EXAMINING SINGLE-SUBJECT RELIABILITY OF FUNCTIONAL MAGNETIC RESONANCE IMAGING IN ESTABLISHED MULTIPLE SCLEROSIS

by

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To my parents

" ...and what exactly will you do with that degree? “

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ABSTRACT

Multiple sclerosis (MS) is the most common chronic inflammatory and neurodegenerative disease affecting the central nervous system. Conventional magnetic resonance imaging (MRI) has an invaluable role in the early diagnosis of MS. However, its use to monitor ongoing disease activity is less effective. This shortcoming is likely related to the limited sensitivity of conventional MRI to subclinical brain pathology and functional compensation. Functional MRI (fMRI) is more sensitive to these pathological changes, and may be of value in clinical settings to better characterize pathology and to monitor disease activity in established MS. A useful way to monitor disease activity in MS is assessing specific cognitive function, an important marker of neurological integrity. A few prior studies applied fMRI to monitor treatment effect in patients by using cognitive paradigms, however, intra-subject reliability of fMRI in MS has not been established. Consequently, we compared single-subject test-retest fMRI reliability between patients with MS and age-matched controls. For this purpose, we have chosen three paradigms based on their relevance in MS, the Symbol Digit Modalities Test (SDMT), the Poffenberger Task, and resting state (RS). The data were collected in three sessions a week apart. Our results showed relatively high and stable cluster overlap reliability (COR) for controls. Further, COR values were overall higher for controls compared to patients. This was particularly true for brain regions which comprise white matter. We also detected associations between disability scores (i.e. Extended Disease Status Scale) and COR, and between COR of RS and reaction time and accuracy (i.e. information processing speed) collected during the SDMT and from the Poffenberger Task. Similar to previous findings, we also observed larger recruited brain areas and inferior cognitive performance in patients compared to controls in response to the tasks. Our data obtained from controls suggest that adequate short-term fMRI reliability can be achieved. Conversely, the instability of COR seen in patients indicates that COR itself could be used as a marker of neuronal integrity in MS. Although there is much work ahead, our results are promising in view of implementing fMRI as a monitoring tool in clinical care of MS.

LIST OF ABBREVIATIONS AND SYMBOLS USED

2,3,4D	two, three, four dimensional
5HT	serotonin
20-HETE	20-hydroxy-eicosatetraenoic acid
γ	gyro magnetic ratio
ω	Larmor frequency
AA	arachidonic acid
ANOVA	analysis of variance
AP	action potential
ATP	adenosine triphosphate
AUC	area under the curve
B_0	static magnetic field
BBB	blood brain barrier
BET	brain extraction tool
BOLD	blood oxygen level dependent contrast
C^{13}	carbon 13 (natural occurring isotope of carbon)
Ca^{2+}	calcium ion
$[Ca^{2+}]_I$	intercellular calcium ion
CBF	cerebral blood flow
CC	corpus callosum
CD4+	cluster of dedifferentiation 4
cGMP	cyclic guanosine monophosphate
CIS	clinically isolated syndrome
CO_2	carbon dioxide
COR	cluster overlap relibility
CNS	central nervous system
CSF	cerebrospinal fluid
CYP27B1	cytochrome P450, family 27
DA	dopamine

dHb	deoxyhemoglobin
DMN	default mode network
DOF	degrees of freedom
DTI	diffusion tensor imaging
EDSS	Expanded Disability Status Scale
EET	epoxyeicosatrienoic acid
e.g.	exempli gratia
et al.	et alia
FEAT	FMRI expert analysis tool
FILM	FMRIB's improved linear model
FLAIR	fluid attenuated inversion recovery
FLAME	FMRIB's local analysis of mixed effects
FLASH	fast low angle shot
FLIRT	FMRIB's linear image registration tool
fMRI	functional magnetic resonance imaging
FMRIB	functional magnetic resonance imaging of the brain
FOV	field of view
FSL FMRIB	software library
FWHM	full width at half maximum
GABA	γ-aminobutyric acid
Gd	gadolinium
GLM	general linear model
Glu	glutamate
GM	grey matter
COR	cluster overlap reliability
H^1	protons (hydrogen)
Hb-O_2	oxyhemoglobin
HLA	human leukocyte antigen
Hz	hertz
IC	independent component

ICA	independent component analysis
ICC	intraclass correlation coefficient
ID	identifier
i.e.	id est
Inc.	Incorporated
K_+	potassium ion
LFP	local field potential
Ltd.	Limited
M	functional map
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
MHz	megahertz
min	minute
mm	millimetre
MPFLASH	magnetization prepared fast low angle shot
MPRAGE	magnetization prepared rapid gradient echo
MRI	magnetic resonance imaging
MS	multiple sclerosis
MWF	myelin water fraction
Mxy	transverse magnetization
Mz	longitudinal magnetisation
Na^+	sodium
NAGM	normal appearing grey matter
NAWM	normal appearing white matter
NE	noradrenaline
NMR	nuclear magnetic resonance
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase

O_2	oxygen
PD	proton density
P^{31}	phosporus 31 (natural occurring isotope of phosphorus)
pH	power of Hydrogen
PLA_2	phospholipase A_2
PG	prostaglandin
PPMS	primary progressive multiple sclerosis
r	reliability
RF	radiofrequency
RIS	radiological isolated syndrome
ROC-r	receiver operating characteristics reliability
ROI	region of interest
RRMS	relapsing remitting multiple sclerosis
RS	resting state
RS-fMRI	resting state functional magnetic resonance imaging
s	second
SD	standard deviation
SDMT	Symbol Digit Modalities Test
SPM	Statistical Parametric Mapping
SPMS	secondary progressive multiple sclerosis
SPSS	Statistical Package for the Social Sciences
t	time
T	Tesla
T1	longitudinal relaxation time (due to spin-lattice interactions)
T2	transverse relaxation time (due to spin-spin interactions)
T2*	transverse relaxation time (due to magnetic field inhomogeneities)
TI	inversion time
TR	repetition time
TRvol	repletion time volume

TE	echo time
Thr	threshold
vs.	versus
WM	white matter

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CHAPTER 1

INTRODUCTION

1.1. Overview

Multiple sclerosis (MS) is the most common chronic inflammatory and neurodegenerative disease affecting the central nervous system (CNS, World Health Organization). Since conventional magnetic resonance imaging (MRI) has been introduced into the formal diagnostic work-up, there has been considerable progress in the early diagnosis of people at risk of developing definitive MS (Filippi & Rocca 2010a,c; 2011a,b; Filippi et al., 2010; Sicotte, 2011; Filippi & Rocca, 2012; Filippi et al., 2012; Rocca et al., 2013a).

While valuable in establishing a diagnosis of MS, the use of conventional MRI to monitor ongoing disease activity is less effective. In definitive MS, radiological findings only moderately correlate with disability scores, including physical and cognitive impairment (Filippi & Rocca, 2007: Zivadinov & Cox, 2007). This shortcoming is likely related to the limited sensitivity of structural MRI to several important features of brain pathology like subclinical abnormalities (e.g. chemical or microscopic changes in the normal appearing white matter (NAWM) and cortical lesions), and also functional compensation present in MS (Allen et al., 2001; Coombs et al., 2004; MacKay et al., 2009; Rocca et al., 2010a; Bonavita et al., 2011; Loitfelder et al., 2011; Moll et al., 2011; Forn et al., 2012). To better monitor disease activity and treatment response in MS, radiological results should be complemented with techniques more sensitive to subclinical pathology and functional changes. Functional MRI (fMRI), which detects changes in blood oxygenation in response to neuronal activity (blood oxygen level dependent, BOLD signal), is one of these techniques, and may be of value in clinical settings to better characterize pathology and monitor disease activity and response to treatment in established MS.

A useful way to monitor disease activity and treatment response in MS is assessing specific cognitive functions and the underlying brain activity. Cognitive dysfunction is one of the most common disabilities in MS, affecting 40-70% of patients (Rao et al.,

1991a,b; Archibald & Fisk, 2000; Benedict et al., 2006; Nocentini et al., 2006; Chiaravalloti & Deluca, 2008; Rovaris, 2008b; Olazaran et al., 2009) and it is already present early in the course of the disease (Benedict et al., 2006; Benedict & Zivadinov 2006; Chiaravalloti & Deluca, 2008; Portaccio et al., 2009a,b). Perhaps, the most commonly occurring cognitive dysfunction in MS is the slowing in information processing speed (Rao et al., 1991a; Amato et al., 2001b; 2010a,b; Pelletier et al., 2001; Chiaravalloti & DeLuca, 2008; Filippi et al., 2009; Bonzano et al., 2011a,b).

A few prior studies applied fMRI to monitoring patients with MS by assessing cognitive function (Parry et al., 2003; Mainero et al., 2004; Morgen et al., 2004; Filippi & Rocca 2010c; 2012; Freedman et al., 2011), however, reliability of this imaging technique in MS has not been established. Owing to its potential in clinical setting, reliability of fMRI has been receiving increased attention, and now there is growing evidence that, with careful planning, satisfactory reliability can be achieved (Bennet & Miller 2010; Gorgolewski et al., 2013a,b; Zanto et al., 2014). However, many of these studies examined healthy controls, and findings of fMRI reliability may not be generalizable to MS. For instance, patients with MS have been shown to have high intra-individual variability in cognitive performance (Wojtowicz et al., 2012; 2013; 2014), which would affect fMRI reliability. Furthermore, most of fMRI reliability studies have focused on group reliability (Bennet & Miller 2010) and not on individual test-retest reliability, which is a key feature of clinical care (Gorgolewski et al., 2013b; Stevens et al., 2013; Kristo et al., 2014).

The objective of the current work is to compare single-subject fMRI reliability between patients with MS and age-matched controls. For this purpose, we have chosen three paradigms based on their relevance in MS. Cognitive impairment is one of the most prevalent and early manifestations of the disorder, and as such it provides a meaningful way to monitor MS and efficacy of early intervention. Among many other cognitive domains, slowing in information processing (Pelletier et al., 2001; Chiaravalloti & DeLuca 2008; Filippi et al., 2009; Bonzano et al., 2011a,b) and transcallosal conduction influencing information processing speed (Rao et al., 1989a; Barkhof et al., 1998a,b; Pelletier et al., 2001; Mesaros et al., 2009; Brown 2010; van der Knaap & van der Ham 2011) are affected in MS and have been tested using fMRI. To examining slowing in

information processing and transcallosal conduction, we have chosen the Symbol Digit Modalities Test (SDMT, Smith, 1982) and the Poffenberger Task (Poffenberger, 1912; Marzi, 1999), respectively. A task-free or 'resting state' fMRI that circumvents performance-related variability was also selected to assess reliability of baseline neuronal activity, as there is evidence of specific pattern of abnormal neuronal activation and its relevance to cognitive impairment in MS.

The structure of this manuscript-based thesis will be as follows. The first chapter will address general features of MS (Section 1.2.), principles of conventional and functional MRI (Section 1.3.), findings of previous research on cognitive performance in MS using structural and functional MRI, and the overview of the functional paradigms and their relevance in MS that will be used in this study (Section 1.4.). The introduction will conclude with a description of specific objectives (Section 1.5.). Chapters 2 and 3 will address the studies of single-subject reliability of the BOLD signal using the SDMT, the Poffenberger Task, and a resting state, paradigm. The current work will conclude with chapter 4 describing limitations of the studies, future goals and summarizing overall conclusions.

1.2. Multiple Sclerosis

1.2.1. Epidemiology

Multiple Sclerosis is a demyelinating and degenerative disorder with complex immunopathology characteristics and a variable clinical course. It affects approximately 2-2.5 million people worldwide (Milo & Kahana 2010) and its prevalence has been reported to vary by continent and geographical latitude. The condition is highly prevalent (>30 per 100,000) in northern parts of Europe and North America. In 2007, the U.S. National Multiple Sclerosis Society estimated 400,000 people affected in the United States. A recent investigation estimated the prevalence of MS in Nova Scotia at a rate of 267 per 100,000, which is among the highest known in Canada and worldwide (Evans et al., 2013; Kingwell et al., 2013). Similar to many autoimmune diseases, MS is more

common in women, with the female to male sex ratio by year of birth increasing for at least 50 years, and now exceeding 3.2:1 in Canada (Orton et al., 2006).

1.2.2. Aetiology

The underlying factors of MS are largely unknown and are still under investigation. Genetic factors, including the HLA locus, the CYP27B1 vitamin D gene, environmental factors, such as lack of sunlight exposure/vitamin D, infectious agents like the Epstein-Barr virus, and the interaction between genetic make-up and the environment (Orton et al., 2006; Cree et al., 2007) are among the suspected causes.

1.2.3. Pathophysiology

1.2.3.1. Hypothesis of Immunopathology

The pathologic hallmarks of MS are demyelinated plaques within the white matter (WM). As such, MS has traditionally been considered primarily an inflammatory demyelinating disease. In the periphery, mature lymphocytes ($CD4^+$ T-cells) become activated and differentiate into proinflammatory cells. The proinflammatory cells can enter the CNS parenchyma through the blood-brain barrier (BBB) and weaken the BBB. Weakening the BBB has several downstream consequences: (i) more immunomodulating agents can get through the damaged BBB; and (ii) proinflammatory T-cells multiply within the CNS parenchyma and consequently, cause the release of new potential CNS autoantigens. These secreted proinflammatory immunomodulating agents in turn amplify the process of myelin disruption and tissue injury. Damage to the CNS tissue initiates activation of CNS resident immune cells, which in turn promote a release of excessive amounts of glutamate (Glu), free radicals, nitric oxide, and enzymes promoting protein breakdown, thereby causing necrotic damage to oligodendrocytes and axons. This cycle is propagated by the recruitment of additional T-cells (Inglese 2006; Bennett & Stuve 2009).

1.2.3.2. Hypothesis of Neurodegeneration

Demyelination, however, neither explains the high frequency of acute axonal injury shown in both the chronic and the early stage of the illness, nor the damage occurring in

normal appearing white matter (Trapp et al., 1998; Allen et al., 2001) and cortical areas (Kidd et al., 1999; Peterson et al., 2001; Chard et al., 2004). A recent study has demonstrated that in certain cases of MS the primary pathology is disruption in myelin lipids that is more related to degenerative processes (Laule et al., 2013). Further, grey matter (GM) lesions reflect different pathology from plaques in WM (i.e. less inflammation, with less macrophage and lymphocyte infiltration) (Peterson et al., 2001; Bo et al., 2003; 2006) that mostly, but not exclusively occur in the progressive phase of the disease (Chard et al., 2004; Dalton et al., 2005). These findings put forward the notion that neurodegeneration rather than inflammation is the primary pathology in some cases of MS.

1.2.4. Course of Multiple Sclerosis

The current MS classification system is based on consensus and relies on the clinical course of the disease (Confavreux et al., 2000). The most common type of established MS (> 85 %) is relapsing-remitting multiple sclerosis (RRMS), which is characterized by cycles of attacks and remission. Symptoms may vary from mild to severe, and relapses and remissions may last for days or months. There is thought to be little or no disease progression during the periods between disease relapses. The second type is the secondary progressive multiple sclerosis (SPMS) affecting ~10% of MS patients. SPMS usually progresses from initial RRMS, and, in fact, eventually develops in most patients with RRMS. This course is characterized by relapses and minor remissions, without complete return to baseline and overall progression over time (Confavreux et al., 2000). Finally, primary progressive multiple sclerosis (PPMS), represents about 10% of cases of MS (Lublin & Reingold 1996). In this form of the disease, there is continuous and usually gradual deterioration of neurologic function. There may be occasional plateaus and slight fluctuations, but not complete relapses. Unlike RRMS and SPMS, both of which predominantly affect females, PPMS tends to be equally prevalent in males and females, and has worse prognosis for ultimate disability compared to patients with RRMS. The fourth type of MS is referred to as progressive-relapsing MS and is the least common form of the disease (Confavreux et al., 2000). Types of established MS are depicted in Figure 1.1.

Prior to developing established MS, many people present with radiological isolated syndrome (RIS), which converts to clinically isolated syndrome (CIS). Radiological isolated syndrome describes incidental findings suggestive of MS without the typical MS symptoms and normal neurological findings. While CIS is the first neurological episode caused by inflammation or demyelination of the CNS with focal or disseminated brain lesions.

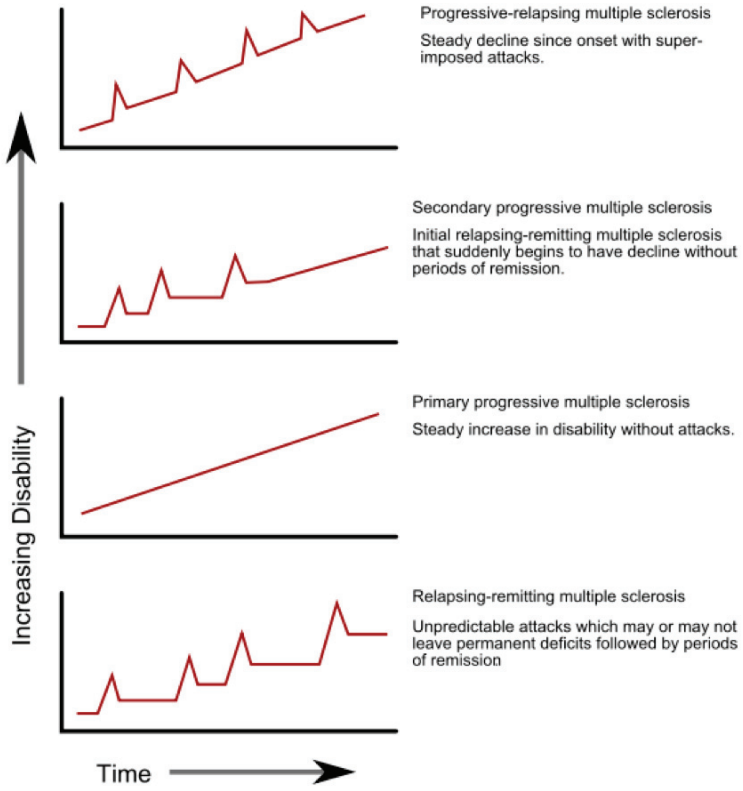


Figure 1.1. Types of Multiple Sclerosis and Course of the Disease. (Figure released to public, http://commons.wikimedia.org/wiki/Category:Multiple_sclerosis).

1.2.5. Signs and Symptoms of Multiple Sclerosis

Pathological CNS changes are associated with several neurological symptoms that are typical to MS. The most common symptoms and signs include fatigue, pain, tremor, optic

neuritis, paraparesis, paraplegia, Lhermitte phenomenon, coordination problems, as well as bowel, bladder, or sexual dysfunction.

The Expanded Disability Status Scale (EDSS) is important in characterizing the degree of MS-related disability. The EDSS is commonly used for monitoring patients with MS (Kurtze 1983). This clinical scale describes clinical disability in eight functional systems (pyramidal, cerebellar, brainstem, sensory, bowel/bladder, visual, cerebral, other) using scores from 0 (i.e. normal examination and function) to 10 (i.e. for death due to MS). The scales are nonlinear, with greater emphasis on ambulation capabilities with scores above 4.

In addition to the physical problems, high rates of depression with a lifetime prevalence of ~50% and an annual incidence of 20%, have been reported in MS (Feinstein 2004; Patten, 2003). It is challenging to disentangle the direct effects of the disorder upon mood from the non-specific effects of chronic illness, but evidence suggests that the prevalence of major depression in MS is elevated compared to that in both healthy people and in other chronic conditions (Patten, 2003).

Cognitive impairment is another common feature of MS (Benedict et al., 2006; Patti 2009; Patti et al., 2009). Its prevalence may vary between 35- 60% (Benedict 2006; Patti et al., 2009) depending on the population studied. The most frequent deficits are in information processing speed sustained attention, and in anterograde episodic memory (DeLuca et al., 2004; 2008), although the nature and the degree of impairment may vary due to the heterogeneity of brain pathology (Chiaravalloti & DeLuca 2008; Wojtowicz et al., 2012; 2013; 2014), which poses considerable challenges in monitoring patients.

Studies addressing the effect of cognitive impairment on activities of daily living reported devastating impact on patients' ability to maintain employment (Amato et al., 2001a; Morrow et al., 2010; Strober et al., 2012), independent living skills, and on maintaining social relationships (Amato et al., 2001a ; Benedict et al., 2005b; Kalmar et al., 2008).

While cognitive impairment is more pronounced in the progressive forms of the disease, it has been demonstrated in all stages and in all subtypes (Amato et al., 2001a,b; De Sonneville et al., 2002; Benedict et al., 2005a; 2006; Benedict & Zivadinov 2006; Chiaravalloti & Deluca 2008; Potagas et al., 2008; Kalmar et al., 2008; Portaccio et al.,

2009a,b). Decline in cognitive performance is also prominent; a previous study found that prevalence of cognitive dysfunction increased from 26 to 56% over the 10-year retest interval, and the extent of cognitive impairment was associated with the degree of physical disability, progressive disease course, and increased age (Amato et al., 2001b).

As such, cognitive dysfunction appears to be an important marker of neurological integrity in MS. To relate brain pathology to cognitive function, at first, conventional MRI data (e.g. lesion volume, plaque volume, brain atrophy) were compared with neuropsychological test scores with significant, but only mild to moderate correlations (Rao et al., 1989b; Comi et al., 1995; Moll et al., 2011; Cerasa et al., 2013). These results suggested an important role of MRI in MS, but also indicated that structural correlates measured by conventional MRI may not provide comprehensive explanation of brain pathology relating to cognitive function. The introduction of advanced MRI in MS research seemed to offer more accurate characterization of tissue injury and compensatory mechanisms in MS affecting cognitive performance.

1.2.6. Role of Magnetic Resonance Imaging in Diagnosis of Definitive Multiple Sclerosis

Although, there is a range of tests to diagnose MS including basic neurological examination for signs and symptoms compatible with the pattern of neurologic deficits seen in MS, recording sensory or visual evoked potentials, testing for the presence of oligoclonal bands in the cerebrospinal fluid (CSF), currently, MRI is the most commonly used tool in diagnosis that provides early and reliable *in vivo* detection of typical CNS lesions. Commonly used imaging sequences include the older, conventional T_2 and T_1 weighted structural imaging with or without the use of contrast agents and fluid attenuated inversion recovery (FLAIR). These techniques will be discussed in section 1.3

MRI was formally included in the 2001 diagnostic criteria for MS (McDonald et al., 2001) with subsequent revisions in 2005 and 2010 (Polman et al., 2005; 2011), and now allows for the diagnosis of probable MS after a single attack. Acquiring series of brain scans after a single attack has been shown to be suggestive of lesion dissemination in time and to be highly specific for predicting the development of clinically definitive MS (Montalban et al., 2009; Rovira et al., 2009). Introduction of the McDonald criteria

(McDonald et al., 2001) has impacted the treatment of MS tremendously, as disease treatment started after the first attack has been shown to reduce disability and the secondary relapse rate (Miller et al., 2004a,b,c; Kennedy 2013).

The increasing availability of MRI has also led to more incidental findings suggestive of MS (i.e. RIS). Several follow-up studies have demonstrated that evolving RIS is prognostic to clinical conversion to CIS and/or MS. According to a recent systematic review, two-thirds of individuals with RIS showed radiological progression, and around one-third showed conversion to CIS and/or MS in cohorts within approximately two to five years (Granberg et al., 2013).

To predict conversion from CIS to definitive MS, nonconventional MR techniques also appear to be useful.. Recently, cortical lesions detectable using double-inversion recovery sequences (Geurts et al., 2012) have been proposed to be incorporated into the new MRI criteria for diagnosis. The proposed new MRI criteria appear to have higher specificity (93%) and accuracy (86%) compared to the available criteria while maintaining a relatively high sensitivity (77%) (Filippi et al., 2010).

