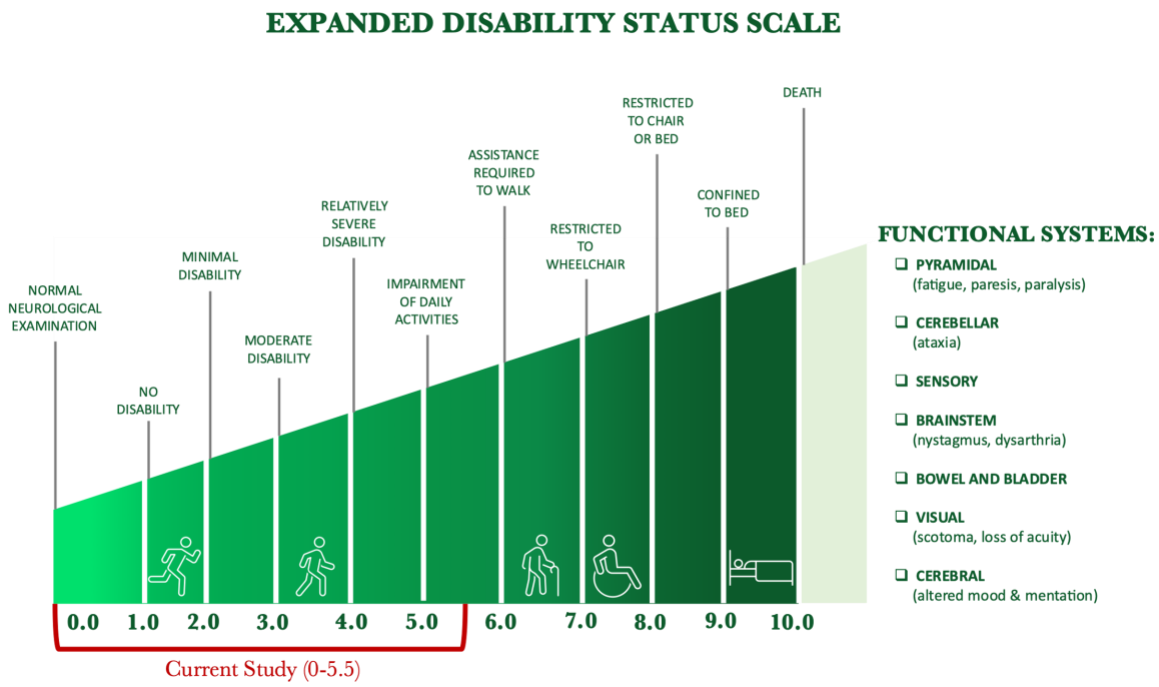
Note: n=33. CI = confidence interval. Only the metrics derived from the PSI-CPM network reached statistical significance as a predictor of EDSS. EDSS = Extended Disability Status Scale (Kurtzke, 1983). TLV = Total Lesion Volume. PSI = Processing Speed Index standardized score, WMI = Working Memory Index standardized score (Wechsler, 1981). Low-PSI CPM = metrics derived from connectome-based predictive modeling of slow processing speed. * $p < .05$

Figure 1. Connectome-based Predictive Modeling.