The current protocol of conventional or diagnostic MRI includes a T_1 -weighted image with and without Gadolinium (Gd) enhancement, a 3D-FLAIR, and a T_2 -weighted or proton density (PD) image. T_2 –weighted, or T_2 FLAIR, sequences have a particularly high sensitivity to focal WM lesions, which appear hyperintense on those images due to the presence of the extravascular fluid. However, the underlying pathology (e.g. edema, demyelination, remyelination) cannot be determined based on these images alone (Filippi & Rocca 2009). Gadolinium enhanced T_1-weighted imaging is sensitive to active lesions that appear shortly before and during clinical relapses, because the enhancement occurs as a result of the increased permeability of the BBB (Molyneux et al., 1998, Kappos et al., 1999). Finally, lesions that appear as ‘black holes’ on T_1 -weighted image before and after enhancement indicate chronic/permanent damage, mostly axonal loss (van Walderveen et al., 2001; Bakshi et al., 2004; Fisher et al., 2008; MacKay et al., 2009).

MS-related lesions can be found throughout the brain, but they typically have a pattern of distribution that includes the periventricular WM, corpus callosum (CC), internal capsule, pons, middle cerebral peduncles, U-fibers, optic nerves, and other parts of the visual pathway. Some of these typical lesions are depicted in Figure (1.2.). The CC

or periventricular areas are especially sensitive to demyelination, possibly due to their close anatomic location to the lateral ventricular roofs and the rich innervation of small penetrating vessels. In addition to the brain, the spinal cord, and particularly the cervical segment, is also affected in MS (Laule et al., 2010). In fact, 5-20% of patients (Thorpe et al., 1996; Agosta & Filippi, 2007) exhibit lesions only in the spinal cord.

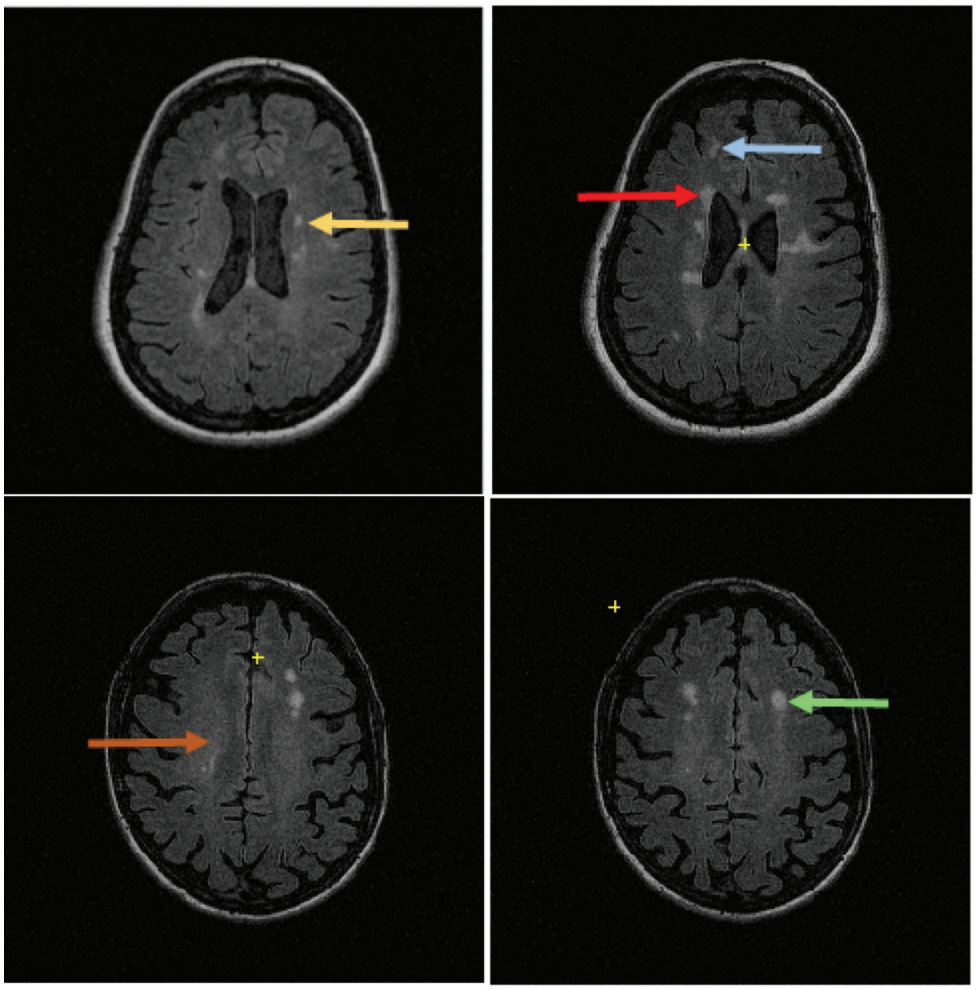


Figure 1.2. Typical Presentation of White Matter Lesions of Two Patients of the Study. Lesions are detectible by the fluid attenuated image (FLAIR) sequence; ovoid lesions perpendicular to the lateral ventricles are called Dawson fingers (yellow arrow), periventricular lesions (red arrow), juxtacortical lesions (light blue arrow), dirty or diffused white matter (rusty color arrow), and lesions on the U-fibers reaching the cortical area (green arrow).

1.3. Magnetic Resonance Imaging

The role of conventional MRI as a diagnostic tool has been discussed above. This section briefly addresses the principles of structural as well as functional MRI, a nonconventional imaging technique applied in our studies. We conclude this section with a description of the neurophysiological basis of fMRI.

1.3.1. Principles of Magnetic Resonance Imaging

Magnetic resonance imaging is a non-invasive medical imaging technique providing highly detailed images of body tissue. MRI makes use of the property of nuclear magnetic resonance (NMR) of atomic nuclei that possess a magnetic moment, such as H^1, C^{13}, and P^{31}. In conventional MRI, the most commonly imaged nucleus is the H^1 due to: i. its abundance in a biological tissue and ii. a particular magnetic property called the nuclear spin. When a human body is placed in a strong magnetic field (B_0), nuclear spins align either parallel or anti-parallel with B_0. As aligning anti-parallel demands a higher energy state, more nuclei align parallel, which results in a net magnetization of the body along the direction of B_0. When the net magnetization of the sample is aligned with the B_0, the magnitude of the magnetization cannot be measured. In order to measure the net magnetization, an electromagnetic (radio frequency, RF) pulse matching the nuclear spins has to be applied that tilts the net magnetization away from B_0 by a certain angle. This matching pulse has to be at the Larmor frequency or ω_0 proportional to the B_0 and a property called the 'gyro magnetic ratio' that is descriptive of the nuclei under study:

$\omega_0 = \gamma B_0$ where ω_0 = precessional or Larmor frequency (MHz)
γ = gyro magnetic ratio (MHz/T)
B_0 = magnetic field strength (T)

This process is called excitation. A 3D x, y, and z vector system can be plotted, where the net magnetization is along the Z direction = B_0 and is referred to as the longitudinal magnetisation or Mz. Following the electromagnetic RF pulse application, magnetization on the Z-axis is rotated into the XY plane, and the magnitude of the magnetization can be measured (McRobbie et al., 2006; MacKay et al., 2009).

When the RF pulse is removed, the net magnetization rotates back or 'relaxes' to its original, Z axis, while emitting radiofrequency waves. This phenomenon is called T_1 relaxation, also referred to as spin-lattice relaxation, as the spins release energy to their surroundings (lattice) (Figure1.3.).

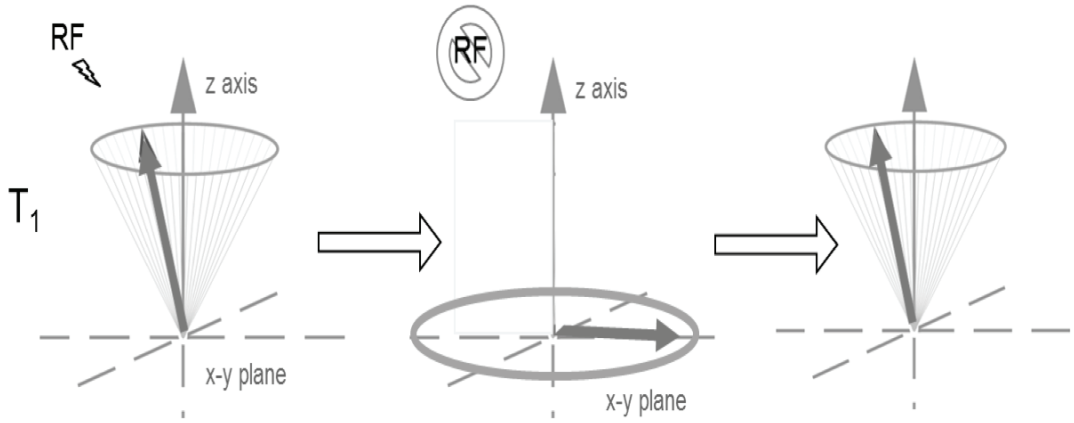


Figure 1.3. Generation of Longitudinal Magnetization and Relaxation of the H^+.

T_1 is different for each tissue, shortest for fat (e.g. WM) and longest for water (e.g. CSF). Obtaining T_1 weighted contrast of the three tissue types of the brain at a given time is depicted in Figure 1.4.

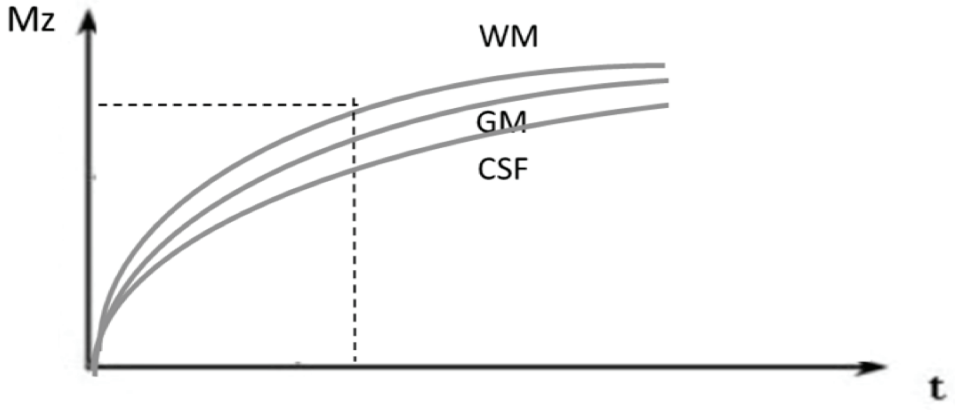


Figure 1.4. T_1 Weighted Relaxation as a Function of Time is Different for Each Tissue.

Along with the longitudinal magnetization and T_1 relaxation, another simultaneous process takes place. Prior to excitation, the nuclear spins have no phase coherence in the XY plane. By applying the RF pulse, the spins not only flip into the XY plane but are also aligned, and start to rotate in phase. This results in a net transverse magnetization, Mxy. During relaxation, nuclear spins start to move out of phase until there is no more phase coherence left. This phenomenon is called T_2 relaxation, which is also referred to as spin-spin relaxation, as the process is dominated by the interaction between nuclei (Figure 1.5.).

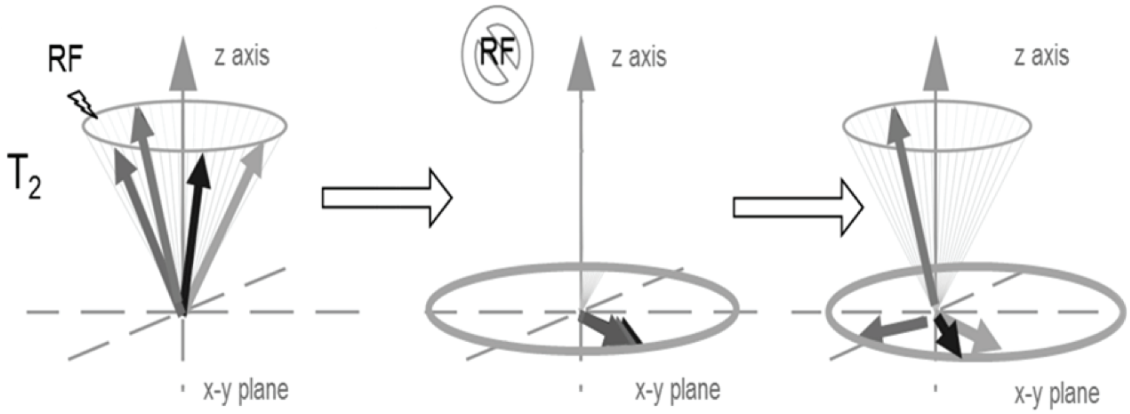


Figure 1.5. Generation of Transverse Magnetization and Relaxation of the H^+.

Similarly to T_1, T_2 relaxation depends on the tissue type. Fat dephases quickly, while water has a long T_2. (Figure 1.6.).

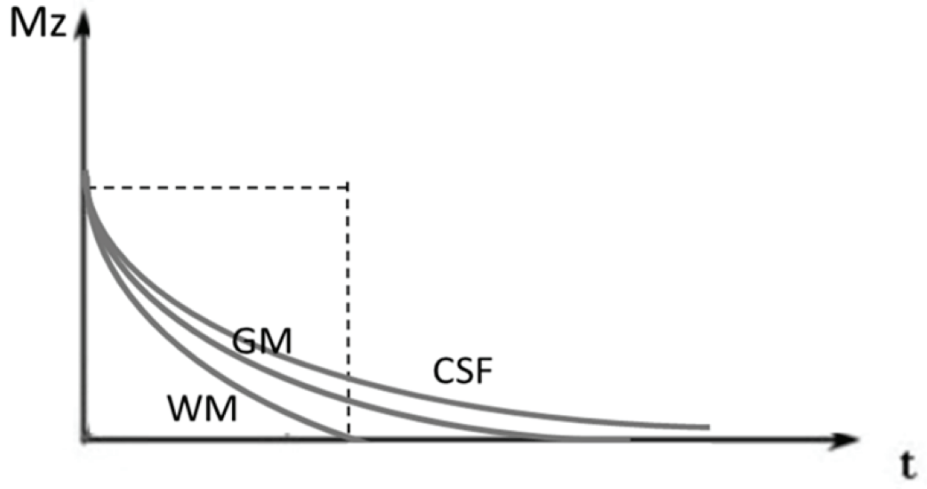


Figure 1.6. T_2 Weighted Relaxation as a Function of Time is Different for Each Tissue.

T_1 and T_2 are both time constants; T_1 is defined as the time it takes for Mz to reach 63% of the original (equilibrium) magnetization, while T_2 is the time it takes for transverse magnetization (Mxy) to decrease to 37% of the original value. While T_1 is longer in a higher magnetic field, T_2 is relatively independent of field strength (McRobbie et al., 2006; MacKay et al., 2009).

Pure T_2 decay is a function of random spin-spin interactions with the assumption of an absolutely homogeneous B_0. However, in reality, there are imperfections in the homogeneity of the B_0 that accelerates the T_2 decay. The phenomenon that depends on both, the spin-spin interaction and the inhomogeneity of the B_0 is called T_2*. The relationship between the three time constants is $T_1 > T_2 > T_2$* (McRobbie et al., 2006; MacKay et al., 2009).

In order to produce an image, the NMR signal has to be located, which can be achieved by spatial encoding. Spatial encoding comprises applying gradients (magnetic fields that vary linearly in space) in three orthogonal directions. Slice selection is achieved by applying a gradient coil in one direction that causes the magnetic field to vary linearly as a function of spatial location within an area. The slice selection gradient is applied simultaneously with the RF pulse that has a particular bandwidth that matches the thickness of the slice to be selected. Applying the gradients produce spatially dependent variations of ω along the direction of the gradient, allowing for the selection a

range of ω that corresponds to a particular section or slice of the sample. Once the slice has been selected, the remaining two in-plane dimensions need to be encoded. Next, a phase encoding gradient is applied for a brief time in the second orthogonal direction, which causes some of the spins to precess faster than others. Once the gradient is removed, the resonant frequency returns to its pre-gradient value, but the spins retain phase differences from the application of the gradient. These phase differences can then be used to localize the signal along the second axis (phase-encode). The third gradient, referred to as frequency-encode, is applied on the third orthogonal axis during the recording of the MR signal. Similar to the slice selection gradient, it causes a range of ω to exist along the third direction and based on the frequency information, the signal can be encoded. The data derived from spatial encoding are digitized and written into a data matrix that is known as a K-space matrix. A mathematical transformation, the Fourier transform of the K-space data is completed by a computer to produce a grey-scale image (McRobbie et al., 2006).

1.3.2. Sequences

1.3.2.1. Spin Echo Sequences

The most commonly used pulse sequence is the spin echo sequence. In this sequence, a 90° slice selective pulse is followed by a 180° rephasing pulse at a time (*t)* later, which causes the Mxy to rephrase. The signal is called an echo because it is rebuilt from a decaying signal following the initial 90° RF pulse. The sequence has two important parameters: i. the echo time (TE, which is the time between the 90° RF pulse and the sampling of the MR signal, which corresponds to the maximum of the echo, and it is equal to 2t, and ii. the repetition time (TR), which is the time between 2 excitations or the time between two 90° RF pulses. Timing of the pulse sequence can be adjusted to acquiring a T_1, PD, or a T_2-weighted image (McRobbie et al., 2006; MacKay et al., 2009).

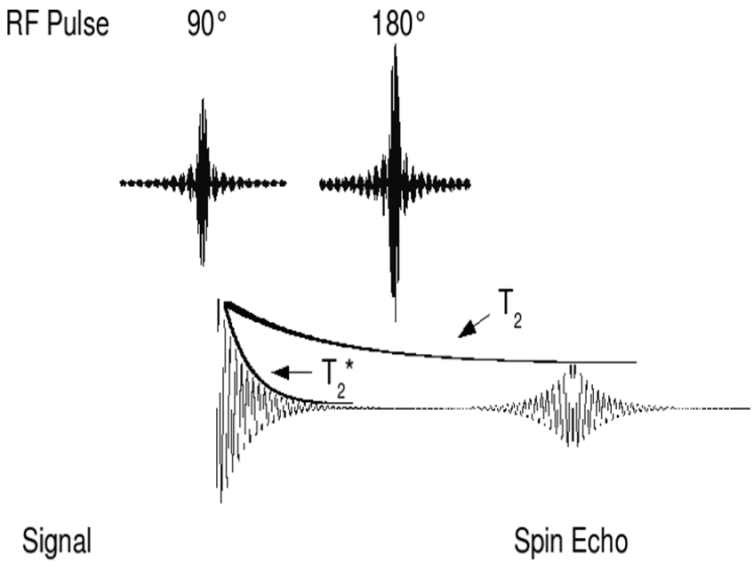


Figure 1.7. Refocusing Spin Magnetization with a 180° Radio Pulse. (Figure released to public, http://commons.wikimedia.org/wiki/Category:Spin_echo).

1.3.2.1.1. Fluid Attenuated Inversion Recovery (FLAIR)

FLAIR is a special inversion recovery sequence designed to suppress water signals. It consists of a spin echo sequence preceded by a 180° RF pulse that inverts the longitudinal magnetization (Mz) by 180° which in turn, grows back according to T_1 relaxation. Following the 180° RF pulse, a 90° RF pulse is applied at the time (inversion time, TI) when there is no Mz to be flipped into transverse magnetization (Mxy) from water. Suppressing the water signal results in water (e.g. CSF) looking black in the image (Figure1.8a). This sequence is commonly used in clinical practice to evaluate cerebral edema, such as periventricular pathology.

1.3.2.2. Gradient Echo and Fast Low Angle Shot (FLASH)

The gradient echo sequences differ from spin echo sequences in two ways: i. instead of applying a 180° RF pulse (i.e. flipping the spins), dephasing or rephasing gradients are applied at the end of the sequence to refocus the spins and ii. while flip angle ~ 90° is used in spin echo sequences, the flip angle can vary between 10° and 80°in gradient echo acquisitions. Refocusing the spins with a gradient takes considerably less time than 180° degree RF pulse refocusing (McRobbie et al., 2006; MacKay et al., 2009). Thus,

variations of this sequence can be used for fast imaging. One such example is the FLASH sequence created for acquiring predominantly T_1 weighted images. (Figure1.8b). This fast imaging sequence is based on the elimination of the residual transverse magnetization at the end of the excitation cycle and thereby reducing T2* weighting and increasing T1 weighting.

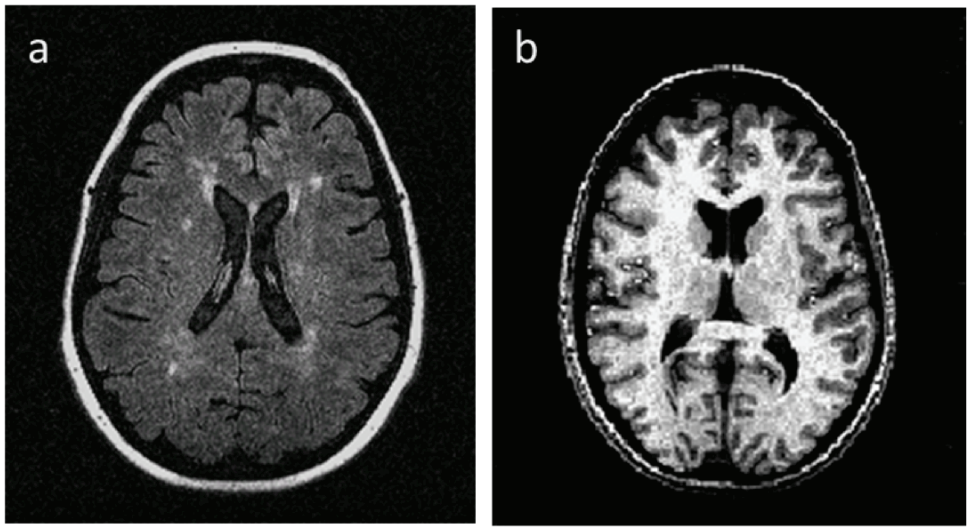


Figure 1.8. Fluid Attenuated Inversion Recovery Image Contrasted to a T_1 Weighted Image Obtained Using a Magnetization Prepared Rapid Gradient Echo Sequence.

1.3.2.3. Functional Magnetic Resonance Imaging

While structural MRI allows for the identification of structural abnormalities, functional MRI is important for assessing the impact of macro- and microscopic, or biochemical changes by measuring how the brain functions.

1.3.2.3.1. The Hemodynamic Response

Functional MRI is a technique for measuring and mapping brain activity in a healthy or diseased brain. It capitalizes on the coupling of cerebral blood flow (CBF), energy demand, and neural activity. The basis of fMRI, a somewhat paradoxical and not an entirely understood concept, is the BOLD signal (Ogawa & Lee, 1990; Ogawa et al., 1990a,b) that depends on a chain of events.

Neural activity triggers a much larger change in CBF than in O_2 metabolism, and this leads to a higher O_2 blood level when neural activity increases. This phenomenon is called hyperemia. Oxygen (O_2) rich blood and O_2 poor blood have different magnetic properties related to the hemoglobin that binds to O_2 in blood. While oxygenated hemoglobin (Hb-O_2) is diamagnetic, just like the surrounding brain tissue (i.e. has a property of repelling an external magnetic field) deoxygenated hemoglobin (dHb) is paramagnetic (i.e. has a property of attracting an external magnetic field). This means that if the blood is more oxygenated, the signal is stronger, whereas if the blood is less oxygenated (i.e. more dHb), the signal will be weaker (Thulborn et al., 1982; Ogawa et al., 1990b). This phenomena is based on the T_2* effect; more dHb results in field inhomogeneities, thus in a faster signal decay and shorter T_2*, which will ultimately cause a less intense signal. Whereas more Hb-O_2 results in less field inhomogeneities, thus in a longer signal decay and longer T_2*, and thereby the image will have a higher intensity (Fox & Raichle, 1886; Kim & Ogawa, 2012). Figure 1.9. represents a whole brain functional image with T_2* contrast.

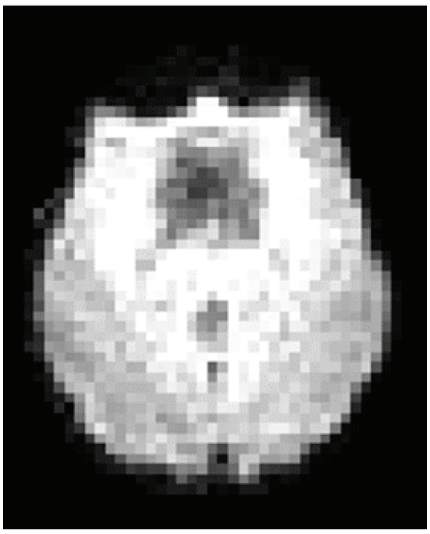


Figure 1.9. Raw Functional Image with a T_2* Weighted Contrast.

1.3.2.3.2. Neurophysiological Basis of Functional Magnetic Resonance Imaging

Understanding the neuronal origin of the BOLD signal is essential for correctly interpreting fMRI results. This area has been the focus of intensive research for well over a decade. However, despite the significant progress, our comprehension of the topic is not complete. Of the remaining questions, one is whether perisynaptic or spiking activity drives the vascular response. Perisynaptic, or local field potentials (LFPs), are considered to be the input to the neurons, while spiking activity or action potentials (APs) are thought to be the output. Earlier studies using simultaneous fMRI and electrophysiological recordings of the cortex noted that the hemodynamic response correlated stronger with LFPs compared to spiking activity (Logothetis et al., 2001, Logothetis, 2003). Others using similar methods of investigation demonstrated a disassociation between spikes and CBF in the cerebellum and in the dentate gyrus of the hippocampus (Lauritzen and Gold, 2003; Viswanathan & Freeman, 2007; Canals et al., 2009) leading to the conclusion that CBF is primarily driven by perisynaptic LFP.

However, the LFP driven hypothesis is not universally accepted. There is support that spiking activity is related to the BOLD response and the associated hemodynamic and metabolic processes (Heeger et al., 2000; Smith et al., 2002_{261}; Mukamel et al., 2005). A recent study reported that spiking activity consistently predicted the BOLD signal in a positive and linear fashion regardless of the brain area, whereas this form of relationship existed only in the cortex when LFPs and the BOLD signal were compared (Devonshire et al., 2012). This result not only attests that spiking activity is related to the hemodynamic response, but it also indicates that the BOLD signal and its neuronal origins have regional dependence (Devonshire et al., 2012).

The view of a region-dependent BOLD signal has further support. Sigalovsky and Melcher, (2006) reported differential BOLD response in cortical and subcortical auditory structures in response to auditory stimuli, whereas others demonstrated a larger LFP response and a smaller BOLD response in the striatum compared to those recorded in the cortex in response to direct stimulation of sensorimotor areas (Sloan et al., 2010).

Evidence of the high correlation between LFP and spiking activity further increase the complexity of the question (Jones et al., 2004; Hewson-Stoate et al., 2005), and

according to previous reports, correlation is present in most studies which examine bottom-up information processing ,Logothetis & Wandell 2004).

To fully appreciate neurovascular coupling, another key area under investigation is the metabolic basis of the BOLD signal. Evidence of a coupling between neuronal activation and energy supply was first introduced in the nineteen century (Roy & Sherrington, 1890). Traditionally, it was thought that CBF was locally controlled by a negative-feedback system. Neural activity led to energy demand following the use of adenosine triphosphate (ATP) to restore ion gradients after the generation of synaptic potentials and APs. Decreasing ATP in the cells was thought to produce a metabolic signal that in turn increased blood flow, allowing O_2 and glucose to enter the site. This metabolic signal could be the lack of O_2 or glucose, or the production of carbon dioxide (CO_2) (Attwell et al., 2001).

Testing blood O_2 and glucose concentration however has led to the conclusion that a decrease in O_2 or glucose does not regulate CBF. Furthermore, during neuronal activity, the local extracellular pH initially becomes alkaline, rather than becoming acidic as would be expected if arteriolar dilation were caused by the accumulation of CO_2 (Attwell et al., 2010).

There is one energy metabolite in the brain that does fit the feedback model of neurovascular coupling, lactate. Important work on the role of glycolysis in brain energy production provided the concept that the rate of glycolysis increases during brain activation (Raichle & Mintum, 2006) and it is linked to CBF modulation via lactate. Lactate concentration has been shown to modulate the BOLD response in the primary visual cortex of non-human primates (von Pfostl et al., 2012).

A variety of unexplained physiological processes linked to neurovascular coupling have promoted further investigation, and led to the neurotransmitter-mediated feedforward hypothesis. According to this hypothesis, neurons either directly or indirectly signal to blood vessels. The indirect signal occurs via activating astrocytes. Both of these signalling routes involve neurotransmitters, predominantly glutamate (Glu) (Attwell et al., 2010; Petzold & Murthy, 2011), explained in Figure 1.10.

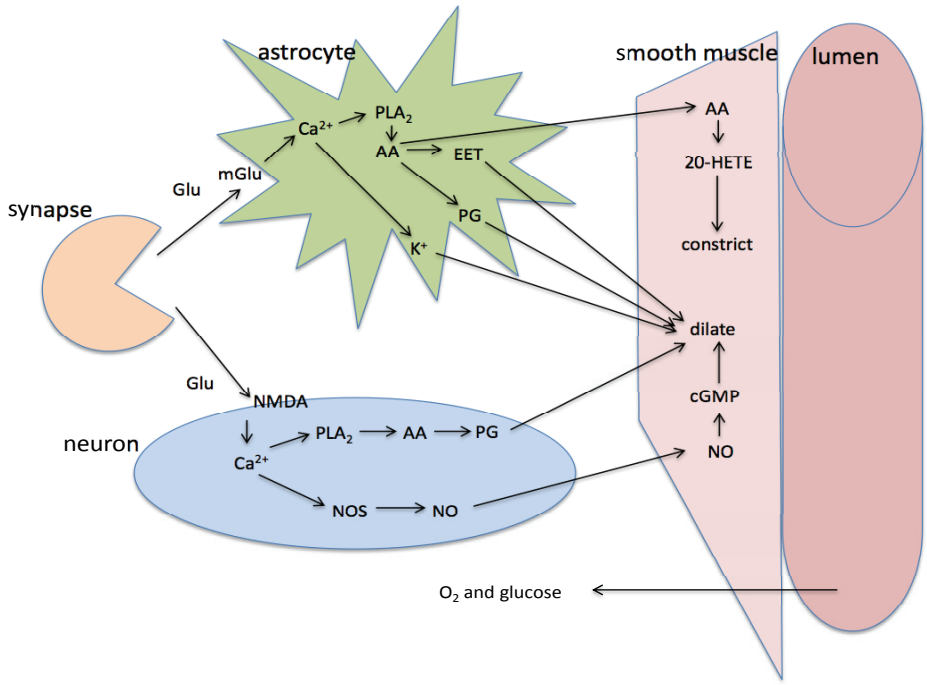


Figure 1.10. Glutamate Mediated Cerebral Blood Flow (based on Attwell and colleagues' work 2010). Synaptic Glu acts on neurons as well as astrocytes. Glu released in the synaptic cleft activates neuronal *N*-methyl-D-aspartate (NMDA) receptors, which results in an increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$). Increased levels of $[Ca^{2+}]_i$ in the neuronal cells have two effects: i. causing neuronal nitric oxide synthase (NOS) to release NO, which in turn generates cyclic guanosine monophosphate (cGMP) in smooth muscle to dilate vessels, ii. generating arachidonic acid (AA) from phospholipase A_2 (PLA_2) which is converted to prostaglandins (PGs) that dilate vessels.
Synaptic Glu also acts on metabotropic Glu receptors in astrocytes resulting in a rise of $[Ca^{2+}]_i$. Similar to the neuronal cascade, increased $[Ca^{2+}]_i$ in astrocytes generates AA and two consecutive vasodilator metabolites, PGs and epoxyeicosatrienoic acids (EETs). In addition, AA diffuses to arterioles and is converted to 20-hydroxy-eicosatetraenoic acid (20-HETE) in smooth muscle where it acts as a vasoconstrictor. A rise of $[Ca^{2+}]_i$ in astrocytes' endfeet may also activate Ca^{2+}-gated K^+ channels, releasing K^+ that in turn also acts as a vasodilator.

Apart from Glu, other neurotransmitters have also been suggested to possess vasoactive properties including: γ-aminobutyric acid (GABA) (Vaucher et al, 2000; Cauli et al, 2004; Kocharyan et al., 2008), serotonin (5-HT), dopamine (DA) (Blanco et al., 2008), and noradrenaline (NE) (Vanhoutte & Rimele, 1982; Mulligan & MacVicar, 2004). Several studies have shown that in addition to its primary role as a modulator of cortical activity, GABA released from specific subsets of interneurons also has an

important role in modulating CBF. Cortical GABA interneurons project to local microvessels and perivascular astrocytes (Vaucher et al, 2000), and are thus positioned to adjust CBF to local changes in neuronal activity (Cauli et al, 2004; Kocharyan et al., 2008). Aminergic innervations, specifically DA and 5-HT may also regulate the extent of the vasoconstrictor or vasodilator effect of Glu pathways mediated by astrocytes (Blanco et al., 2008). Noradrenaline has also been shown to act directly on blood vessels via biding to α_1 and α_2 receptors (Vanhoutte & Rimele, 1982) or to express a constrictive effect through the 20-HETE pathway (Mulligan & MacVicar, 2004).

Lastly, contractile cells located around the endothelial cells of capillaries, called pericytes, have been shown to have similar vasoactive properties as arteriole smooth muscle cells. As yet, however, there is no conclusive *in vivo* evidence regarding their importance in neurovascular coupling (Hamilton et al., 2010).

In summary, for the last 15 years there has been significant development in our understanding of the physiological correlates of the BOLD signal, which appears to be far more complex than was formerly believed. There is evidence of both origins, LFP and spiking activity, of the BOLD signal. Neurovascular coupling may also be task and region dependent. The metabolic foundation of the hemodynamic response is also multifaceted. There is support that a metabolic need generates an increase in CBF, while several neurotransmitters also seem to play a direct or indirect role in hyperemia. Indirect regulation may involve neurons and/or astrocytes. Lastly, there is evidence that the control of CBF occurs not only at the arteriole, but also at the capillary level. This area of research is still active and essential as a greater understanding of the physiological means of the BOLD signal can provide the opportunity to manipulate CBF by acting on relevant vasocative agents. This could lead to more controlled testing of brain function as well as open doors for developing new therapies. In spite of the uncertainty, fMRI remains one of the most powerful imaging techniques available today. There are over 300,000 published research articles using this technique to study brain function in diseased and healthy populations.

1.3.2.3.3. Task-Driven and Task-Free Functional Magnetic Resonance Imaging

Several fMRI designs are available for studying brain function; this work focused on two: a block design and a task-free, or resting state (RS), design. The block design is the oldest design used in functional imaging. It consists of two or more separable, alternating conditions (e.g., stimulus vs. rest) with a frequency of BOLD response around the task frequency. This design has an advantage of high statistical power, but has a limited use, as it does not answer certain research questions, for example, separating brain activation based on correct and erroneous responses.

At times, the execution of a task is hard or there is no model for testing the system of interest (e.g. sleep). In recent years there has been an increased application of brain activation at rest or resting state fMRI (RS-fMRI). At rest, brain regions exhibit spontaneous fluctuations at low frequencies (0.01–0.1 Hz) (Biswal et al., 1995). It has been demonstrated that different brain areas show temporally correlated signals, regarded as functional networks. Several functional networks have been identified using RS-fMRI (Damoiseaux et al., 2006; Buckner et al., 2008; Greiciuset al., 2009). The default mode network (DMN) is one of the most studied networks (Raichle et al., 2001). The DMN comprises the medial prefrontal cortex, the medial temporal lobe, the posterior cingulate cortex, the precuneus, and the inferior parietal cortex. It has been thought of as a brain network which is active during introspection or as a network responsible for baseline processing and information maintenance, and it is only active when an individual is not engaged in an explicit active task (Raichle et al., 2001, Buckner et al., 2008).

1.3.2.3.4. Reliability of Functional Magnetic Resonance Imaging

Reliability is fundamental in any scientific measurement. Therefore, understanding and determining reliability of the BOLD signal is critical for both scientific studies and clinical applications, such as pre-surgical mapping. Interest in the reliability of the BOLD signal has increased in recent years, and owing to studies addressing this issue, we are now aware of numerous modifiable factors influencing reliability. Some of these factors are hardware noise, MRI sequence acquisition, physiological noise, task and task design, test-retest interval, underlying cognitive process, learning, fatigue, endogenous and

exogenous vasoactive substances, data processing, and the method of reliability analysis itself, just to list a few (Bennett & Miller, 2010; Gorgolewski et al., 2013a).

At least, a hundred of studies reported BOLD signal reliability at the group level (Bennett & Miller, 2010), which is important for learning general features of the healthy or diseased brain, but clinical application of fMRI requires single-subject level assessment. Single-subject reliability poses different data analysis and interpretation challenges compared to group level reliability. While group-level analysis largely discards within-subject variances by relying on each subject's contrast maps and is thus mostly affected by inter-subject variability, single-subject analysis relies mostly on within-subject variances (i.e. on *t*-maps, which can be expressed as contrast maps weighted by errors), which is the variable of interest (Gorgolewski et al., 2013b).

There are two common methods of measuring reliability. One is the intraclass correlation coefficient (ICC, two –way model with no interaction) that measures between-subject vs. between-session (within-subject) variances, while allowing for constant between-session effects (e.g. learning). When using this computation at the group level, the determination of between-session variances can be problematic as ICC can be influenced by between as well as within-subject variances. At the single-subject level, between-subject variances become insignificant, but this method of analysis is still vulnerable to poor model fitting of the observed values in the general linear model (GLM) analysis (Caceres et al., 2009; Gorgolewski et al., 2013b). Caceres and colleges (2009) found that certain areas of the brain do not respond linearly or directly to stimuli, thereby do not follow the hemodynamic respond function. The consequence of this "off" response is a falsely low ICC reliability while the signal is relatively consistent within subjects.

The second method is the cluster overlap reliability (COR) that measures how many active voxels of the *t*-maps are obtained at different sessions at the same location. This is a simple method comparing the *t*-maps (variance of contrast maps weighted by error), which are the final product of the fMRI data analysis instead of comparing between-subject contrast maps. This is true for group as well as for single-subject level analysis (Gorgolewski et al., 2013b). Similar to the case of ICC, this method also has weak

characteristics, such as COR is greatly affected by threshold, which will be discussed in the following section of this chapter.

Bennett & Miller (2010) reviewed the body of literature addressing reliability and presented several concluding remarks. One of the main points of the review was that reliability depended on the method of evaluation. Considering all the studies included in the review, the average lowest COR reached by all studies was 0.314, the average highest was 0.670, and the grand average COR was 0.476. When single studies were examined, many of them reached COR $\geq$ 0.80. For the ICC method, the corresponding numbers (average lowest, highest and grand average among all studies) were 0.17, 0.75, and 0.50, and several studies reach ICC $\geq$ 0.80, when considered separately.

From the review, it was also clear that reliability is task and cognitive processing dependent. Simple tasks tend to have higher reliability (Bennett & Miller 2010; Gorgolewski et al., 2013a), while the comparison of task-driven with task-free fMRI results seems to be more conflicting. Kristo and colleagues (2014) found that the finger-tapping task resulted in a higher ICC compared to task-free fMRI, while Mannfolk and colleagues (2011) noted comparable results between finger-tapping task and task-free fMRI paradigm.

Test-retest intervals also seem to make an impact on reliability, as shorter intervals (days/week) tend to have a higher reliability compared to longer intervals (weeks) (Bennett & Miller 2010). The region of interest (ROI) chosen also seems to influence reliability. Gorgolewski and colleagues (2013a) found that whole brain analysis typically results in lower reliability than a single functional area, such as the motor or the auditory cortex.

1.3.2.3.5. Methods to Increase Reliability of Functional Magnetic Resonance Imaging

A variety of elements can impact reliability of the BOLD signal. Some of them are noted in the previous section. This section will address solutions that help to overcome these factors, most of which have been applied in the studies introduced in this work.

The practice effect as well as habituation can impact the variability of the BOLD signal. This has particular relevance if the task is complex or the study population is patients with cognitive impairment. To reach a task performance that is not confounded

by learning or habituation, adequate practice before each session is recommended. Similarly, the effect of learning and habituation can also change if the test-rest intervals are not the same. Testing at the same time of the day can also increase reliability, as the hemodynamic response depends on numerous vasoactive substances and hormones influenced by circadian rhythms, and cognitive performance fluctuates throughout the day (Buysse et al., 2004; Vandewalle et al., 2009; Shannon et al., 2013). Another potential method of increasing reliability is to ask participants not to drink coffee or smoke for a couple of hours prior to the assessment. Finally, reliability varies with task design, data analysis, and repetition. Repeating the scan three or four times can increase reliability by up to a factor of two to three (Maitra et al., 2002; Friedman & Glover, 2006; Friedman et al., 2008). Block design tends to elicit a more robust response compared to event-related design, and as such, produce higher reliability. Regarding data processing, motion and inconsistent or bad registration have perhaps the most detrimental effect on reliability (Gorgolewski and colleagues 2013b), but can be improved by securing the head position during the scanning session, applying post-processing motion correction, and using linear and non-linear registration techniques, respectively. Finally, visual inspection at every stage of the data analysis is very important, particularly when pipeline processing is exercised.

As discussed above, there are various techniques to estimate fMRI reliability. Two of the most commonly used methods have been introduced here while highlighting their shortcomings when single-subject reliability is in question (Gorgolewski et al., 2013b; Stevens et al., 2013). Application of a predetermined threshold has been a common practice to separate active voxels from various artifacts in the fMRI data. However, the appropriate threshold choice may differ significantly from one individual to the other. This is particularly true for patients with various degrees of CNS impairment. Further, the BOLD response can be altered by non-task related factors, discussed above, such as learning and habituation. Consequently, applying a predetermined threshold is not only arbitrary when single-subject reliability is in question, but it also hinders reliability. Recently, Stevens and colleagues (2013) introduced a data driven method (receiver operating characteristics reliability, ROC-r) for assessing the reliability vs. threshold relationship. Stevens and colleagues (2013) argued that reliability varies significantly

with the threshold and threshold can significantly vary between individuals. The method employs COR and optimization of individual thresholds instead of relying on a predetermined one, thereby trading activation magnitude for reliability. A detailed description of the method is available elsewhere (Stevens et al., 2013). In short, the algorithm calculates overlapping and non-overlapping areas between two functional maps at a time, keeping one functional map (M1) at a given threshold and comparing that with the second functional map (M2) at all thresholds, producing an ROC curve from which the area under the curve (AUC) can be calculated. This computation is repeated for all M1 thresholds, producing a plot of AUC vs. M1 threshold. The role of the maps is then reversed, providing a plot of AUC vs. M2 threshold. Using the same threshold optimization method, AUC vs. M3 threshold can be plotted as well by comparing with M1 and M2. As most of the inactive voxels are removed from the functional maps by increasing the threshold, the rate of increase of the AUC (e.g. M1 threshold) declines. To obtain a high test-rest AUC (i.e. high reliability) and to maintain sensitivity to activation, the algorithm takes both the AUC value and its rate of increase with the threshold into account (Stevens et al., 2013). A typical presentation of the ROC-r threshold maps of the three visits is depicted as the function of the AUC vs. threshold for a control participant of the current study in Figure 1.11a. Following threshold optimization for the whole brain, threshold was estimated for the created ROI masks, by registering cortical and subcortical ROIs to individual fMRI space. A typical presentation of the ROC-r functional maps of two ROIs is depicted as AUC vs. threshold for a control participant in Figure 1.11b.

a b

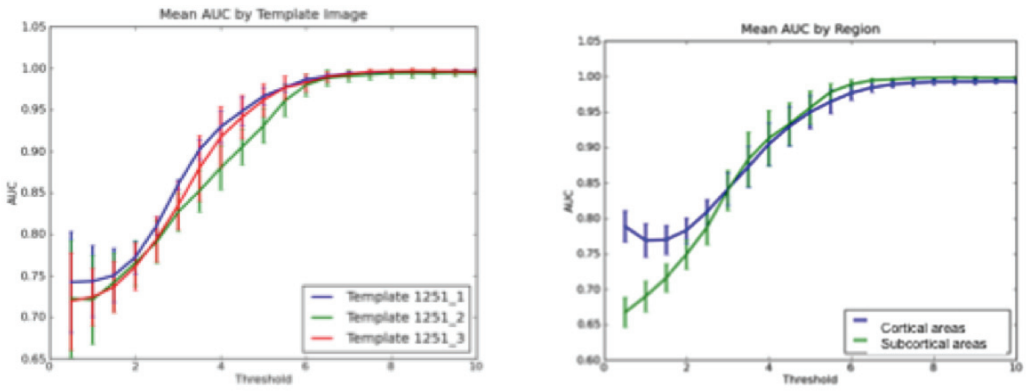


Figure 1.11. Presentation of the Receiver Operating Characteristics-Reliability Threshold Maps of the Three Visits Depicted as Area Under Curve vs. Threshold for a Control Participant. (a). Presentation of the ROC-r threshold maps of the two ROIs depicted as AUC vs. threshold for a control participant (b).

The method of Stevens and colleague (2013) has a number of advantages: i. it allows meaningful test-rest assessment of single-subject brain activation using a personalized fMRI baseline; ii. the method is compatible with group analysis; iii. the method produces task-related activation maps; and iv. the method is automated, and is thus efficient and not prone to user error.

Taking advantages of this new technique, the ROC-r analysis was also applied in the current work to increase reliability of fMRI.

1.4. Role of Magnetic Resonance Imaging in Established Multiple Sclerosis: Disease Monitoring and Prognostic Value

Studies providing evidence of the prognostic value of conventional MRI to develop definitive MS, following the initial attack, have dramatically changed the diagnosis of MS and early treatment initiation (McDonald et al., 2001; Polman et al., 2005; Charil et al., 2006; Swanton et al., 2006; Swanton et al., 2007; Montalban et al., 2009; Rovira et al., 2009).

In contrast to diagnostic applications of MRI in MS, its role in monitoring established MS is not as clear. For instance, as noted previously, associations between cognitive

dysfunction, an important marker of neurological integrity and progression in MS, EDSS, and conventional MRI are significant, though moderate at best (Rao et al., 1989b; Comi et al., 1995; Li et al., 2006; Zivadinov & Cox, 2007; MacKay et a;l., 2009; Sormani et al., 2009; Freedmand et al. 2011; Hayton et al., 2012; Lund et al., 2012; Cerasa et al., 2013). This can be explained by the limited ability of conventional MRI to characterize subclinical pathology, such as normal-appearing GM (NAGM) and NAWM (Allen et al., 2001; Zivadinov et al., 2004; Coombs et al., 2004; Narayana, 2005; Rovaris et al., 2005a; Bakshi, 2005; MacKay et a;l., 2009; Moll et al., 2011), or the adaptive compensatory neuronal rewiring that occurs throughout the course of the illness (Werring et al., 2000; Rocca et al., 2002; 2004; 2012; Roosendaal et al., 2010b; Tomassini et al., 2012[280,281]; Basile et al., 2013; Sacco et al., 2013).