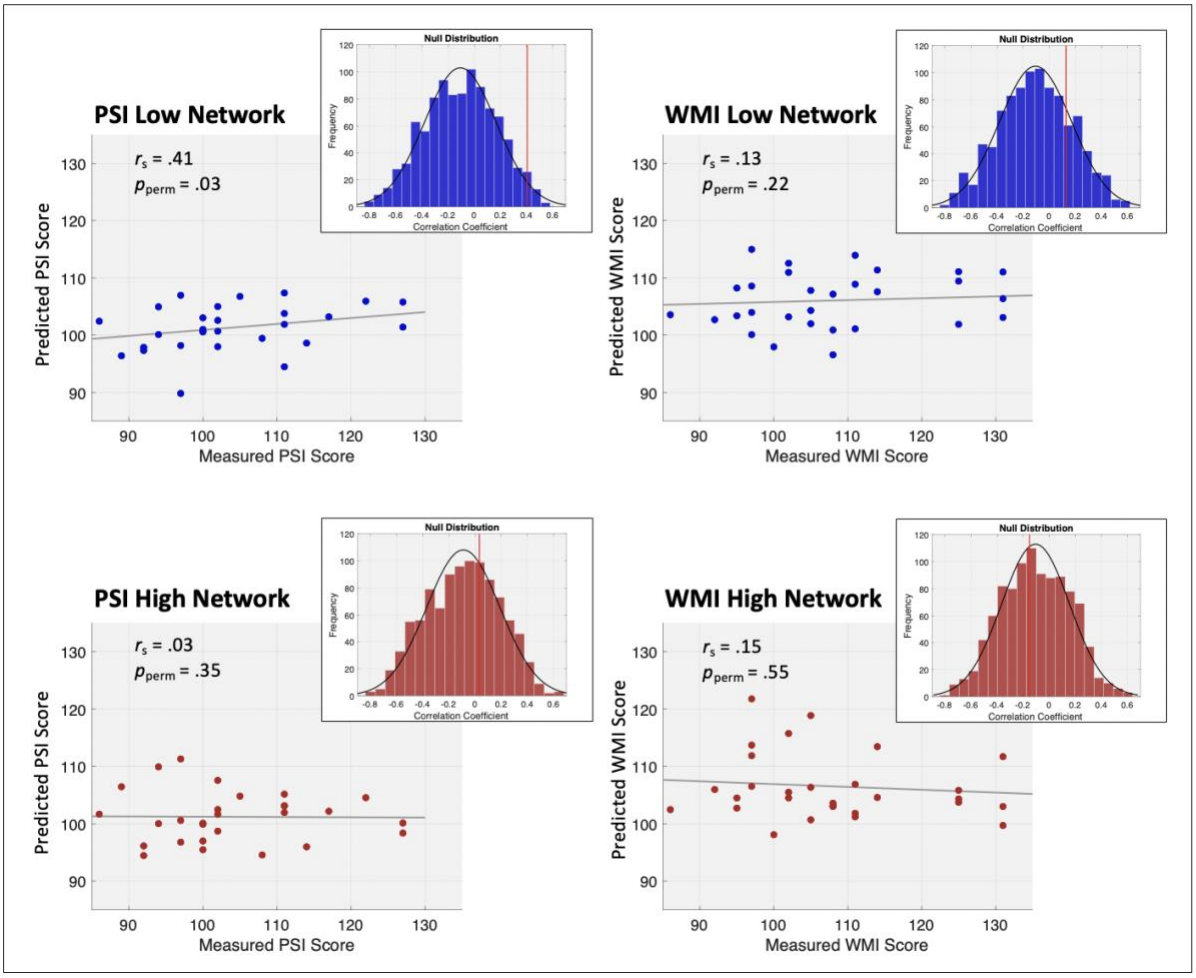
Note. Schematic visual of connectome-based predictive modeling (CPM).