Linking MRI measures and neuropsychological assessments is important to assessing and monitoring underlying brain pathology and clinical manifestation of the disorder. Advance MRI techniques (e.g. Myelin Water Imaging, Magnetization Transfer Imaging, Diffusion-Weighted and Diffusion-Tensor MRI, and fMRI) have potentials to overcome the limitations of conventional MRI, and their application in MS has been studied in the past. The following section addresses findings and challenges of conventional and advanced MRI studies in relation with cognitive impairment in MS and reviews cognitive tests applied in this work.

1.4.1. Cognitive Performance and Structural Correlates

Structural MRI correlates of cognitive performance include lesion burden, brain atrophy, and diffuse brain damage. Studies have noted correlation with lesions in the frontal lobe and performance on executive functions (Swirsky-Sacchetti et al., 1992; Arnett et al. 1994), lesions in the parietal-occipital lobes and deficits in both verbal learning and complex visual-integrative skills (Swirsky-Sacchetti et al., 1992), lesions in the U-fibers and performance in executive control and memory (Miki et al., 1998), and correlation with posterior fossa lesion volume and slowed information processing (Archibald et al., 2004). Further, accumulation of cortical lesions over a period of three years has been shown to be predictive of cognitive performance (Roosendaal et al., 2009; others found this predictor value less reliable (Filippi & Rocca, 2007; Zivadinov & Cox,

2007). With respect to overall brain atrophy, several studies have demonstrated that processing speed deficit is more correlated with whole or regional volume loss than lesion burden (Rao et al., 1989b; Bermel et al., 2002; Benedict & Zivadinov, 2006; Lazeron et al., 2006; Houtchens et al., 2007). In addition, a recent follow-up study has demonstrated that the most significant predictors of cognitive deteriorations were diffuse brain damage, as well as global and central brain atrophy (Deloire et al., 2011).

1.4.2. Cognitive Performance and Correlates of Advanced Magnetic Resonance Imaging

Advanced structural MR techniques available for the study of MS pathology, including structural and functional MR imaging, have provided new markers of disease activity and progression. Both, cross sectional and longitudinal studies using double inversion recovery sequence and voxel based morphometry demonstrated the formation of new cortical lesions and lesion burden in RRMS associated with progression of disability and severity of cognitive impairment. (Benedict et al., 2006; Calabrese et al., 2010b,c; Prinster et al., 2010). Further, recent research suggests that deep GM abnormalities may explain physical and cognitive decline in patients with MS better than WM abnormalities can (Rovaris et al., 2008b; Geurts et al., 2012). Another longitudinal study found that GM atrophy rate increases with disease stage, increasing from 3.4 times the normal (healthy control) rate in CIS patients who convert to RRMS, to 14 times the normal rate in SPMS (Fisher et al., 2000; 2008).

Abnormalities of water distribution in NAWM have been demonstrated by different advanced imaging techniques assessing myelin cohesion (Laule et al., 2004; Vavasour et al., 2009; Preziosa et al., 2011; Mazerolle et al., 2013b), including Myelin Water Imaging (MacKay et al., 1994) and Diffusion Tensor Imaging (Basser et al., 1994). These abnormalities seem to be associated with disease activity (Laule et al., 2004; Vavasour et al., 2009) and with motor and cognitive disability (Preziosa et al., 2011). Further, abnormalities in water diffusion appeared to be reflective of the disease stage, notably, they have been found to be greatest in SPMS and least in benign MS (Preziosa et al., 2011). A follow-up study reported that changes in GM magnetization transfer ratio after 12 months were a good predictor of accumulation of long-term cognitive disability

(Filippi et al., 2013b). A recent study has noted significant relations between reduced integrity of WM microstructure and intra-individual variability in information processing speed. Further, the authors concluded that intra-individual variability might be a more sensitive measure to the overall burden of microstructural WM than measuring mean-level performance (Mazerolle et al., 2013b).

Several studies applied fMRI to investigate functional changes in MS in response to various task-driven paradigms probing different functional systems. The main conclusions of these studies were: i. the pattern of activation (i.e. cortical reorganization and over recruitment) is different depending on MS type; ii. functional changes have a specific pattern over the course of the disease; and iii. there is a relationship between the extent of functional activation and the extent of global and regional tissue damage in MS (Filippi & Rocca, 2009).

Following tissue damage, the brain is able to self-repair by remyelination and cortical reorganization to compensate for some of the damage (Vavasour et al., 2009). Cortical reorganization takes place typically at the early stage of the disease (Werring et al., 2000; Staffen et al., 2002; Rocca et al., 2002; 2007; 2012; Au Duong et al., 2005a,b; Filippi et al., 2005; Loitfelder et al., 2011), but it has been also observed in the progressive phase (Rocca et al., 2012). The degree of tissue injury in NAGM, NAWM, in WM the tract, such as the CC, as well as the number of T_2 lesions seem to have a modulating role in cortical reorganization as well as in increased brain recruitment (Reddy et al., 2000; Rocca et al., 2002; 2007; Lenzi et al., 2007). Increased brain recruitment also accompanies altered functional connectivity (Werring et al., 2000; Rocca et al., 2002; 2012, Filippi et al., 2009; Roosendaal et al., 2010a,b; Loitfelder et al., 2011; Basile et al., 2013; Sacco et al., 2013), and similarly to the cortical reorganization phenomenon, overactive brain response is typically present at early stage (Loitfelder et al., 2011; Forn et al., 2012; Rocca et al., 2012), and have been hypothesized to be the result of an adaptive compensatory mechanism that maintains cognitive performance (Audoin et al., 2003; 2006; Roosendaal et al., 2009; Rocca et al., 2012; Basile et al., 2013; Sacco et al., 2013).

Patients exhibit altered and overactive brain response that supports intact cognitive performance by relying on additional neuronal networks (Loitfelder et al., 2011; Forn et

al., 2012; Rocca et al., 2012). Bonnet and colleagues (2010) reported recruitment of high cortical association areas in patients with MS compered to controls who simply relied on the cerebellum to perform Go/No-go task.

With progression of MS, more areas are being recruited to maintain an adequate level of processing (Loitfelder et al., 2011; Rocca et al., 2012) and at the later (progressive) stage of MS, when the brain is no longer able to compensate for brain tissue damage, cognitive performance starts to decline (Rocca & Filippi 2007; Rocca et al., 2010b,c; Loitfelder et al., 2011). Interestingly, fatigue has been associated with reduction in cortical activation and poorer performance, suggesting that fatigue has a similar effect as a reduction in brain compensatory mechanisms (Filippi et al., 2002).

1.4.3. Testing Cognitive Performance

As noted previously, the heterogeneity of the pathology in MS results in variable presentations of cognitive deficits, however, a core deficit in MS is the slowing of information processing (Pelletier et al., 2001; Chiaravalloti & DeLuca, 2008; Filippi & Rocca, 2010d; Bonzano et al., 2011a,b).

1.4.3.1. Symbol Digit Modalities Test

Although several cognitive paradigms have been incorporated into neuropsychological batteries, the SDMT (Smith, 1982) shows the most robust results in evaluating information processing speed and working memory (Nocentini et al., 2006). The test measures the time to pair abstract symbols with specific numbers capitalize on elements of attention, visuoperceptual processing and psychomotor speed. The original version of the SDMT has a strong test-rest reliability score (r = .70 to .91) for six-year interval (Smith, 1991), while reliability scores of alternative versions reported to be exceeding r = .80 (Benedict et al., 2008). Substandard score of the SDMT has been shown to be an early marker of general cognitive dysfunction (Deloire et al., 2006, Parmenter et al., 2007a,b), to be sensitive to cognitive decline (Benedict et al., 2008; 2012; Drake et al., 2010), to be a valuable measure of cognitive decline in daily activities (Morrow et al., 2010), and to be a predictor of employment status (Strober et al., 2012). Further, it has been validated for the use in fMRI in MS (Forn et al., 2009; Akbar et al.,

2011). With regards to neuroradiological measures, performance in the SDMT showed strong correlation with brain lesions (Benedict 2005a,b; 2006a; Stankiewicz et al., 2011), atrophy (Lazeron et al., 2005; 2006), and diffusion tensor indices of NAWM (Warlop et al., 2009; Mazerolle et al., 2013b).

1.4.3.2. Poffenberger Task

To investigate interhemispheric processing, several paradigms have been developed. Perhaps the oldest one is the Poffenberger Task (Poffenberger, 1912; Marzi, 1999). The paradigm consists of unimanual response to lateralized visual stimuli presented at the same and the opposite hemifield and entails visuomotor interhemispheric transfer through the corpus callosum (CC). Beyond to be valuable for investigating the basic function of the CC, measuring information speed processing has been demonstrated previously (van der Knaap & van der Ham, 2011).

Slowing in information processing can also result from compromised transcallosal conduction, which has been reported in MS (Rao, 1989a; Barkhof et al., 1998b, Pelletier et al., 2001; Mesaros et al., 2009; Brown et al., 2010; van der Knaap & van der Ham, 2011). The CC is the largest cerebral commissure connecting the left to the right brain hemisphere, and its basic function is transferring information between the two brain hemispheres. It plays an important role in the organization of complex commands involving bilateral tasks with precise timing of information transfer between halves of the brain (Bloom & Hynd, 2005). As is noted above, the CC is a common target in MS, frequently showing focal demyelinating lesions and atrophy since early stages of the disease (Barkhof & Scheltend, 2002; Coombs et al., 2004; Traboulsee et al., 2005; Traboulsee & Li, 2006, 2008). Callosal lesions are seen in 55% to 93% of MS patients (Simon et al., 1986), consequently, it is not surprising that dysfunction in interhemispheric processing has been documented in MS. In particular, associations between severity of CC atrophy, focal lesions, diffuse fibre bundle injury, EDSS score, interhemispheric processing measures (i.e. reaction time, and accuracy have been noted in MS patients (Barkhof et al., 1998a,b; Rao 1989a,b; Pelletier et al., 2001; Brown et al., 2010). The extent of CC damage has also been associated with cognitive impairment in benign MS (Mesaros et al., 2009). Though it has not been validated in MS specifically,

taking this information together, the Poffenberger Task might be an informative task to assess information processing speed in MS.

Interestingly, most of these data stem from MRI studies measuring atrophy or microstructural integrity of the WM expressed by water diffusion in the CC, but there is scarce evidence originating from fMRI studies permitting direct assessment of the function of the CC (Gawryluk et al., 2011a).

1.4.4. Cognitive Performance and Baseline Brain Activity

There is an increasing interest in the application of RS-fMRI to explore changes in functional connectivity of the brain associated with cognitive performance in MS (Audoin et al., 2006; Cader et al., 2006; Rocca et al., 2010c; Bonavita et al., 2011; Forn et al., 2012). Similar to task-driven fMRI response, altered connectivity within functional areas, broader brain region recruitment, and furthermore, inability of disengaging the DMN have been reported (Filippi et al., 2013b; Basile et al., 2013; Roosendaal et al., 2010b; Sacco et al., 2013). As it has been noted, RS is characterized by synchronization of neuronal oscillation within areas serving the same functional network. Synchronization can be increased as well as decreased in MS depending on the interaction between brain tissue damage and the ability of the brain to repair itself (Esposito et al., 2006; Fox & Raichle, 2007). Previous studies concluded that altered connectivity between functional areas and overactive brain recruitment seen in CIS and in RRMS disappears later on as the disease progresses (Roosendaal et al., 2010b; Basile et al., 2013; Sacco et al., 2013). Furthermore, greater RS connectivity within the DMN has been associated with more stable performance on processing speed task (Wojtowitcz et al., 2014). These observations suggest that cortical reorganization and overactive brain recruitment may be a marker of early MS, while the lack or the decreased functional connectivity suggests more severe brain pathology and symptoms in progressive stages (Roosendaal et al., 2009; Rocca et al., 2010b,c).

Relationship between RS connectivity and cognitive impairment has been the focus of several studies. Among the various discovered RS neuronal networks, the DMN has received the most attention (Rocca et al., 2010a,b,c; Bonavita et al., 2011; Loitfelder et al., 2012) and a potential role of RS connectivity of the DMN in cognitive rehabilitation

has been suggested. Following cognitive rehabilitation, associations between increased connectivity of the DMN network and improvement in cognitive performance as well as in severity of depression have been reported in several studies (Leavitt, 2012; Parisi et al., 2012; 2014). Additionally, dysfunction of the DMN at the level of the anterior cingulate cortex has been proposed to be specific to MS (Rocca et al., 2010c; Bonavita et al., 2011).

In addition to recruiting more brain areas during rest, patients have also shown a deficit in engaging or/and disengaging the DMN (Loitfelder et al., 2012; Forn et al., 2013b; Sacco et al., 2013). As is noted above, the DMN is only active during rest, and it is switched off when the individual actively engages in a task. Inability to switch the DMN on or off has been shown in several other neurologic and neuropsychiatric disorders, such as Alzheimer disease (Buckner et al., 2008), autism (Kennedy et al., 2006; Buckner et al., 2008), and attention deficit disorder (Liddle et al., 2011), and it has been linked to hindered cognitive performance.

In the same way as functional connectivity, the ability of turning on and off the DMN seems to be disease state dependent. Previous studies reported that patients with CIS exhibited an enhanced deactivation of the posterior cingulate gyrus (part of the DMN) in comparison to healthy controls during rest periods (Forn et al., 2012), whereas patients with RRMS had decreased deactivation compared to controls, but much stronger deactivation than patients with SPMS (Petsas et al., 2013). These results suggest that at early state of MS, patients show an enhanced pattern of brain deactivations during cognitive performances to facilitate normal neuropsychological status, which disappears as the disease progresses (Forn et al., 2012; Petsas et al., 2013).

1.4.5. Application of Functional Magnetic Resonance Imaging in Monitoring Patients with Multiple Sclerosis

Although its application is still limited, there is evidence for the value of fMRI in monitoring patients with MS. Sensitivity of the fMRI to detecting treatment effects was reported by a few studies (Parry et al., 2003; Mainero et al., 2004; Morgen et al., 2004; Filippi & Rocca, 2010c; Parisi et al., 2012). One study showed a relative normalization of abnormal Stroop task- associated brain activation when compared pre- and post-treatment

with rivastigmine, an acetylcholinesterase inhibitor used in MS (Parry et al., 2003). Another study showed an increase in activation of the ipsilateral sensorimotor cortex and supplementary motor area during a simple finger-tapping task in patients receiving 3,4-diaminopyridine compared to those receiving a placebo (Mainero et al., 2004). Others reported improvement in attention and information processing and an increase in functional connectivity at the anterior cingulate cortex in response to several month long cognitive training (Parisi et al., 2012). Finally, there is also evidence for a normalised cortical recruitment following motor rehabilitation in established MS (Morgen et al., 2004).

1.5. Objectives of the Study

Diagnosis of MS has advanced since the early 2000's, particularly with regard to early diagnosis, detection of individuals at risk of developing definitive MS. However, using conventional MRI does not adequately allow for monitoring established MS, as it has low sensitivity to characterize functional compensation due to clinical and subclinical CNS pathology. Given its sensitive nature to changes in brain function, fMRI may convey valuable information regarding monitoring the disease, treatment response, and rehabilitation. Slowing in information processing is a common and early manifestation of MS that has been linked to structural and functional changes of the brain, and therefore, its application with fMRI may allow monitoring early pathological changes and response to treatment.

Previously, there have been attempts to use fMRI to monitor the disorder. However, reliability of fMRI has not been addressed in MS. Previously, satisfactory fMRI reliability has been reached, but mainly in healthy population with stable cognitive performance. On the other hand, patients with MS have been shown to have high intra-individual variability in cognitive performance, which can impact BOLD signal reliability. Further, most of fMRI test-retest reliability studies have focused on group reliability, but not on individual reliability, which is essential to establish for clinical care.

This manuscript-based thesis attempts to carry out the first single-subject fMRI reliability studies in MS. In order to increase our reliability value, we have applied a new

method; the ROC-r analysis (Stevens et al., 2013) that improves reliability of the single-subject fMRI. As reliability task dependent, we have chosen two fairly simple task-driven and one task-free fMRI paradigms with relevance in MS. Notably, the two task-driven paradigms are the SDMT and the Poffenberger Task that probe slowing in information processing speed (Nocentini et al., 2006; van der Knaap & van der Ham, 2011). The task-free paradigm is the RS-fMRI with attention to the DMN.

To interpret our finding better, we have examined fMRI reliability in patients with RRMS using EDSS score as a marker of clinical disability and compared the results to those acquired from age-matched controls.

CHAPTER 2

EXAMINING SINGLE-SUBJECT RELIABILITY OF FUNCTIONAL MAGNETIC RESONANCE IMAGING IN ESTABLISHED MULTIPLE SCLEROSIS: THE SYMBOL DIGIT MODALITIES TEST AND THE POFFENBERGER TASK

2.1. Introduction

Disease monitoring requires higher sensitivity for subtle neuropathological and compensatory changes (Werring et al., 2000; Laule et al., 2004; Rocca et al., 2004; 2012; Filippi et al., 2005; 2013a,b; Vavasour et al., 2009; Roosendaal et al., 2010b; Tomassini et al., 2012[280,281]; Vigeveno et al., 2012; Basile et al., 2013; Sacco et al., 2013). Functional MRI using neuropsychological tests probing the dysfunction present in MS, such as information processing speed (Rao et al., 1991b; Pelletier et al., 2001; Amato et al., 2001b; 2010a,b; Chiaravalloti & DeLuca, 2008; Filippi et al., 2009; Bonzano et al., 2011a,b), and underlying compensatory mechanism may provide valuable insight into disease progression or response to treatment. In the past, there have been a few attempts to monitor treatment or rehabilitation of MS symptoms using fMRI (Parry et al., 2003; Mainero et al., 2004; Morgen et al., 2004; Filippi & Rocca, 2010a), but reliability of this technique has not been evaluated in MS. Further, there is evidence of considerable intra-individual variability in cognitive performance and underlying brain pathology in patients with MS (Bodling et al., 2012; Wojtowicz et al., 2012; 2013, 2014), which may influence fMRI test-retest reliability. Therefore, reliability of fMRI derived from studies examining healthy controls and other patient populations may not be generalizable to MS patients.

In addition, the majority of the work completed on reliability of fMRI were group-level studies, while single-subject fMRI reliability, critical for clinical application, has received much less attention. There are several ways to increase fMRI reliability (Bennett & Miller, 2010). Our research group has shown that applying individualized threshold (ROC-r analysis) can also increase reliability of the fMRI and provide meaningful test-rest assessment of single-subject brain activation (Stevens et al., 2013).

While several tests probe information-processing speed, existing literature suggests that the SDMT (Smith, 1982) could be the best measure of choice (Deloire et al., 2006; Parmenter et al., 2007b; Benedict et al., 2008; 2012; Forn et al., 2009; 2013a; Drake et al., 2010; Akbar et al., 2011). Along with the SDMT, we also wanted to include the Poffenberger Task (Poffenberger, 1912) that can be used to measure transcallosal activity affecting information processing speed (van der Knaap & van der Ham, 2011). As advanced imaging studies confirmed that neurological disability in MS measured by the EDSS are likely the consequence of both, apparent and normal appearing damaged tissue (Rovaris et al., 2000; 2005a,b; 2008a; Laule et al., 2004; Dalton et al., 2005; Agosta et al., 2006; Fisniku et al., 2008a,b; Vavasour et al., 2009; Laule et al., 2010; 2013; Calabrese et al., 2011a,b,c; 2010a; 2012), and it may affect COR, we also included EDSS scores in our analysis.

Although there is ample of evidence that GM pathology plays a crucial part in MS symptomology (Fisher et al., 2000; 2008; Bo et al., 2003; 2006; Chard et al., 2004; Dalton et al., 2005; Agosta et al., 2006; Rovaris et al., 2006; Calabrese et al., 2011a,b,c; 2010a; 2012; ; Vavasour et al., 2009; Laule et al., 2010; 2013; Geurts et al., 2012), the RRMS is still considered primarily a WM disease. To separate the two tissue types and enhance COR score by creating smaller ROIs, reliability of the fMRI was measured in two regions, 'cortical' and 'subcortical' ROIs, mainly consisting of GM and WM, respectively.

Reliability is a central characteristic of every clinical tool thus, the main goal of this study was to test and compare single-subject fMRI reliability in patients with MS and age-matched controls in response to the SDMT and the Poffenberger Tasks.

2.2. Hypotheses

With regards to the imaging results, our hypotheses were similar to the general hypotheses. We hypothesized: i. COR would be higher for the control group compared to the patient group due to the greater intra-subject variability within the MS group, and these differences would be particularly obvious when the 'subcortical' region is considered; ii. EDSS functional scores would correlate with COR values in patients with

MS.; and iii. patients would recruit larger brain areas (sign of inefficiency) to execute both, the SDMT and the Poffenberger Task, compared to the control group.

With regards to the behavioral results, we expect that i. patients would show slowing in information processing speed compared to the control group (i.e. longer reaction time and lower timed accuracy).

2.3. Methods

2.3.1. Participants and Procedures

2.3.1.1. Participants

Fifteen participants with clinically definite RRMS (14 females, one male) were recruited through the MS Society of Canada, Atlantic Division of the Self- Help Network and the Multiple Sclerosis Research Unit – Dalhousie University, Halifax, Nova Scotia. Participants with MS had the inclusion criteria: i. no experience of a relapse or a worsening of clinical symptoms during the study; ii. an EDSS score (Kurtzke, 1983) of 6 or less to ensure an ability to get in and stay still in the scanner; and iii. since mood disorders and pain affect the performance, participants with a history of depression or anxiety and those with chronic pain, such as arthritis, were included in the study only if they were not experiencing active symptoms in the clinical range. Due to changes in disease status, 4 patients had to be excluded or not re-invited to complete the study.

The remaining patients had an age range of 37-58 years with a mean age of 51.0 years and with years of education of 15.55 ± 3.35. One patient was left-handed and two were smokers. The mean duration of illness and treatment was 9.718 ± 6.19 and 7.91 ± 4.61 years, respectively. The average EDSS score was 3 ranging from 1 to 5.5. Everyone from the patient group was on disease modifying medication.

The control group consisted of 6 participants without MS matched for age and education. The age range for the control group was of 42-52 years with a mean age of 47.57 years, while the mean of years of education was 18.837 ± 3.57. One control was left-handed and a smoker. The two study groups did not differ in age ($F = 1.123$, $p = 0.306$) and education ($F = 3.757$, $p = 0.072$).

2.3.1.2. Procedures

All participants attended three scanning sessions approximately a week apart, at the same time of the day. Every visit started with safety screening, followed by practicing all cognitive tasks presented through the fMRI sessions to minimize the learning effect across the three visits. Participants were asked not to take caffeine or smoke four hours before the tests. If needed, vision was corrected with MRI compatible goggles (Safe Vision Inc.). Hand dominance was determined by the Edinburgh Handedness Inventory (Oldfield, 1971). All scanning sessions started with the functional runs (i.e. SDMT and the Poffenberger Tasks), followed by acquiring the structural images. For anatomic reference, registration, and tissue segmentation purposes, T_1 weighted structural scans were acquired at the first visit. To ensure stable lesion load for patients as well as to check for presence of lesions for controls, spin-echo FLAIR sequences were collected at the first and at the last visits from the patient group and only at the first visit from the control group. To minimize head motion, foam pads were placed around the head. The study was approved by the local ethics boards. Each participant provided written informed consent prior to their participation.

2.3.2. Imaging Sequence

The data were collected using a 4T Oxford magnet with a Varian INOVA console. A body coil provided gradients (Tesla Engineering Ltd.) and a transverse electromagnetic head coil was used to transmit and receive the signal (Bioengineering Inc.). Acquisition for the functional runs was the following; two shots, repetition time volume (TRvol) = 2s (SDMT) and 3s (Poffenberger), echo time (TE) = 15s (SDMT) and 30s (Poffenberger), flip angle = 60°, 64 x 64 matrix, readout direction = 240, phase direction =240, number of slices =31, slice thickness = 2.9mm, gap = 0.3mm, volume (number of images taken) = 192 (SDMT) and 103 (Poffenberger). For anatomic reference, registration, and tissue segmentation, high resolution structural images were collected using a 3D magnetization prepared FLASH sequence (TR /TE = 10/5ms, flip angle = 11°, matrix 200 x 200, 240 x 240 x 135mm3 FOV, gap = 0, volume 1). To assess WM lesions, FLAIR sequences were collected (TR /TE/ TI = 12s/90ms/2.55s, matrix 256 x 256, 240 x 240 x 35mm3 FOV,

slice thickness =3 mm, gap = 0.3, volume 1). All images were acquired on the axial plane.

2.3.3. Experimental Design

All the stimuli were displayed on E-Prime 2 software (Psychological Software tools, Inc.), back-projected onto a screen mounted inside the magnet bore, and viewed through a mirror mounted on the head coil. The sequence order remained the same throughout the study.

The first functional run was the Poffenberger Task (Poffenberger, 1912) using a block design, 10 (12s long) blocks with 8 stimuli/block, alternated with 18s long rest blocks. In this task, checkerboard stimuli appear randomly to the left and right visual fields. Instructions were given prior to each block indicating whether the responses were to be made with the same or opposite hand in reference to the side of the visual stimulus. Varying the response hand in this way created two different conditions: crossed (for example, left visual hemifield stimulation requiring right hand responding) and uncrossed (for example, left visual hemifield stimulation requiring left hand responding). Each block of stimuli contained only one condition, and the block order was randomized.

The second functional task was a visual version of the SDMT (Forn et al., 2009; 2011; 2013_a). The stimulus consisted of a legend containing a sequence of 9 symbols, 9 matching numbers, ranging in natural order (1-9), and one symbol-number combination (Figure 2.1.). The stimuli were presented in random order from 60 different cues. Participants were asked to indicate whether they found the presented symbol-number combination in the legend ("match") or not ("not a match") using a hand-held response pad. The adapted version of the SDMT consisted of 9 (16s long) and 8 (30s long) altering rest and active blocks, yielding a time of approximately 5 minutes per task. The active blocks consisted of 12 2s long stimuli.

For both tasks, during the resting blocks, participants were asked to fixate on a cross presented in the center of the screen. Prior to the tasks, participants were instructed to be as accurate and fast as possible. Response timed accuracy (and reaction time were recorded.

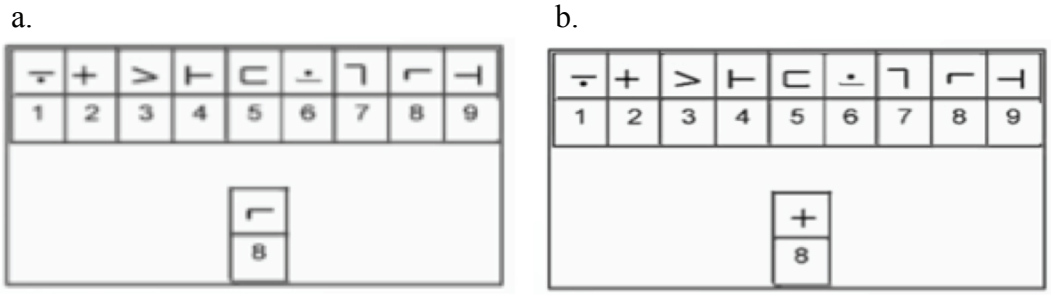


Figure 2.1. Stimuli of the Symbol Digit Modalities Test are Represented in the Picture; Conditions 'Match' (a) and 'Not Match' (b).

2.3.4. Functional Magnetic Resonance Imaging- Individual Level Analyses

2.3.4.1. Region of Interest Selection

At an individual level, region of interest (ROI) analysis was completed using the FreeSurfer software program (Martinos Center for Biomedical Imaging). This software was prepared to measure various morphometric properties of the brain including, 'cortical' and 'subcortical' regional volumes and surface thickness. The tissue classification technique used in this software employs a registration procedure that is sensitive to anatomical variability associated with neurological diseases, such as ventricular enlargement, and it is comparable with manual segmentation (Fischl et al., 2002; 2004). We used this software to segment T_1 weighted individual anatomical images into 'cortical' GM and deep WM, and these ROIs were subsequently inflated in order to cover most of the brain. The inflated GM 'cortical' and deep WM 'subcortical' areas for a representative participant are shown in Figure 2.2. Note that the 'cortical' areas included mostly, but not exclusively 'cortical' GM, while the 'subcortical' areas consisted of mostly, but not exclusively WM tracts.

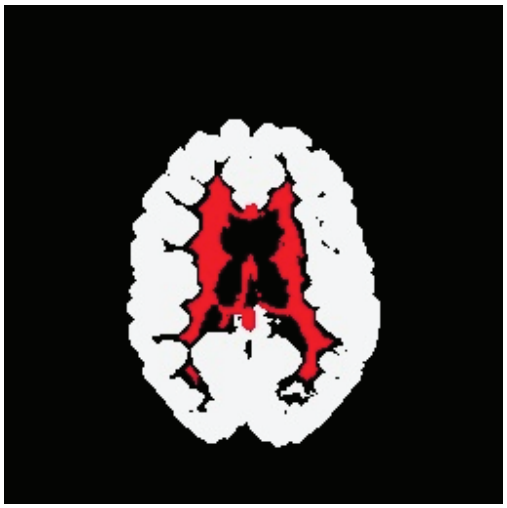


Figure 2.2. Inflated Grey Matter Areas 'Cortical' Region of Interest and Deep White Matter Areas 'Subcortical' Region of Interest for a Representative Participant.

2.3.4.2. Preprocessing and General Linear Model Analyses

Preprocessing was performed with the fMRI expert analysis tool (FEAT) version 6.0 in FMRIB Software Library (FSL; Smith et al., 2004). Pre-statistics processing included the following steps: motion correction using Statistical Parametric Mapping (SPM; Worsley & Friston, 1995), non-brain tissue removal using Brain Extraction Tool (BET, Smith 2002_{262}), mean-based intensity normalization, high-pass temporal filtering (0.01 Hz), and spatial smoothing using a Gaussian kernel, 5mm full width at half maximum (FWHM). Additional to motion correction, volumes (number of images taken during the functional scan) exceeding 2mm displacement were removed to minimize motion related artifacts.

Single-subject statistical analyses were carried out using FMRIB's Improved Linear Model (FILM) with local autocorrelation correction (Woolrich et al., 2001). This model fit the signal changes observed in the data more closely than the standard hemodynamic response function. Activation was modeled by using the custom input (3 column format) waveform and convolving a boxcar function representing the task with the double gamma hemodynamic response function in FEAT. Statistical maps were calculated with and without thresholding. Thresholded *Z*- statistic images were obtained to analyze group data using a threshold for clusters determined by $Z > 2.3$ and a corrected for multiple comparisons cluster significance threshold of $p < 0.05$ (Worsley et al., 1992; 2002).

While non-thresholded *t*-statistical maps were obtained in preparation for the ROC-r analysis (Stevens et al., 2013). To co-register the functional and the structural images using 6 degrees of freedom (DOF) and normal search function, we used FLIRT (Jenkinson & Smith, 2001; Jenkinson et al., 2002). The registrations were inspected visually to ensure quality. Registration of a representative individual is depicted in Figure 2.3. Note that most of the cerebellum is not included in the functional image.

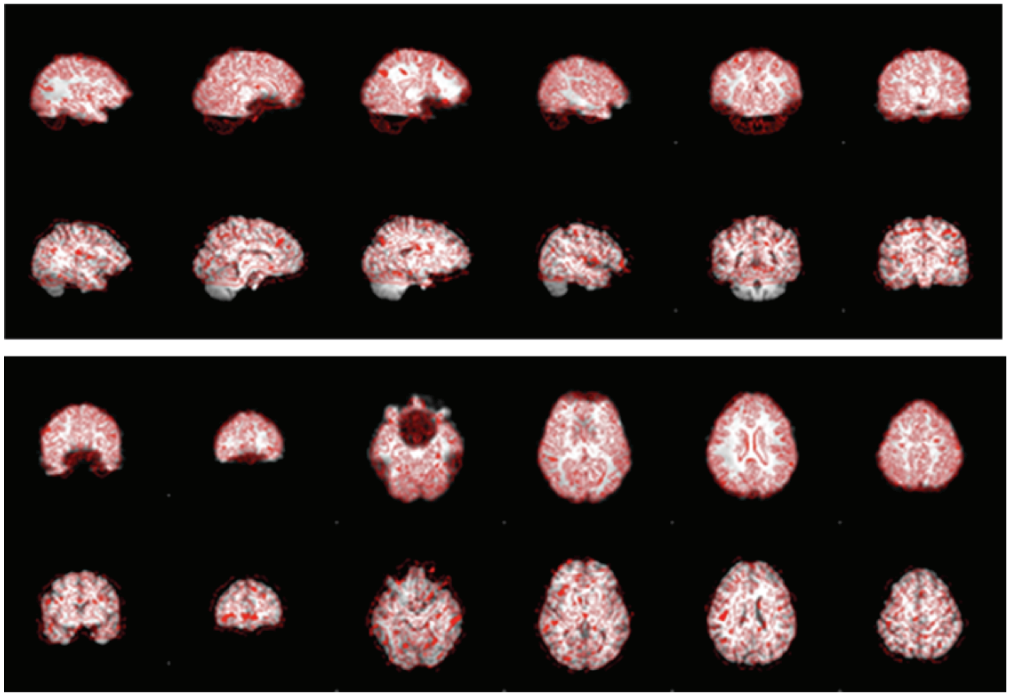


Figure 2.3. Registration of a Functional Image to a Structural High-Resolution Image at Individual Level.

2.3.4.3. Receiver Operated Characteristic Reliability Analysis

To optimize the threshold for individual functional maps, ROC-r analysis was completed (Stevens et al., 2013). Please see the description of method in chapter one. Stevens and colleagues (2013) argued that reliability varied significantly with changing the threshold and threshold can considerably vary between individuals. Stevens and colleagues (2013) showed that using ROC-r analysis increased reliability remarkably at individual levels.

2.3.4.4. Cluster Overlap Estimation

To estimate the number of overlapping voxels of the maps, the thresholded registered maps were binarized (value of 1 for voxel with signal, 0 for voxel without signal), and two maps at a time were compared. Thus, active voxels had a value of 1, inactive voxels had a value of 0, and overlapping voxels had a value of 2. Overlap coefficients were calculated by the Rombouts method (Rombouts et al., 1997) expressing reliability measures with the following calculation: COR = 2 x overlap / (M1 + M2).

2.3.5. Functional Magnetic Resonance Imaging- Group Level Analyses

2.3.5.1. Region of Interest Selection

Group level analyses of the functional maps were obtained by considering the whole cerebrum, the thalamus, and most of the pons.

2.3.5.2. Group-Level General Linear Model Analyses

To gain more representative group *Z* statistical maps, individual functional maps from the three visits were first averaged and then fed into FSL higher level analysis program, FMRIB's Local Analysis of Mixed Effects stage 1 and 2 (FLAME 1 and 2; Beckmann et al., 2003; Woolrich et al., 2004a,b; Woolrich, 2008). This statistical model allows for estimating the true random-effects variance and DOF at each voxel. *Z* statistic images were reported using a threshold for clusters determined by $Z > 2.3$ and a (corrected) cluster significance threshold of $p < 0.05$ (Worsley et al., 1995). Registration was completed in two steps. First, individual functional maps were registered to individual structural images (DOF = 6) and, secondly, these registered images were then put into a common space (Montreal Neurological Institute template, DOF = 12). All registration steps were completed using FLIRT (Jenkinson & Smith, 2001; Jenkinson et al., 2002). Group activation map differences were obtained by contrast analyses using FEAT.

2.3.5.3. Statistical Analyses of the Behavioural Data

Behavioural and imaging data were analyzed using SPSS version 15.0.1 (SPSS Inc.). A one-way analysis of variance (ANOVA) was completed to check for differences in age and education between the groups. Behavioral measures, including reaction time and

timed Acc were compared within and between groups using repeated General Linear Model (GLM) analyses and COR of 'cortical' and 'subcortical' regions were determined using the same method. Association between COR of patients and EDSS scores were also compared by regression curve fitting analysis by computing the average COR for 'cortical' and 'subcortical' areas of the SDMT and the Poffenberger Task (i.e. collapsing sessions) and treating the categorical values (EDSS) as the independent, while the continuous values (COR) as the dependent variables. On account of the unequal sample size between the two groups, homogeneity of variance (Leven's test) or Mauchly's Test of Sphericity of the data was tested and upon violation of these assumptions, a random sample selection was used to adjust for unequal sample size.

2.4. Results

2.4.1. Imaging Results

2.4.1.1. Individual Reliability

Individual COR of the 'cortical' and 'subcortical' areas are represented in Tables 2.1. and 2.2. for both tasks. Note that the COR is expressed comparing two functional maps at a time. Figures 2.5. and 2.6. show the functional maps of the SDMT and the Poffenberger Task for a representative individual, respectively.

Comparing functional maps elicited by the SDMT revealed no difference in COR of the 'cortical' areas ($F = 0.916$ $p = 0.356$), but a statistically significant difference was seen for 'subcortical' regions ($F = 11.616$, $p = 0.005$) when between measures (patients vs. controls) were examined. Cluster overlapping reliability did not change significantly over the three visits when 'cortical' ($F = 0.196$, $p = 0.666$) or when 'subcortical' areas ($F = 0.224$ $p = 0.664$) were measured.

The Poffenberger Task resulted a statistically significant difference when comparing COR of 'cortical' ($F = 14.956$, $p = 0.002$), while there was no difference between 'subcortical' ($F = 2.999$, $p = 0.144$) regions. It is important to note however, that only 7 out of 11 patients and 5 out of 6 controls had activation in 'subcortical' areas. Similarly to the SDMT task, COR did not change significantly over the three visits when 'cortical' ($F = 0.255$, $p = 0.621$) and when 'subcortical' areas ($F = 0.224$ $p = 0.664$) were measured.

Cluster overlap reliability values were treated as missing values when a map was excluded. While COR = 0 describes cases when activation was observed in only one of the ROI maps or activation was seen in both of the ROI maps, but active clusters did not overlap. We included all the data for all the statistical analyses of both tasks, as sphericity was not violated.

Table 2.1. Individual Cluster Overlap Correlation Coefficients of the 'Cortical' and 'Subcortical' Region of Interest for the Group with MS and for Controls Elicited by the Symbol Digit Modalities Test

MS group	ID	Cortical			Subcortical		
		Visits			Visits		
		1 vs. 2	1 vs. 3	2 vs. 3	1 vs. 2	1 vs. 3	2 vs. 3
	1112	0.513	0.558	0.629	0.485	0.483	0.638
	1113	0.584	0.454	0.923	0.099	0.087	0.39
	1119	0.654	0.582	0.593	0.486	0.383	0.413
	1222	0.613	0.687	0.733	0.378	0.385	0.462
	1204	0.538	0.509	0.715	0.208	0.222	0.444
	1231	0.335	0.663	0.536	0	0.237	0
	1232	0.397	0.438	0.442	0.171	0.181	0.070
	1233	0.633	0.449	0.5	0.083	0	0
	1234	0.991	0.959	0.385	0.237	0.125	0.166
	1245	0.317	0.443	0.45	0.07	0.128	0.183
	1247	0.371	-	-	0.134	-	-
Mean		0.522	0.544	0.621	0.214	0.223	0.276
(SD)		(0.112)	(0.089)	(0.137)	(0.172)	(0.143)	(0.098)
Controls	1239	0.603	0.676	0.605	0.605	0.374	0.666
	1250	0.602	0.56	0.686	0.594	0.48	0.64
	1251	0.727	0.675	0.737	0.423	0.288	0.307
	1252	0.861	0.687	0.58	0.949	0.767	0.77
	1253	0.566	0.38	0.775	0.64	0.6	0.584
	1283	0.844	0.578	0.472	0.956	0.734	0.801
Mean		0.700	0.593	0.643	0.694	0.540	0.628
(SD)		(0.129)	(0.117)	(0.112)	(0.213)	(0.193)	(0.177)

Table 2.2. Individual Cluster Overlap Correlation Coefficients of the 'Cortical' and 'Subcortical' Region of Interest for the Group with MS and for Controls Elicited by the Poffenberger Task

MS group	ID	Cortical			Subcortical		
		Visits			Visits		
		1 vs. 2	1 vs. 3	2 vs. 3	1 vs. 2	1 vs. 3	2 vs. 3
	1112	0.444	0.487	0.46	-	-	-
	1113	0.584	0.466	0.53	0.489	0.404	0.521
	1119	0.683	0.413	0.529	0.543	0.445	0.622
	1222	0.228	0.467	0.406	-	-	0
	1204	0.643	0.683	0.894	0.345	0.357	0.444
	1231	0.25	0.294	0.109	0	0	0
	1232	0.241	0.251	0.345	-	-	-
	1233	0.273	0.413	0.391	-	0	-
	1234	0.113	0.103	0.201	-	-	-
	1245	0.304	0.441	0.495	0	0	0
	1247	0.287	0.104	0.115	-	-	0
Mean		0.368	0.401	0.427	0.275	0.201	0.239
(SD)		(0.197)	(0.126)	(0.225)	(0.261)	(0.222)	(0.296)
Controls	1239	0.691	0.608	0.515	-	-	-
	1250	0.607	0.615	0.591	0.451	0.421	0.483
	1251	0.727	0.704	0.737	0.763	0.726	0.606
	1252	0.805	0.768	0.849	0.681	0.606	0.538
	1253	0.566	0.402	0.501	-	-	0.583
	1283	0.863	0.66	0.719	0.605	0.561	0.655
Mean		0.709	0.626	0.662	0.625	0.578	0.573
(SD)		(0.113)	(0.127)	(0.138)	(0.338)	(0.314)	(0.241)

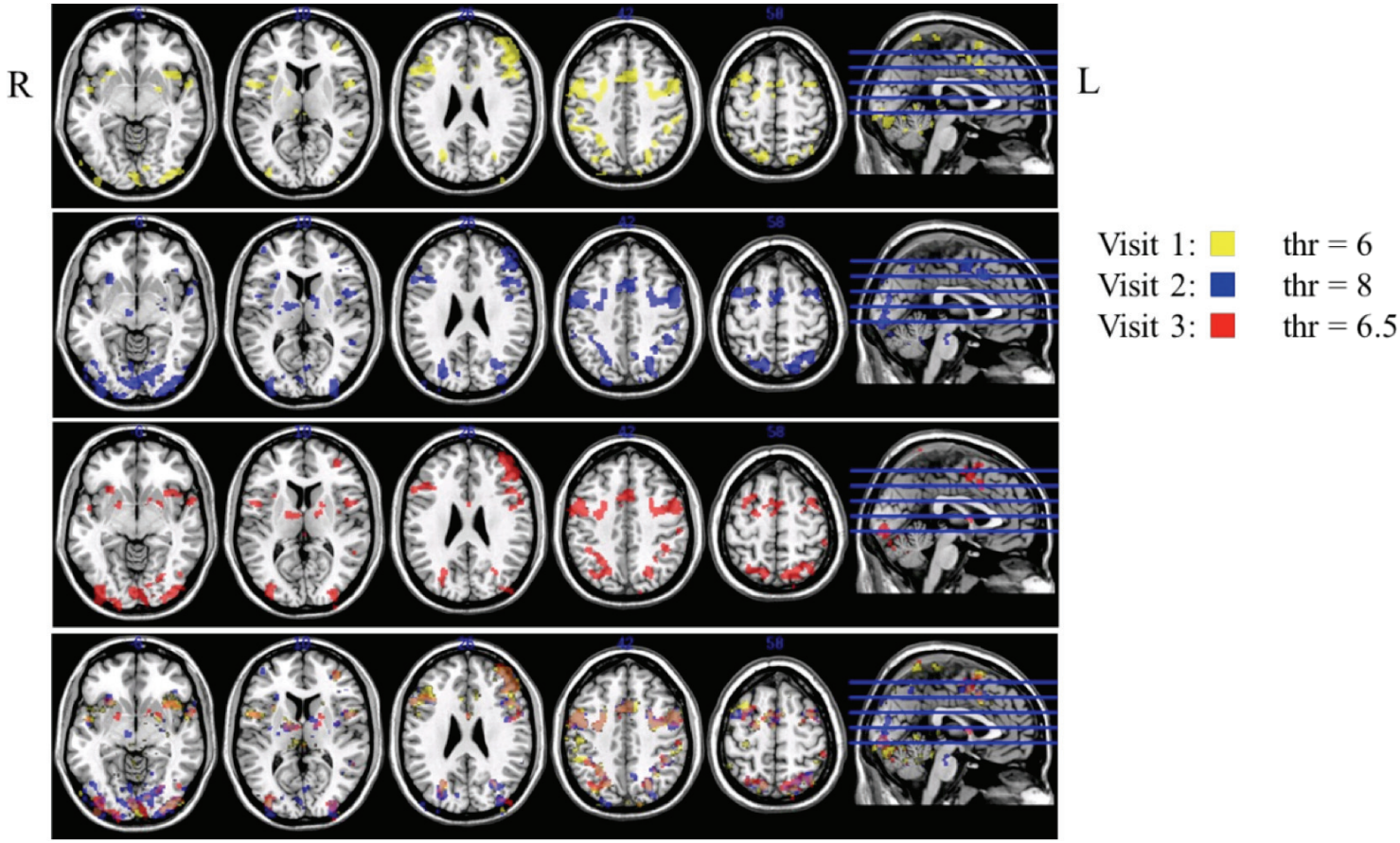


Figure 2.4. The Three Functional Maps of the Symbol Digit Modalities Test Collected on the Three Visits from an Individual with Multiple Sclerosis. The Three Functional Maps are Depicted Separately As Well As Overlaid on Each Other (Threshold = thr).