Figure 2. Expanded Disability Status Scale



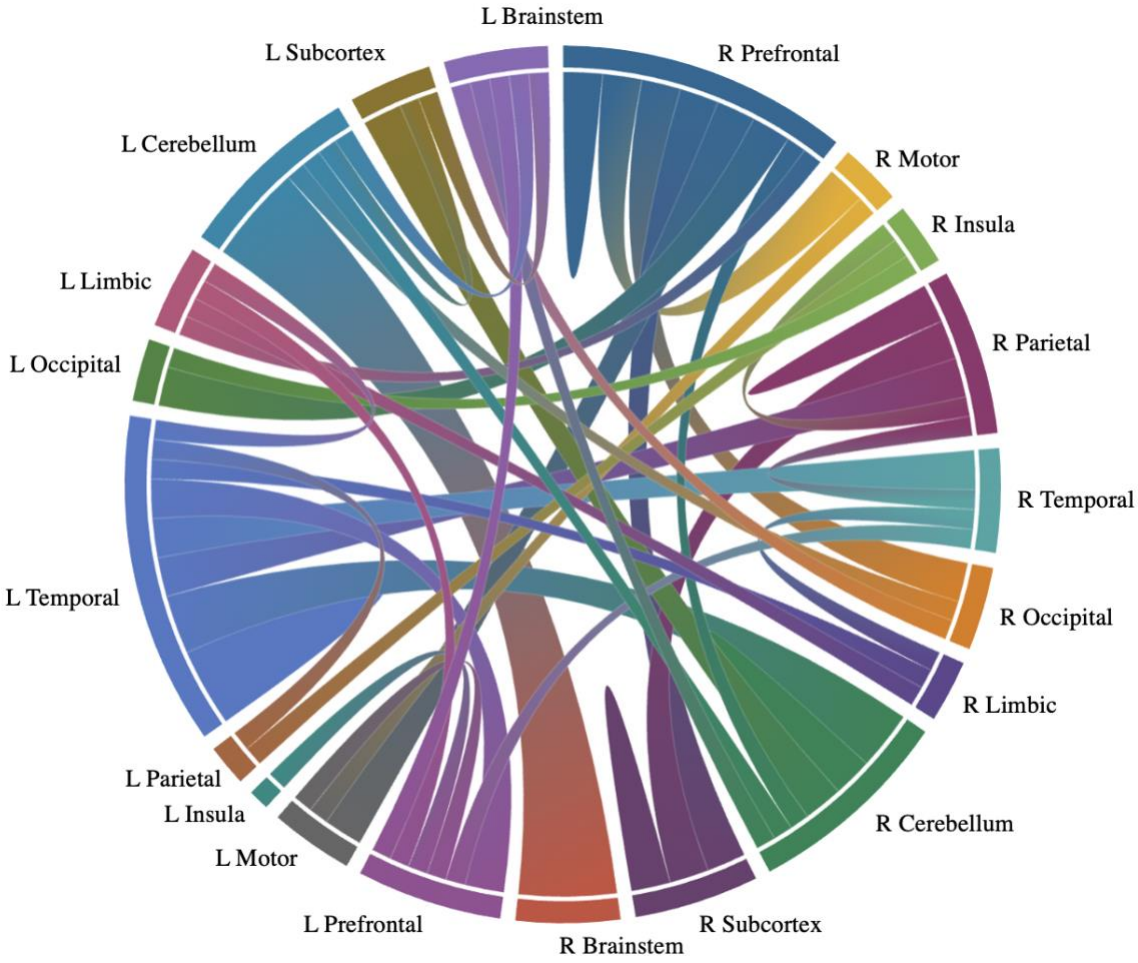
Note. This study included exclusion criteria that limited EDSS to a score of 5.5. EDSS = Extended Disability Status Scale (Kurtzke, 1983). Figure adapted from [https://www.epresspack.net/temso/media-backgrounder-multiple-sclerosis-a-serious-and-unpredictable-neurological-disease//]

Figure 3. In-sample Fit of Processing Speed and Working Memory Models



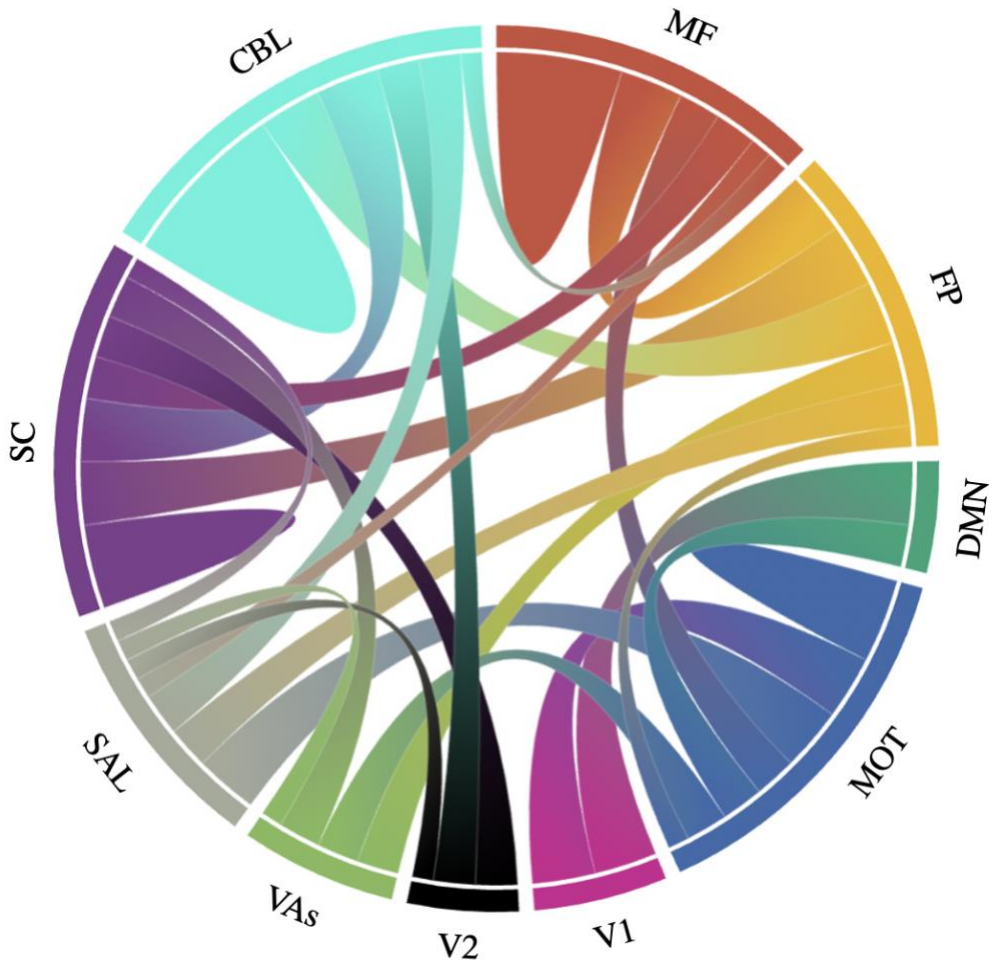
Note. Visual depiction of the in-sample fit for the network of edges negatively correlated with WAIS-IV Processing Speed Index. Histograms depict the null distribution CPM results after breaking the brain-behavior relationship for permutation testing, with red line indicating where the true model falls. PSI = Processing Speed Index standardized score, WMI = Working Memory Index standardized score (Wechsler, 1981).

Figure 4. Edges Common to All Iterations of the low-PSI Network by Macroscale Brain Region