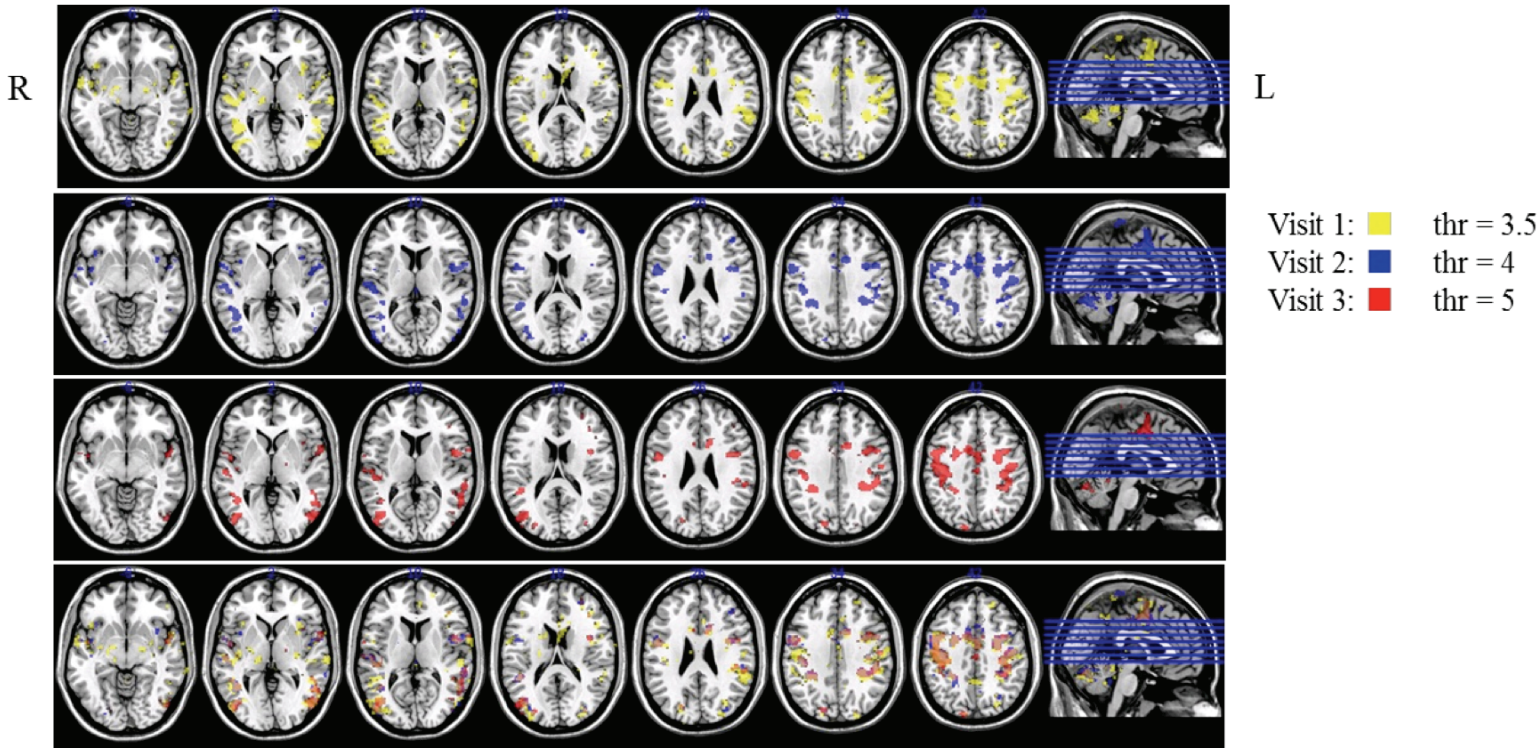


Figure 2.5. The Three functional Maps of the Poffenberger Task Collected on the Three Visits from an Individual with Multiple Sclerosis Three Functional Maps are Depicted separately As Well As Overlaid on Each Other. (Threshold = thr).

2.4.1.2. Reliability and Expanded Disease Status Scale Scores

We explored the relationship between EDSS score and COR for both tasks by using linear regression. Moderate to high significant correlations were found between EDSS scores and CORs of the SDMT, which was not the case for the Poffenberger task. The specific results were the following: $R^2 = 0.574$, $p = 0.007$ for 'cortical' and $R^2 = 0.482$, $p = 0.038$ for 'subcortical' COR of the SDMT, and $R^2 = 0.090$, $p = 0.369$ for 'cortical' COR of the Poffenberger Task. Linear regression was not run for the 'subcortical' COR of the Poffenberger task, as after removing points COR = 0 only three points were left. The linear regression between EDSS scores and the average COR (collapsing the three visits) of the two taks for the patient group is shown in Figure 2.6.

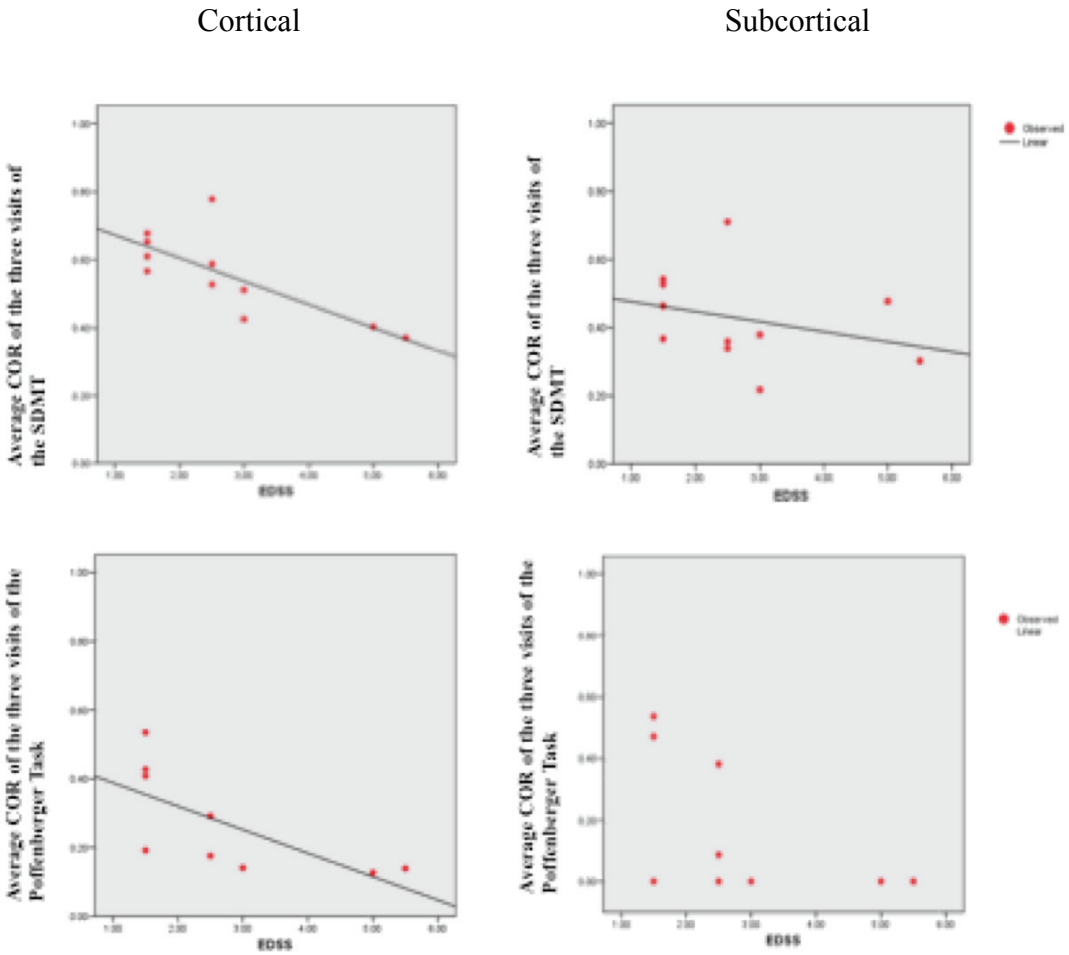


Figure 2.6. Relationship between the Expanded Disease Status Scale Scores of the Patient Group and the Average Cluster Overlap Reliability of the Three Visits of the Symbol Digit Modalities Test and the Poffenberger Task

2.4.2. Group Activation

We completed group analyses for both tasks using equal sample sizes (n =6). As expected the patient group recruited larger brain areas in response to both tasks compared to the controls (Figure 2.7. and 2.8.), but contrast analyses did not show statistically significant group differences for either task. The SDMT elicited a similar activation pattern as reported in the literature (Genova et al., 2009), notably the superior, middle, and the inferior frontal gyri, the precentral gyrus, the supplementary motor area, the anterior cingulate gyrus, the insula, the thalamus, the inferior temporal gyrus, including the fusiform area, the precuneus, the lateral occipital cortex, the cuneus, and the occipital pole.

Group map of the Poffenberger Task was also in agreement with previously noted brain regions (Tettamanti et al., 2002), notably activation of the superior, medial and inferior frontal gyri, the pre- and postcentral gyri, the supplementary motor area, the anterior cingulate gyrus, the operculum along with the supramarginal gyrus, and the lateral visual cortex.

a b

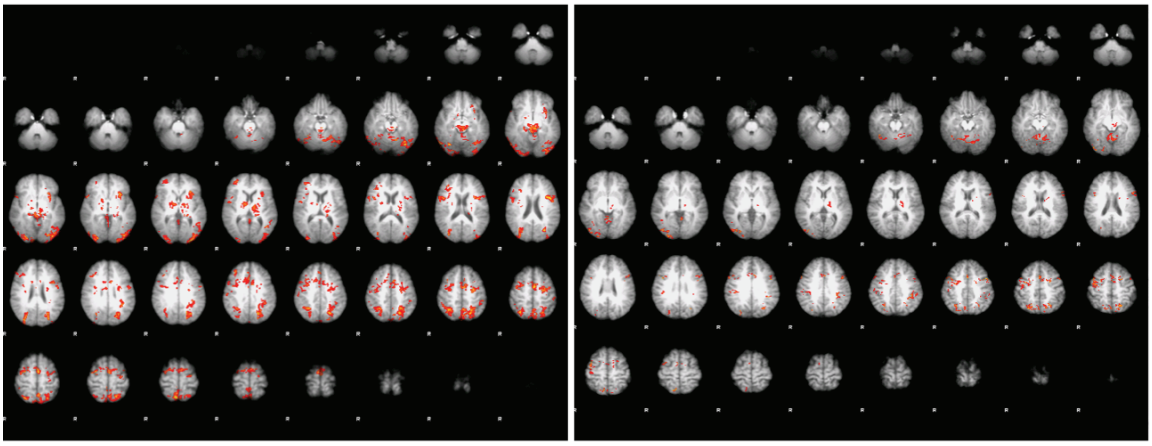


Figure 2.7. Group Maps of the Symbol Digit Modalities Test for Patients (a) and Controls (b).

a b

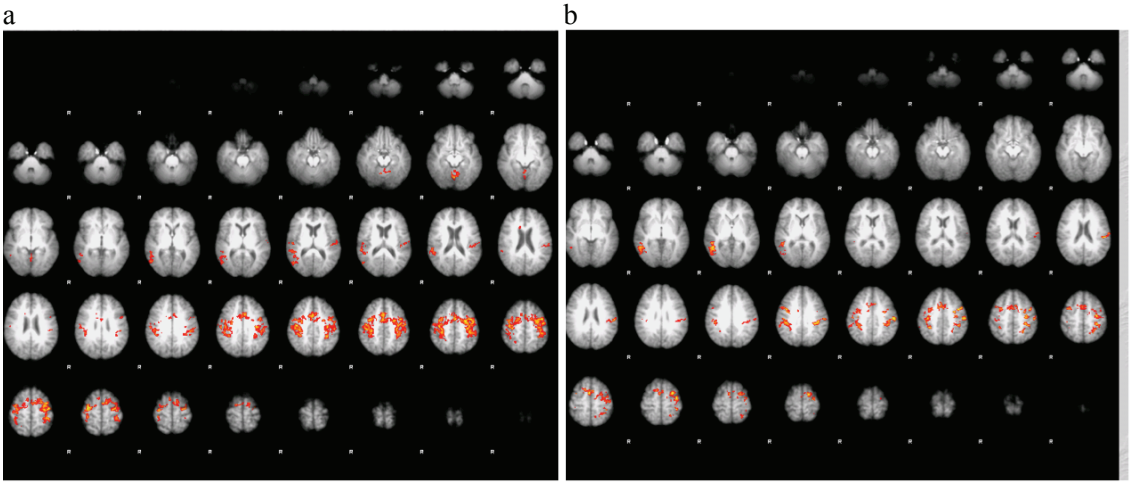


Figure 2.8. Group Maps of the Poffenberger Task for Patients (a) and Controls (b).

2.4.3. Behavioral Measures

2.4.3.1. The Symbol Digit Modalities Test

There was no statistically significant difference in reaction time when the two groups ($F = 2.703$, $p = 0.122$), or when visits within the groups were compared ($F = 0.146$, $p = 0.856$), although patients had longer reaction time scores compared to the controls, and reaction time shortened across the three visits for both groups.

Apart from the higher variability in the patients group, results for timed accuracy were similar compared to the results of reaction time. Examining the groups, patients had lower timed accuracy, but it did not reach statistical significance ($F = 2.311$, $p = 0.151$). The repeated measures showed similar non-statistically significant results ($F = 1.498$, $p = 0.241$), although accuracy improved over the three visits for both groups. Figure 2.9. shows between and within group measures of timed accuracy and reaction time for patients and controls

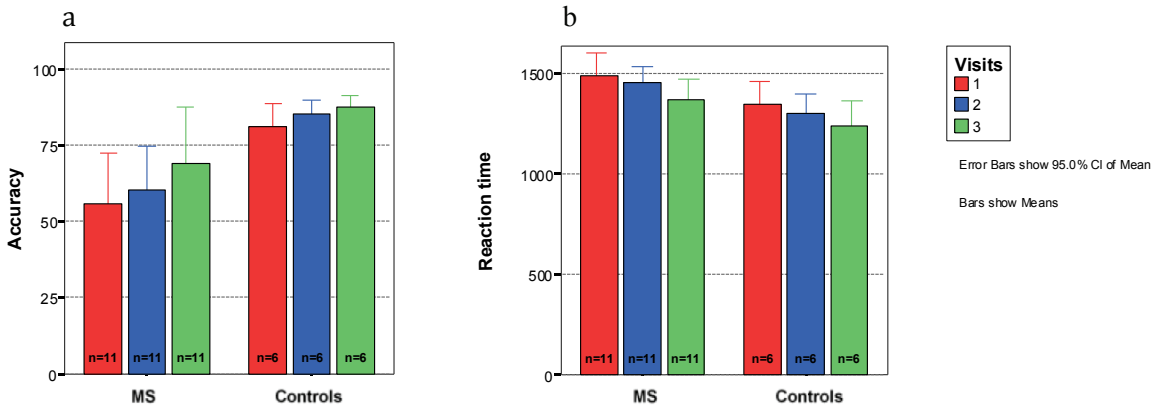


Figure 2.9. Timed Accuracy (a) and Reaction Time (b) Measures of the Symbol Digit Modalities Test Separated by Study Groups and Visits.

2.4.3.2. The Poffenberger Task

Patients had longer reaction time compared to controls, which was also statistically significant ($F = 5.476$, $p = 0.035$) overall. After correcting for multiple comparisons ($p = 0.016$), a significant difference was discovered comparing reaction time of the second ($t = 11.997$, $p = 0.007$) between the groups. The reaction time was stable across visits within groups ($F = 0.004$, $p = 0.949$),

When timed accuracy was examined, there was no statistically significant difference between ($F = 0.423$, $p = 0.526$) or within the two groups ($F = 1.558$, $p = 0.232$), although interestingly patients showed a decline in timed accuracy across visits. Figure 2.10. shows between and within group measures of timed accuracy and reaction time for patients and controls.

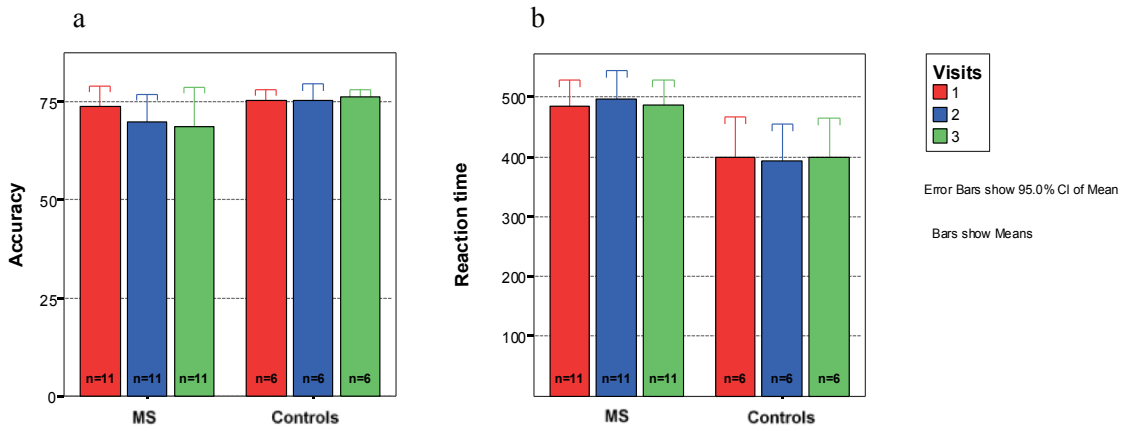


Figure 2.10. Timed Accuracy (a) and Reaction Time (b) Measures of the Poffenberger Task Separated by Study Groups and Visits.

2.5. Discussion

Short- and long-term longitudinal assessment of changes in brain activation using fMRI is a meaningful way to monitor patients with established MS. However, single-subject test-retest reliability of the BOLD signal has never been addressed before to our knowledge. Accordingly, the main objective of this study was to examine single-subject COR in patients with MS and compare those with COR values of age matched controls. To increase our COR, we have applied a technique that uses individualized thresholding (ROC-r, Stevens et al., 2013). In RRMS the central problem is WM injury. In order to address this, we created 'cortical' and 'subcortical' ROIs mainly comprising GM and WM, respectively. As reliability of the BOLD signal task dependent (Bennett & Milller, 2010), tasks were chosen based on their reliability and sensitivity to assess information processing speed (Forn et al., 2009; Akbar et al., 2011) and their adaptability to fMRI (Forn et al., 2009; Akbar et al., 2011; Gawryluk et al., 2011a).

2.5.1 Cluster Overlap Coefficient in Patients with Multiple Sclerosis and Controls

Our main finding was consistent with our first hypothesis: patients with MS had generally lower COR compared to the age-matched control group, and this was particularly true for the 'subcortical' ROI. This finding is noteworthy, as the test-retest interval was short (one week) and patients were expected to be stable, thus showing only

somewhat lower COR values compared to controls. The fact that brain activation of patients was so different from one week to the other can be interpreted that an inherent neuronal instability is present even during remitting periods, and COR is sensitive enough to measure that. The low reliability of 'subcortical' COR seen in patients is also consistent with the WM disease concept in RRMS (Reddy et al., 2000; Rocca et al., 2002; 2007; Filippi et al., 2010). Therefore our results indicate that functional instability, which may be characteristic of MS, associated with neuronal pathology in areas mainly occupied by WM can be measured by COR.

Reliability results of the SDMT comparing patients and controls support this hypothesis better than data obtained from the Poffenberger Task. One potential explanation for the significantly lower 'cortical' COR of the Poffenberger Task is that this task sets different demand compared to the SDMT. This includes short-term memory, interhemispheric transfer, and callosal inhibitory conduction, which are also affected in MS (Rao et al., 1989a; 1991a; DeLuca et al., 1998; Archibald & Fisk, 2000; Benedict et al., 2006; Manson et al., 2006; Chiaravalloti & Deluca 2008). The comparable results between the groups regarding the 'subcortical' COR is however, probably the result of low power due to data exclusion and the lack of active clusters in brain areas covered by the ROI. Explanation for this lack of response is not yet clear, as WM activation in response to the Poffenberger Task has been demonstrated in healthy young individuals (Mazerolle et al., 2010; Gawryluk et al., 2011a,b). One possible rationale is using a relatively high threshold (*t*- scores) in some cases that may have eliminated the BOLD response in WM (Wise et al., 2004). Nevertheless, as this task measures trans-callosal performance commonly affected in MS as well it shows robust COR in the control group its potential role should be further investigated in MS.

Despite a considerable amount of individual variability of COR, especially in the patients group, reliability values did not change significantly across the visits. This was true for both, the SDMT and the Poffenberger Task. This finding is important as it indicates that in short test-retest interval (one week) single-subject reliability of the fMRI is fairly stable for both tasks.

On the other hand, the lower COR with higher variability seen in the patient group is also valuable information, and may point us to the direction that focusing on the

variability of test-retest reliability of functional maps may be a meaningful way to monitor patients with MS. To support this premise, recent findings suggested fluctuation in neuronal recruitment as a valuable descriptor of disease activity in MS (Wojtowicz 2012; 2013; 2014; Mazerolle et al., 2013b). Low COR may represent functional instability due to competing functions of a certain brain area when a new function is taken over from a damged brain region. This neuronal instability can be measured by COR, and based on results targeted therapy and rehabilitation can be scheduled. Rehabilitation should help brain regions to take over and reinforce new functions and stabilize neuronal networks, thus enhance cognitive efficiency.

It would be interesting and important to examine whether very short-term (same day) test-retest interval yields similarly low COR with similar variability in patients compared to controls and whether these observed differences of COR between the groups remain for long (e.g. one year) test-retest interval periods as well. Finally, as COR will likely decrease with a longer test-rest interval (Bennett & Miller, 2010), it would be important to verify stable reliability for both tasks in population with no neuronal pathology in order to establish COR as a marker in MS.

2.5.2. Relationship between the Expanded Disease Scale Status Scores and Cluster Overlap Coefficient Values in Patients with Multiple Sclerosis

Regarding our second hypothesis, the SDMT also revealed interesting, significant negative associations between COR ('cortical and subcortical') vs. EDSS values. The EDSS measures a wide scale of neurological functions and it has been suggested that neurological disability measured by the EDSS are associated with apparent or normal appearing neuronal injury (Rovaris et al., 2000; 2005a,b; Allen et al., 2001; Audoin et al., 2003; 2006; Agosta et al., 2006; Fisniku et al., 2008a,b; Calabrese et al., 2011a,b,c; 2010a; 2012; Roosendaal et al., 2010a,b; Rocca et al., 2012; Basile et al., 2013; Sacco et al., 2013). Although this clinical measure is less sensitive to cognitive impairment, there is evidence of association between the EDSS scores and lesion load in areas relevant to the SDMT task, including the anterior cingulate gyrus, the insula, and the association cortices (Charil et al., 2006). In the current study, we showed moderate to high correlation between EDSS scores and COR of 'cortical' and 'subcortical' areas of the

SDMT, meaning that the larger the disability, the lower the COR is. With keeping the small sample of the study in mind, this relationship also supports that COR values themselves, measuring stability of activation, could be used as a marker of neuronal pathology.

The Poffenberger Task on the other hand, did not suggest any relationship between COR and EDSS values. Whether the results deviated from our hypothesis only as a consequence of the low power or whether the function of the task differs from the function of the SDMT, needs to be explored

2.5.3. Comparing Recruited Brain Areas

Although group analysis was not the focus of the study, based on reported findings (Forn et al., 2009; 2013a), we expected to see larger brain areas recruited by patients compared to controls congruent with the theory of compensatory 'cortical' activation in MS that states that larger recruited brain areas are needed to better support cognitive performance (Staffen et al., 2002; Mainero et al., 2004; Prakash et al., 2008).

Our results did not show statistical difference between the groups, which is likely due to the large variance in the patient group, and would likely change by either increasing the sample size or making our patients sample to be more homogenous (i.e. recruit patients with similar EDSS score). The latter assumption is supported by visual inspection of the functional maps, as there are obvious group differences in active brain areas during both tasks (Figure 2.7 and 2.8).

Compensatory recruitment was particularly noticeable in the following regions in response to the SDMT (Figure 2.7.); i. the frontal gyri, superior (coordination of the sensory system), the middle (attention and working memory), and the inferior (inhibiting proponent response); ii. the anterior cingulate (error detection and conflict monitoring); iii. the precentral gyrus (motor response); iv. the operculum (light touch, production of speech); v. the thalamus (relay of motor and somatosensory information) vi. the visual cortex including the cuneus (primary visual processing, attention, working memory); and vii. in the fusiform area (within category identification).

The group map of the Poffenberger Task also showed compensatory neuronal networks (Figure 2.8.). These areas were: i. the frontal gyri, superior (coordination of the

sensory system), the middle (attention and working memory), and the inferior (inhibiting proponent response); ii. the anterior cingulate gyrus (error detection and conflict monitoring); iii. the supplementary area and the precentral gyrus (motor planning and response); iv. postcentral gyrus (sensory reception); v. the operculum along with the supramarginal gyrus (light touch, production of speech).

While some of these overworking areas, such as the postcentral gyrus may reflect paresthesias, common symptoms in MS, the majority of the enhanced activation present in patients indicates compensatory mechanisms to surmount limitation of damaged brain areas.

2.5.4. Slowed Information Processing in Patients with Multiple Sclerosis

The behavioral outcome of the two tasks was also corresponding with our expectation and findings in the literature (Nocentini et al., 2006). Although significant difference between the performances of the two group was only seen when RTs of the Poffeneberger task were compared, the noticeably higher reaction time and lower timed accuracy for both tasks correspond with the nature of the disorder (i.e. slowing information processing).

The lower timed accuracy may seem to be conflicting with earlier findings as patients with RRMS showing less difference compared to controls with regard to accuracy relative to reaction time (Sonneville et al., 2002). It is important to remember that each pair matching of the fMRI adopted SDMT is timed unlike the paper-pencil version (Smith, 1982). Most of the SDMT related behavioral finding were based on the paper-pencil version, which allows as much time as needed per pair matching within 90s. Thus, patients more likely have lower number of matched pairs compared to controls on the paper-pencil version, but are expected to have few incorrectly matched pairs. Here, participants had only two seconds to match each pair, and they were scored wrong not only for an incorrect answer, but also for not being able to answer.

2.5.5. "What COR Value Is "Reliable Enough"?

As fMRI is a valuable method of in vivo investigation of cognitive processes of the healthy and diseased brain and its potential application in clinical settings, reliability of the fMRI has been of interest during the past couple of years. However, consensus regarding the acceptable reliability has not been reached. It is a challenging topic for various reasons noted in another section. One of those explanations is related to the method of measuring reliability. We have chosen measuring COR, which has been reviewed previously and its values range from 0.314 to 0.670 with the grand average of 0.476 for all reviewed studies using this method (Bennett & Miller, 2010).

Our results show for the two tasks an average COR ~ 0.63 for controls. While these numbers do not seem to be impressive when clinical decision-making is in question, it is important to remember that COR is a rather rigorous method. First, this method of reliability measures the activated networks relative to the ROIs ('cortical' or 'subcortical' regions) using a voxel-wise assessment. This means the order of a couple of thousands of voxels to a couple of millions. Second, because the method compares active overlapping to non-overlapping voxels as well as to non-active voxels, the discrepancy in the subsequent activation maps by even a couple of hundred voxels within the same functional area can reduce reliability significantly.

Comparing fMRI reliability of other paradigms, such as simple motor or visual tasks, 0.63 of COR is also encouraging as both of these tasks are relatively complex, involving visuomotor coordination, working memory, error detection, and conflict monitoring. Consequently, intra-subject variability in recruiting supporting neuronal networks can be considerably high (Tomassini et al., 2011). Changes in active clusters over the scanning sessions are typically seen during learning and this phenomenon sustains itself longer if the task is complex (Tomassini et al., 2011). Although the administered practice sessions aimed to eliminate these interim activations, the neuronal fine-tuning due to learning may have been observable over the visits even in healthy brains (Kincses et al., 2008). This outcome cautions us about the importance of determining individual practice time of a particular task or average multiple runs of a single imaging session to eliminate learning effect.

2.6. Conclusions

Our main goal was testing single-subject test-retest reliability of the SDMT and the Poffenberger Task comparing patients with MS and age-matched controls. Overall we showed a fairly good reliability score in controls, which should be tested with a larger sample size and longer test-retest intervals. We also demonstrated lower COR scores in patients with MS compared to controls, and COR was particularly low in 'subcortical' areas. In addistion, we demonstrated a negative relationship between reliability and EDSS values of the SDMT. In the light of these findings, the current study suggests that patients with MS have inherent neuronal instability, even during remitting period, that can be measured by COR values. Further, COR values suggest disease stage, meaning the higher the disability, the lower the COR is. Regarding the sensitivity of fMRI to neuronal integrity, it is also possible that COR values reflect transient, hence more repairable neuronal injury compared to visible clinical pathology that often indicates permanent injury. Taken together, COR may be a valuable marker of subclinical disease activity in patients with MS, which in turn can help design early, more effective rehabilitation or treatment plan.

Considering the established role of the SDMT in MS and our results, perhaps this task is a more suitable measure in MS compared to the Poffenberger Task. Nevertheless, the Poffenberger Task may have value testing patients with MS, which should be further investigated.

CHAPTER 3

EXAMINING SINGLE-SUBJECT RELIABILITY OF FUNCTIONAL MAGNETIC RESONANCE IMAGING IN ESTABLISHED MULTIPLE SCLEROSIS: THE DEFAULT MODE NETWORK

3. 1. Introduction

Understanding neuronal networks associated with performance in a specific cognitive domain is important in order to plan well-targeted therapy or rehabilitation. However, task-driven fMRI analysis can be confounded by co-morbid symptoms of MS, such as fatigue, joint pain, or depression, or demographic variables that influence performance on cognitive tasks like age, education, and work history. Resting-state fMRI circumvents this limitation by exploring baseline neuronal activity of functionally related brain regions or networks, and thus may be more effective in isolating abnormalities originating specifically from neuronal injury. Further, RS fMRI is sensitive to large-scale functional changes in the brain reflecting diffuse neuropathology, which has been suggested to be a key contributor of MS symptomology (Filippi et al., 2002; Rocca et al., 2002; 2010c; 2012; Bonavita, 2011).

The most studied RS network is the DMN, and it is linked to cognition (Esposito et al., 2006; Fox & Raichle, 2007). Regarding its value and relevance to MS, the DMN has been the focus of interest (Rocca et al., 2010a,b,c; Roosendaal et al., 2010b; Sumowski et al., 2010, 2013c; Bonavita et al., 2011; Faivre et al., 2012; Loitfelder et al., 2012; Basile et al., 2013; Forn et al., 2013b; Sacco et al., 2013). Main findings of previous studies include enhanced functional connectivity at early stages of MS, which diminishes as the illness progresses (Roosendaal et al., 2009; 2010b; Rocca et al., 2010b,c; Bonavita et al., 2011; Faivre et al., 2012; Basile et al., 2013), reverse association between functional connectivity and cognitive impairment (Rocca et al., 2010b,c; Sumowski et al., 2010; 2013a,c; Bonavita et al., 2011; Basile et al., 2013), deficiency of turning on/off the DMN (Loitfelder et al., 2012; Forn et al., 2013b; Sacco et al., 2013; Sumowski et al., 2013c), and MS specific dysregulation at the medial prefontal cortex (Rocca et al., 2010c; Bonavita et al., 2011; Parisi et al., 2012). In addition, partial restoration of functional connectivity of the DMN by cognitive rehabilitation has also been reported (Leavitt et al.,

2012; Parisi et al., 2012). Taken together, examining activation and functional connectivity of the DMN may have potential value in monitoring patients with established MS.

Previously, short- (same day to a week) and long-term (several months to a year) RS-fMRI reliability has been investigated mainly in healthy population with the evidence of moderate to good reliability (Damoiseaux et al., 2006; Shehzad et al., 2009; Meindl et al., 2010; Zuo et al., 2010; Schwarz & McGonigle, 201; Kristo et al., 2014). To our knowledge, however, RS-fMRI reliability has not been addressed in MS.

The main goal of this study was to test reliability of RS-fMRI in MS at single-subject level and compare that to reliability scores acquired from age-matched controls. We also explored the relationship between cognitive performance obtained from the task-driven paradigms (reaction time and timed accuracy) and test-retest reliability of the DMN. Finally, as previously, we measured group level recruitment of the DMN and contrasted the functional connectivity of the DMN in patients with MS and in age-matched controls.

3.2. Hypotheses

We hypothesized: i. COR would be lower for the patient group compared to the control group due to greater intra-subject variability of the recruited neuronal links in patients; ii. COR values would show a negative and a positive relationship with reaction time and accuracy , respectively, obtained during the task-driven fMRI paradigm; and iii. consistent with the compensatory hypothesis, patients would show an increase in recruited areas during rest, and different functional connectivity compared to the control group.

3.3. Methods

3.3.1. Participants and Procedures

3.3.1.1. Participants

Description and recruitment criteria of the participants are noted in the previous chapter with a couple of exceptions. For this task, we only had 6 out of the 14

participants with clinically definite RRMS (5 females, one male) matched with the same 6 controls for age and education.

Patients had an age range of 38-59 years with a mean age of 51.61 years and with years of education of 15.1 ± 3.351. Every patient was right-handed and one was a smoker. The mean duration of illness and treatment was 8.062 ± 3.56 and 6.04 ± 3.74 years, respectively. The average EDSS score was 3.6 ranging from 2.5 to 5.5. Everyone from the patient group was on disease modifying medication.

The control group consisted of the same people participated in the task-driven experiments. The age range for the control group was of 42-52 years with a mean age of 47.57 years. One control was left-handed and a smoker. The two study groups did not differ in age ($F = 1.184$, $p = 0.300$).

3.3.1.2. Procedures

Procedures were the same as the previous experiments, except the functional run was a RS-fMRI.

3.3.2. Imaging Sequence

The data were collected using a 4T Oxford magnet with a Varian INOVA console. Body coil provided gradients (Tesla Engineering Ltd.) and a transverse electromagnetic head coil was used for transmit/receive signal (Bioengineering Inc.). Acquisition for the RS runs was the following; two shots, TRvol = 2s, TE = 15s, flip angle = 60°, 64 x 64 matrix, readout direction = 240, phase direction = 240, number of slices 31, slice thickness = 2.9mm, gap = 0.3mm, volume = 192. For anatomic reference, registration, and tissue segmentation, we used the same high resolution structural images described in the previous chapter. To assess WM lesions, FLAIR sequences, also described previously, were applied. Similar to the previous experiment, all images were acquired on the axial plane.

3.3.3. Experimental Design

The stimulus for the functional run was displayed on E-Prime2 software (Psychological Software tools, Inc.). During the functional run, participants were asked to focus on a cross presented in the center of the screen fixation point, stay still, and relax.

3.3.4. Functional Magnetic Resonance Imaging Analyses

3.3.4.1. Region of Interest Selection

At individual level, ROI analysis was completed using the same software as for previous tissue segmentations, the FreeSurfer software program (Martinos Center for Biomedical Imaging). The ROI entailed the whole brain delineated on T_1 weighted individual anatomical images. The whole brain ROI mask is shown in Figure 3.1. for a representative participant.

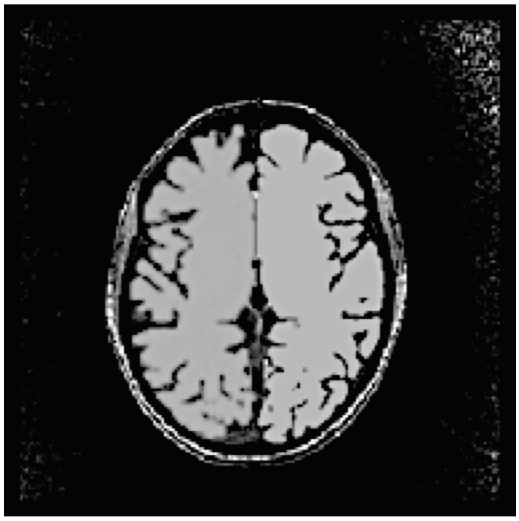


Figure 3.1. The Whole Brain Area Segmented on Individual a T_1 Weighted Image for a Representative Participant.

3.3.4.2. Preprocessing, Independent Component Analysis and General Linear Model Analyses

Similar preprocessing steps were followed as for the previous experiments, including motion correction, non-brain tissue removal, mean-based intensity normalization, high-

pass temporal filtering (0.01 Hz), and spatial smoothing using a Gaussian kernel, 5mm FWHM.

First, running the data in ROC-r required preparing a model describing the BOLD signal for the task-free RS data. Flowchart summarizing the procedures described in the 3.3.4. section is presented in Figure 3.2. The preprocessed data were analyzed using Independent Component Analysis (ICA) (Beckmann & Smith, 2004) with the Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) 3.13 tool box of FSL (Smith et al., 2004). Independent component analysis is a statistical method for decomposing a complex 4D data set into statistically independent spatial component maps with associated time courses. This process simplifies the interpretation of the data, as each final independent component is more likely to be due to only one physical or physiological process (Beckmann & Smith, 2004). Independent component analysis offers several advantages. First, it is a multivariate data-driven approach, and thus it does not require priory hypothesis. Second, by identifying simultaneous voxel-voxel relationships, ICA can assess large scale neuronal interactions relevant to disease pathology in MS. Lastly, extracting noise is also data-driven limiting the dangers of filtering out signal or filtering in noise.

To identify the component of interest (i.e. DMN) for each subject and for each visit, the entire dataset was registered in a common space (Montreal Neurological Institute template) using MELODIC which performs registration on input data as part of an analysis using FEAT functionality. Similar to FEAT registration, individual functional maps were first registered to individual structural images (DOF = 6) and then put into a common space using DOF = 12. The threshold levels of the ICA maps were set to the most conservative value 0.99, meaning that only 1% chance to false-positive discovery was allowed.

The registered dataset (each subject and each visit) was then concatenated temporally to form a 2D space x concatenated-time data matrix (Beckmann & Smith, 2005). This step is essential to reduce the number of independent components. Using the concatenated dataset, ICA was applied to identify large-scale functional connectivity patters common in the data. The concatenated-ICA is a useful method when similar spatial patterns, but not similar time courses, are assumed across individuals and visits,

which is the case with the RS-fMRI. Running ICA with the concatenated dataset reduced the number of components to 52, which allowed easy identification of the DMN (Smith et al., 2010). The independent component of the DMN obtained by running the concatenated-ICA is shown in Figure 3.3. Identifying the DMN based on its spatial map permitted the isolation of its associated time course. The time course was treated as the model of the BOLD signal in the GLM.

Following similar procedures as described in the previous chapter, single-subject GLM analyses were completed using FEAT version 6.0 FSL (Smith et al., 2004). Individual non-thresholded *t*-statistical maps were obtained in preparation for the ROC-r analysis (Stevens et al., 2013), while thresholded $Z > 2.3$ statistic images were averaged for each individual, and these mean values were used for group data (patients vs. controls) analyses (Worsley et al., 1995). Finally, to compare functional connectivity between the two groups, concatenated-ICAs were completed for all visits separating patients and controls and the independent component (IC) labeling the DMN was chosen for both groups using visual inspection of the spatial maps.

3.3.4.3. Receiver Operated Characteristic-Reliability Analysis and Cluster Overlap Estimation

To estimate COR, we used the ROC-r analysis (Stevens et al., 2013) as described previously. Based on the ROC-r analysis, reliability of the three visits of the whole brain was graphed as a function of threshold, and the thresholds for each map were chosen where the AUC was the highest (Figure 1. 11.). The functional maps were then thresholded, binarized, and overlaid on each other to calculate COR with the Rombouts method (Rombouts et al., 1997).

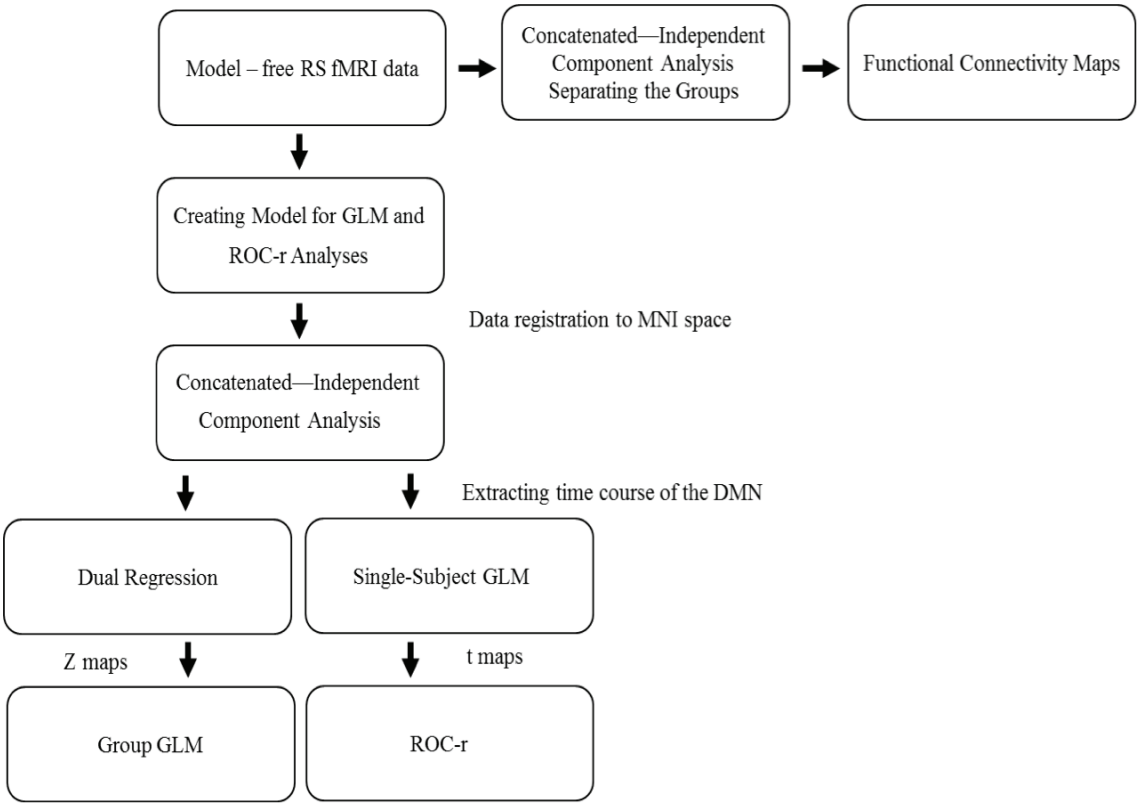


Figure 3.2. Flowchart Describing the Procedures of Creating a Model for the Task-Free Functional Magnetic Resonance Imaging and Using Independent Component Analysis to Assess Functional Connectivity of the Two Study Groups. Note that Z-maps and ROC-r analyses denote area recruited, while group ICA shows functional connectivity.

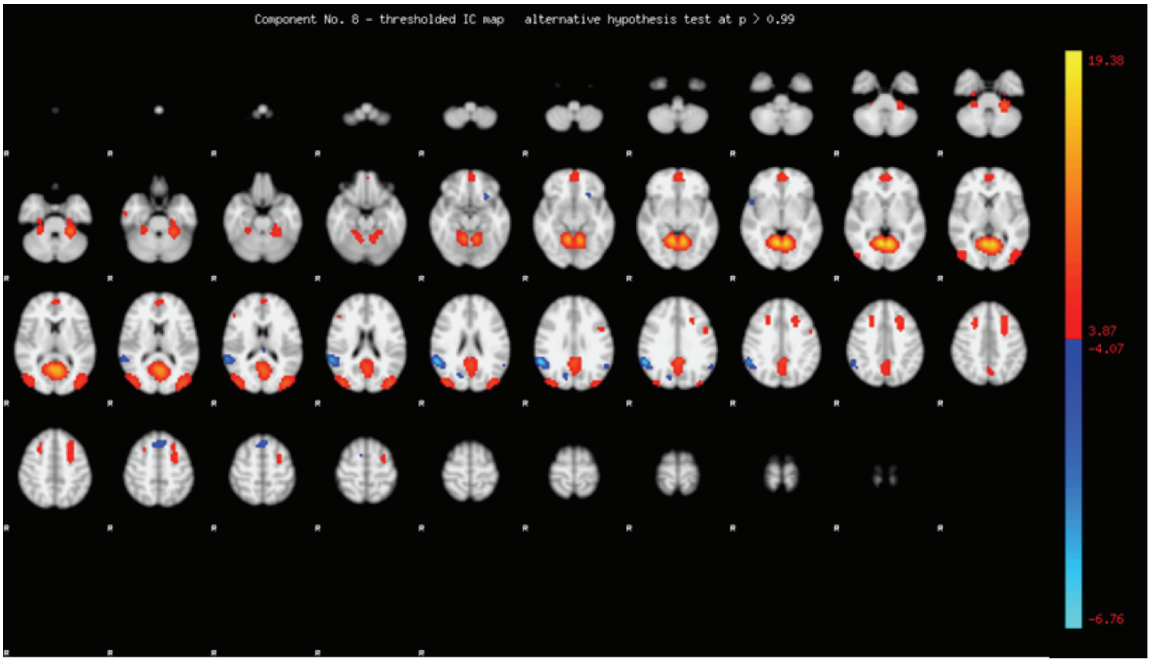


Figure 3.3. The Functional Connectivity of the Default Network Obtained from the Entire Dataset Using Concatenated Independent Component Analysis. Activation shows areas with a synchronous time course of the default network (red-yellow) and areas inversely correlated with the default network (blue-light blue).

3.3.4.4. Relationship between Cluster Overlap Reliability and Behavioral Indices of the Symbol Digit Modalities Test and the Poffenberger Task

Association between COR of patients and behavioral indices were compared by regression curve fitting analysis by computing the average COR of brain areas occupying the DMN (i.e. collapsing the three sessions).

3.4. Results

3.4.1. Imaging Results

3.4.1.1. Individual Reliability

Cluster overlap correlation coefficients, comparing two functional maps at a time, of the DMN are represented in Tables 3.1. Note that there were a lot of functional maps that had to be excluded due to movement or other type of noise. Cluster overlap reliability values were treated as missing values when a map was excluded. Cluster overlap reliability = 0 describes when activation was observed in only one of the ROI maps or when activation was seen in both of the ROI maps, but active clusters did not overlap.

Figure 3.4. shows, for the three visits of a representative individual, the functional maps of the DMN separately and overlaid.

On account of the number of exclusions, the reliability of the functional maps was examined only between the groups. Using the average of COR for the three visits, the two groups did not differ in COR values ($F = 3.568$ $p = 0.132$).

Table 3.1. Individual Cluster Overlap Correlation Coefficients of the Default Mode Network for the Group with Multiple Sclerosis and for Controls

	ID	Cortical		
		Visit 1 vs. 2	Visit 1 vs. 3	Visit 2 vs. 3
Patients	1231	-	0.428	-
	1232	-	0.359	-
	1233	0.575	-	-
	1234	-	-	0.394
	1245	0.543	0.416	0.540
	1247	0.340	0.288	0.578
Mean		0.485	0.373	0.504
(SD)		(0.127)	(0.064)	(0.097)
Controls	1239	0.453	0.459	0.546
	1250	0.506	-	-
	1251	0.642	0.699	0.594
	1252	0.509	0.571	0.527
	1253	0.549	0.573	0.620
	1283	0.635	-	-
Mean		0.568	0.575	0.572
(SD)		(0.075)	(0.097)	(0.043)

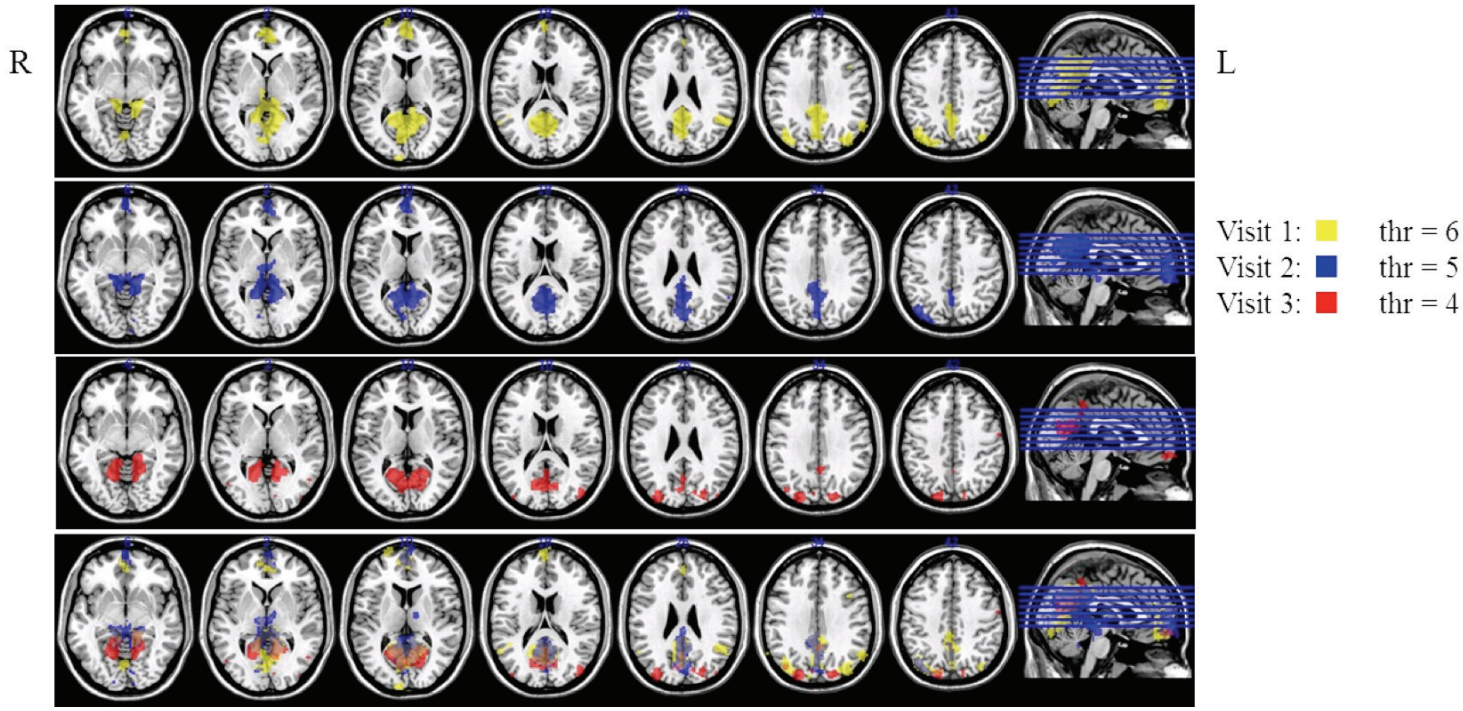


Figure 3.4. The Three Functional Maps of the Default Mode Network Collected on the Three Visits From an Individual with Multiple Sclerosis. The Three Functional Maps are Depicted Separately As Well As Overlaid on Each Other. (Threshold = thr).