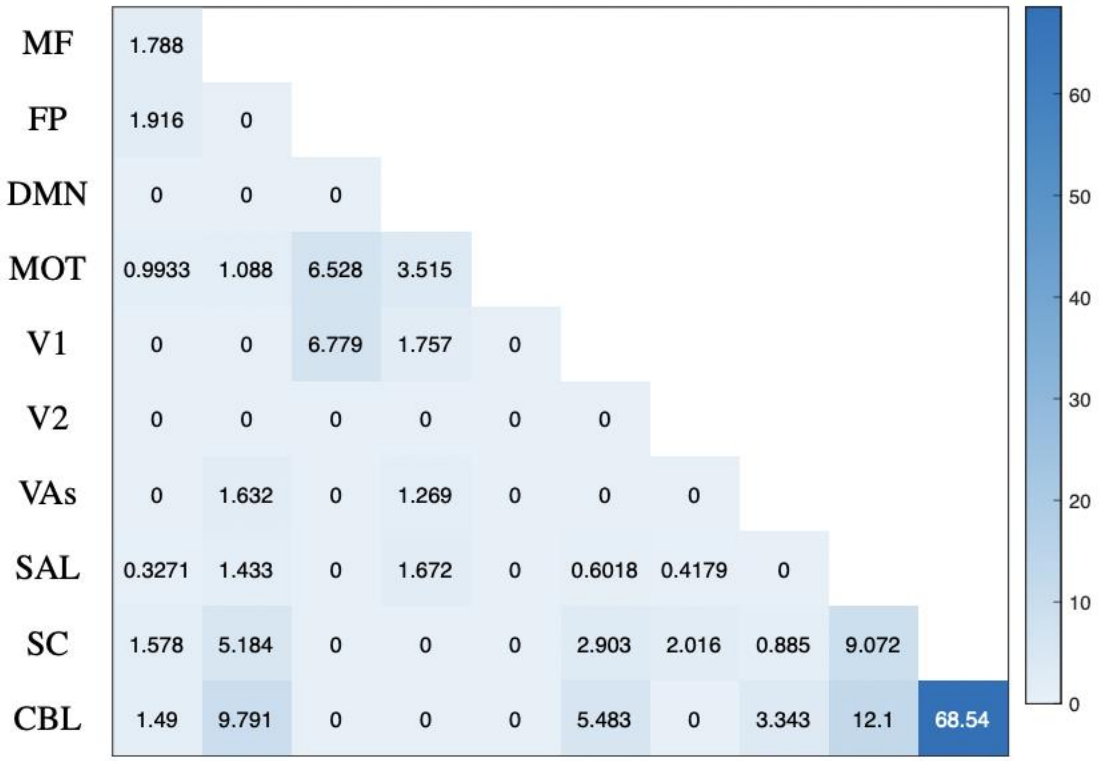
Note. This visual depicts the involvement of the anatomical distribution of the low-PSI network, using regions from the Shen atlas (Xilin Shen et al., 2017)

Figure 5. Edges Common to All Iterations of the low-PSI Network by Canonical Network



Note. Network localization of the low-PSI network. This visual depicts the involvement of the 10 canonical networks. ‘CBL’ = Cerebellar. ‘MF’ = Medial Frontal. ‘FP’ = Frontoparietal. ‘DMN’ = Default Mode Network. ‘Mot’ = Motor. ‘VI’ = Visual 1. ‘VII’ = Visual 2. ‘VAs’ = Visual Association. ‘SAL’ = Salience. ‘SC’ = Subcortical.

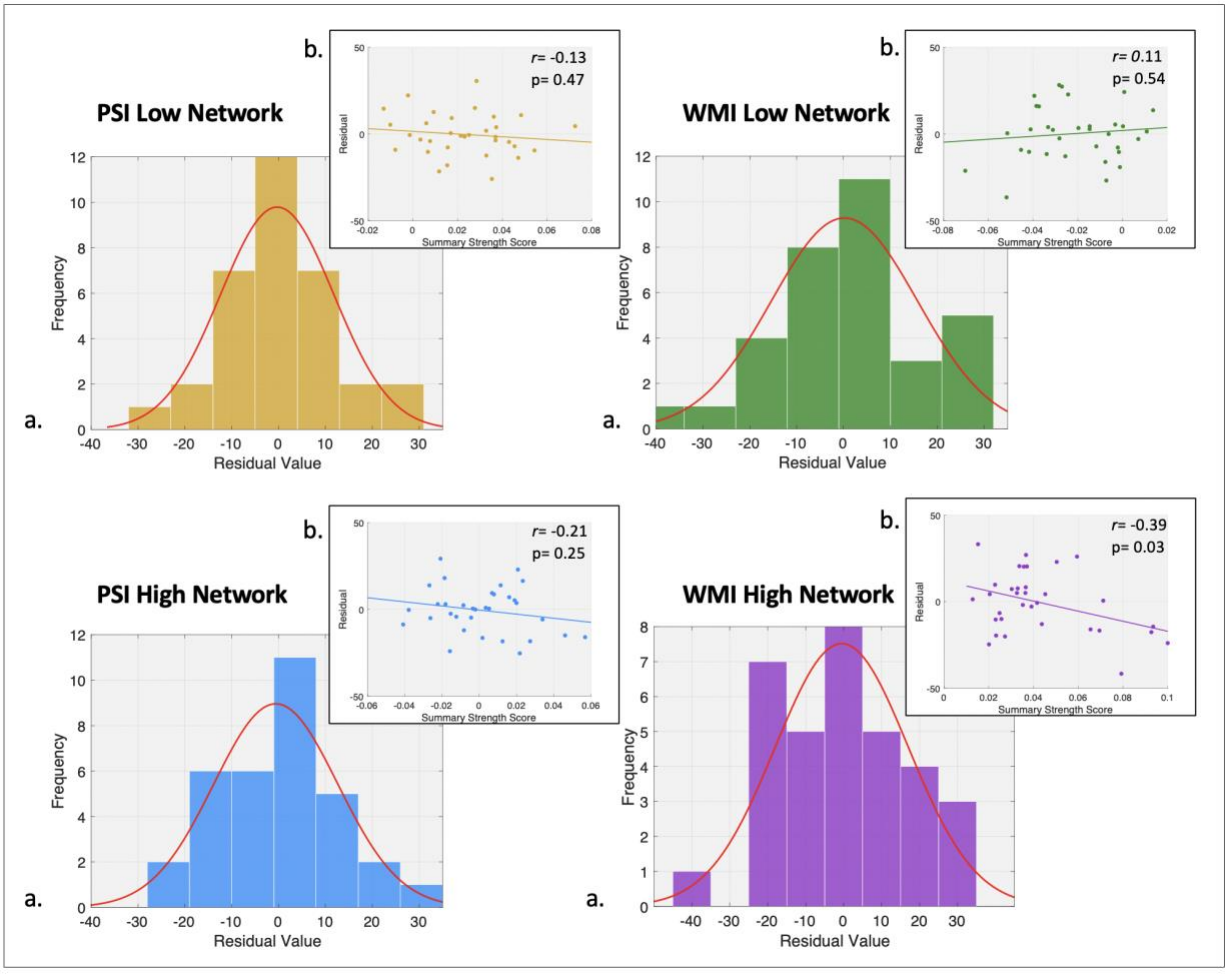
Figure 6. Contributions of Inter- and Intra- Network Connections Relative to Network Size



$$Contribution_{A,B} = \frac{lowPSI_{A,B}/lowPSI_{total}}{E_{A,B}/E_{total}}$$

Note. Contributions of within and between network connections were calculated as the ratio of the proportion of model edges from network A to B, to the proportion of total edges in the brain from network A to B. 'CBL' = Cerebellar. 'MF' = Medial Frontal. 'FP' = Frontoparietal. 'DMN' = Default Mode Network. 'Mot' = Motor. 'VI' = Visual 1. 'VII' = Visual 2. 'VAs' = Visual Association. 'SAL' = Salience. 'SC' = Subcortical.

Figure 7. Assessing Linear Model Assumptions



Note. (a) Normality is assessed by ensuring the residuals are evenly centered around 0. All network's residuals were centered around 0 and normally distributed by visual inspection. (b) Homoscedasticity and linearity were assessed by examining the relationship between the residuals and summary strength scores. Only the high WMI network demonstrated a significant negative trend, suggesting this relationship may not be linear; homoscedasticity appeared to be violated as the model consistently underestimated the WMI score of participants with higher summary strength scores. PSI = Processing Speed Index standardized score, WMI = Working Memory Index standardized score (Wechsler, 1981).