3.4.1.2. Relationship between Cluster Overlap Reliability and Behavioral Indices of the Symbol Digit Modalities Test and the Poffenberger Task

Curve fitting analyses showed a linear relationship between reaction time and timed accuracy for the SDMT and COR of the DMN when all participants were considered. On the other hand, these associations regarding of the Poffenberger Task are rather arguable. The association between reaction time of the Poffenberger Task and COR of the DMN was significant $R^2 = 0.378$, $p = 0.034$. However, it is very likely that this relationship would not survive with a modification of a single data point, while timed accuracy of the Poffenberger Task and COR did not reach significance $R^2 = 0.293$, $p = 0.069$ (Figure 3.5.). For the SDMT, both the reaction time and timed accuracy reached significance when compared to COR of the DMN, $R^2 = 0.609$, $p = 0.003$ and $R^2 = 0.458$, $p = 0.016$, respectively (Figure 3.6.).

3.4.1.3. Group Activation and Functional Connectivity of the Default Mode Network

Patients had different distribution of DMN activation compared to the control group. Contrast analysis of the group *Z* statistical maps indicated a significant increase in area recruitment of the right dorsal area of the prefrontal cortex ($Z = 2.98$ $p = 0.035$) in patients compared to controls (Figure 3.7.).

On the other hand, the ICA maps showed an increase in functional connectivity in the posterior cingulate cortex and in the precuneus patients when MS compared to controls (Figure 3.8.) Further, while controls showed inverse, bilateral functional connectivity between the cuneus and the precuneus, angular, and the marginal gyri, in patients, these inverse correlations almost disappeared or completely shifted to areas occupying the associative visual and the primary somatosensory cortices (Figure 3.8.).

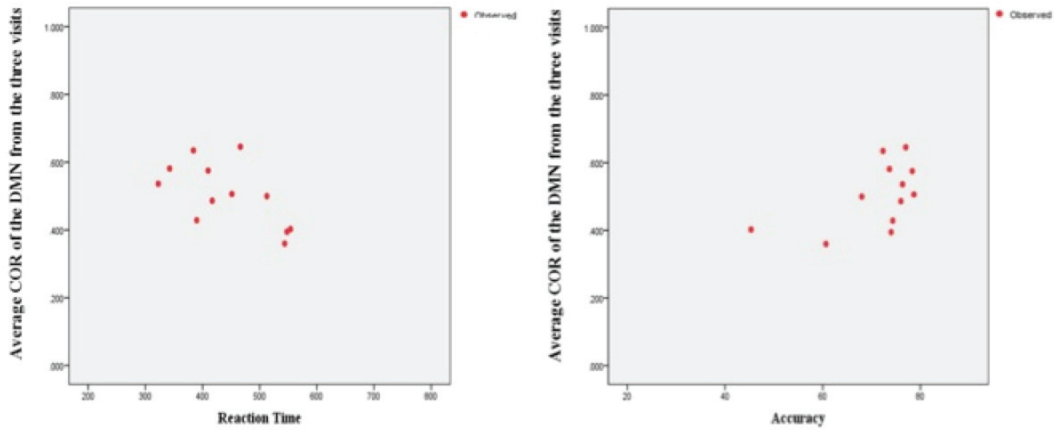


Figure 3.5. Graphs Showing Relationship between the Average of the Reliability Values of the Three Visits for the Default Mode Network vs. Reaction Time and Timed Accuracy of the Poffenberger Task for All Participants.

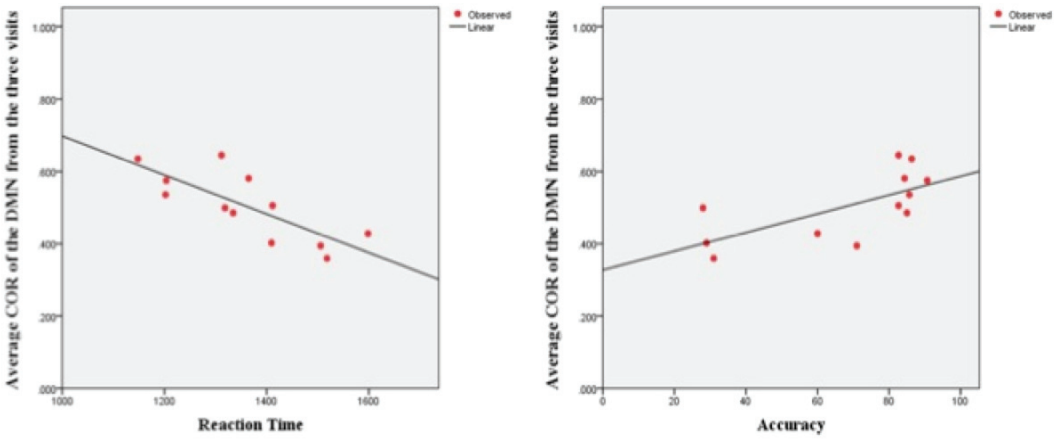


Figure 3.6. Regression Analyses Showing a Linear Relationship between the Average of the Reliability Values of the Three Visits for the Default Mode Network vs. Reaction Time and Timed Accuracy of the Symbol Digit Modalities Test for All Participants.

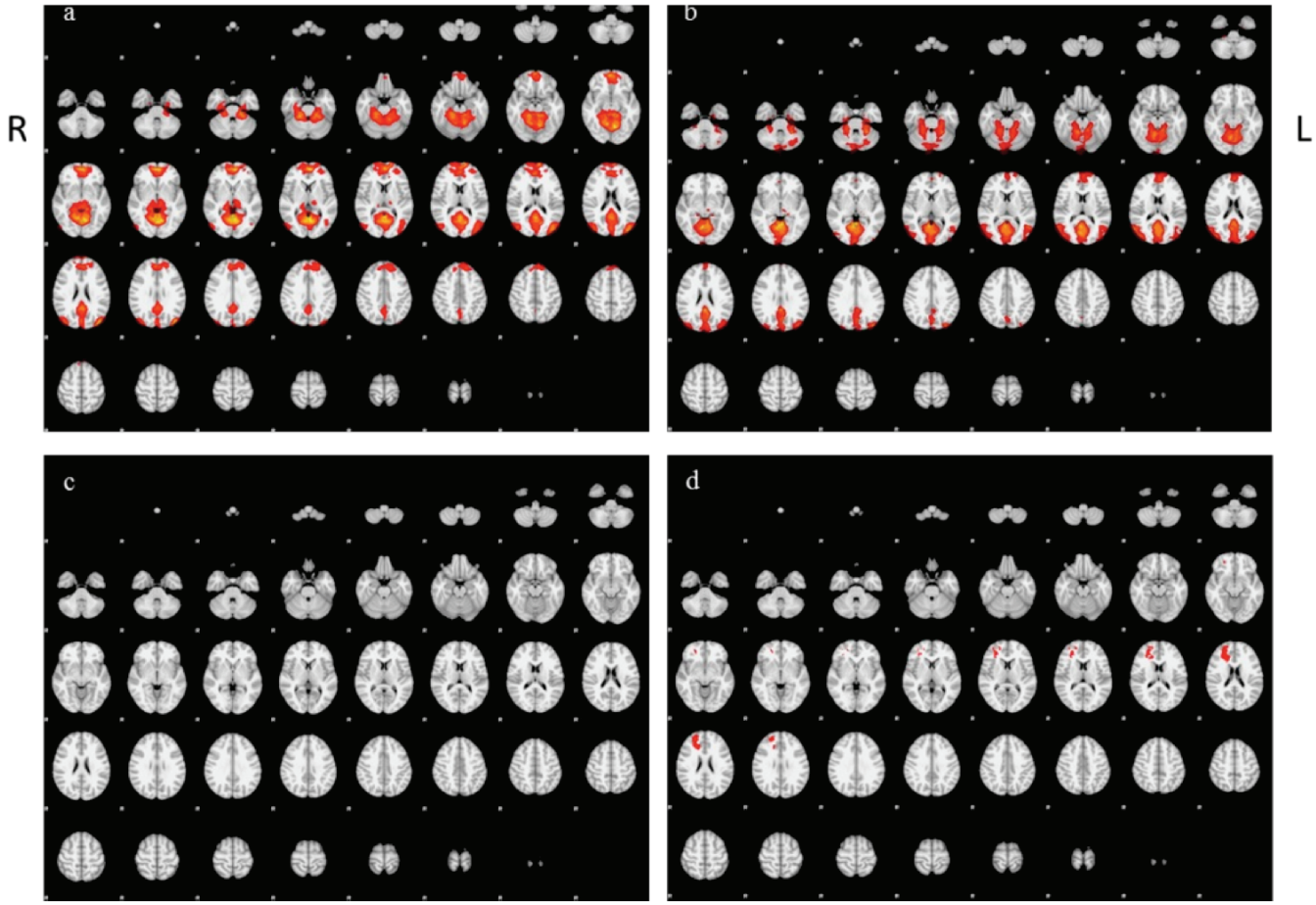
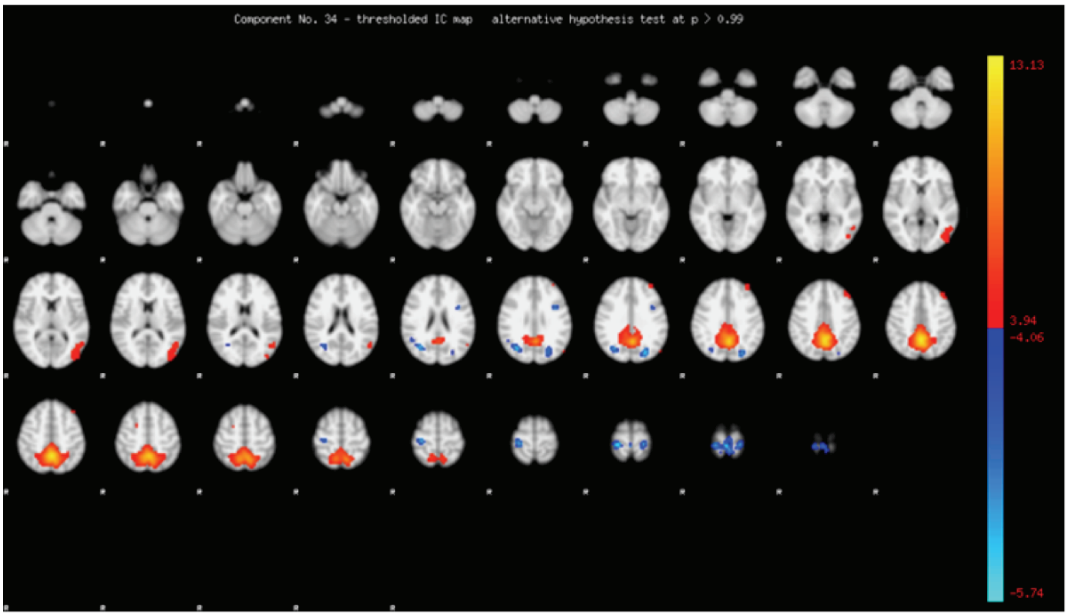


Figure 3.7. Thresholded Z-Statistic Group Maps of the Default Mode Network, Patients (a), Controls (b), and the Contrasts between the Two Groups; a < b (c) and a > b (d).

a



b

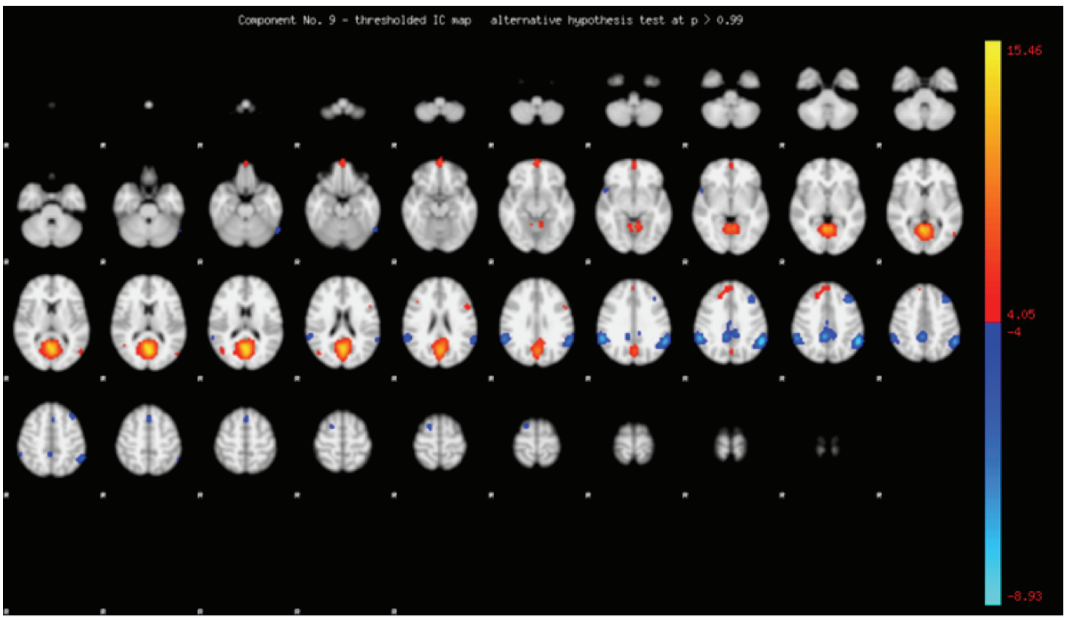


Figure 3.8. Independent Component Analysis Group Maps Depicting Functionally Correlated Areas, Default Mode Network (Red-Yellow) and Inversely Correlated (Blue-Light Blue) Areas for Patients (a) and Controls (b).

3.5. Discussion

The main goal of the current study was to assess single-subject level test-retest reliability of the DMN. Previously, altered functional connectivity depending on the stage of MS has been reported (Rocca et al., 2010c; Roosendaal et al., 2010b; Bonavita et al., 2011 Faivre et al., 2012; Basile et al., 2013). Functional connectivity seems to have a reverse association with the degree of cognitive impairment (Rocca et al., 2010b,c; Bonavita et al., 2011; Basile et al., 2013), but cognitive rehabilitation has been shown to partially restore functional connectivity of the DMN (Filippi & Rocca, 2012; Leavitt et al., 2012; Parisi et al., 2012). Consequently, examining activation and functional connectivity of the DMN may have potential value in monitoring patients with established MS.

3.5.1. Comparing Cluster Overlapping Reliability between Patients and Controls

Our main finding showed 57% COR for the control group and 45% for the patient group. This result is consistent with our hypothesis of higher COR values for controls compared to patients. However, this difference did not reach significance. As this is the first study to examine reliability of resting state in MS, we cannot compare our findings to previous ones, but our reliability of the DMN for the control group is consistent with those reported previously (Damoiseaux et al., 2006; Shehzad et al., 2009; Meindl et al., 2010). Short (same day to two weeks) reliability of RS-fMRI was tested in healthy cohorts, these studies reported similar reliability scores to our values (0.4-0.77; Damoiseaux et al., 2006; Shehzad et al., 2009; Meindl et al., 2010).

The lower reliability score for MS patients is interesting and should be further investigated, as it may be reflective to neuropathology underlying cognitive deficit. In support, former studies demonstrated that reliability of the DMN was greatly influenced by cognitive ability (Damoiseuax et al., 2012; Blautzik et al., 2013; Heister et al., 2013) and that the RS network connectivity weakened in neurological conditions associated with declining cognitive performance (Damoiseuax et al., 2012; Blautzik et al., 2013). The DMN reliability also appeared to be a good precursor of conversion from healthy aging to mild cognitive impairment, from mild cognitive impairment to dementia (Blautzik et al., 2013), and of the progression of Alzheimer's disease (Damoiseuax et al.,

2012). Consequently, it is possible that the variability of the DMN activation resulting low COR values could be a measure of the degree of neuropathology affecting cognitive performance, similarly to COR of task-driven fMRI, and as such it could be a meaningful way to monitor patients with established MS.

3.5.2. Comparing Cluster Overlapping Reliability and Cognitive Performance

To investigate the question whether COR of the DMN is related to slowing of information processing speed, one of the most common and the earliest cognitive impairment in MS (Roosendaal et al., 2010a,b; Bonavita et al., 2011; Wojtovicz et al., 2014), we explored whether there was an association between COR values of the DMN and the behavioural data from the previous study (i.e. reaction time and timed accuracy of the SDMT) and reaction time and timed accuracy of Poffenberger Task). We found a strong relationship between behavioral indices of the SDMT and COR of DMN activity (Figure 3.6.). Notably, we have found that the higher the reliability of the DMN (more stable DMN activity), the higher the cognitive performance (greater cerebral efficiency) in MS. This result is consistent with previously reported positive correlation between activity of the DMN and cognitive reserve (Sumowski et al., 2010, 2013a,b). Patients with a less intact DMN activation (i.e. more prefrontal recruitment) had more brain pathology and less cognitive reserve at earlier stages of MS (Morgen et al., 2007; Rocca et al., 2010b,c; Roosendaal et al., 2010a,b; Sumowski et al., 2010; Bonavita et al., 2011 Forn et al., 2013b). The current finding also strengthens our hypothesis as reliability of the activity of DMN may be a useful measure of neuronal damage. Specifically, it may be related to or mediating information processing. Another advantage of using RS-fMRI is that it can serve as a control scan to assess confounding factors or variances inherent to MS, such as cognitive fatigue also one of the main complaints in MS (DeLuca et al., 2008; Genova et al., 2013; Cook et al., 2007), ultimately influencing task-driven fMRI performance.

Timed accuracy of the Poffenberger Task did not show significant correlation with COR values, and the association between COR and reaction time of the Poffenberger Task was somewhat arguable (Figure 3.5.). Results of the Poffenberger Task may be due

to statistical power, but as noted previously, it would be important to further examine the role of the task in MS, as callosal damage is one of the core features of the disease.

3.5.3. Group Functional Activation and Connectivity between Patients with Multiple Sclerosis and Controls

As before, we also compared fMRI activity between the two groups, controls and patients with MS. Regarding the DMN activity, we have found a significant increase in the dorsal area of the prefrontal gyrus that has been connected to executive function and retrieval of contextual details (Petrides, 2000; Pochon et al., 2001; Leung et al., 2002). Our result supports previously reported findings, notably the compensatory hypothesis (Morgen et al., 2007; Rocca et al., 2010b,c; Bonavita et al., 2011). Although the contrast analysis did not show any significance, greater neuronal recruitment in other areas of the prefrontal cortex is also noticeable when compared patients to controls (Figure 3.7.).

We have also compared functional connectivity of the DMN between the two groups using ICA. Patients showed a different connectivity pattern compared to controls. The most striking difference was the shift of activation of the posterior cingulate cortex to the activation of the precuneus (Figure 3.8.). Further, controls showed bilateral activation of the cuneus, precuneus, angular, and the marginal gyri inversely correlated with the DMN. These inversely correlated areas completely disappeared in patients, and instead, inverse activation appeared in the associative visual and the primary somatosensory cortices (Figure 3.8.).

Inversely correlated functional networks with the DMN were demonstrated by Fox et al. (2005). They have been thought to increase their activity during attention demanding tasks and to denote the ability to increase task related and to suppress unrelated processes (Fox et al., 2005). Failure to suppress competitive neuronal networks has been shown to significantly impact behavioral performance, for example, failure to suppress activity in the posterior node of the DMN results in attentional lapses (Weissman, et al. 2006). Thus, the observed redistributed neuronal fluctuations might reflect compensatory or shifted mechanisms of the DMN or a dysregulation of switching on/off RS networks noted in earlier findings (Bukner et al., 2008) due to apparent and normal appearing neuropathology.

3.6. Conclusions

The current study highlights three key points; i. RS network reliability seems to be higher in controls compared to patients with MS and may be COR itself could be used as a measure of neuropathology; ii. low COR values appear to be associated with slowed information processing, and RS-fMRI may work as a good control task to rule out performance related variability in brain activation; and iii. in RRMS, there is an increased brain recruitment at rest involving prefrontal areas and altered functional connectivity involving the precuneus and primary somatosensory cortices, potentially associated with compensatory mechanism, or a dysregulation of switching on/off RS networks.

CHAPTER 4
GENERAL DISCUSSION

4.1. Limitations of the studies

There are several limitations of the current studies, with perhaps the most important one being the small sample size. This drawback impacted interpretation of the RS-fMRI data the most, and therefore, this reliability study should be repeated with a larger sample. Further, the fact that a number of scans had to be rejected due to movement is a reminder that patients as well as MRI naïve controls do have a time limit for staying still in the scanner. It is important to consider at what point prolonging the sequence or session starts to hinder the result in contrast to improve it. Of note, time constraint was the reason for not repeating functional runs and averaging them in each visit, which, naturally, would have increased our reliability values (Friedman & Glover, 2006; Bennett & Miller, 2010).

Unfortunately, many patients did not have data other than EDSS scores available, but maybe other measures used at clinical settings, including the Multiple Sclerosis Functional Composite that assesses ambulation, visuomotor, and cognitive performance would be a better fit for contrasting clinical disability, and COR in the future. As the EDSS is criticized for its emphasis on ambulation and the absence of adequate cognitive components.

The thalamus appears to be heavily involved in MS. There is evidence for functional changes related to cognitive performance as well as changes at rest (Mainero et al., 2004; Houtchens et al., 2007; Jones et al., 2013; Zhou et al., 2013; Tona et al., 2014). Provided by ROIs delineated by FreeSurfer software program (Martinos Center for Biomedical Imaging), we did not include thalamic areas in our ROI examining the COR of the SDMT and the Poffenberger Task. However, that is unlikely that reliability would have changed significantly by including the thalamus.

We also did not address cognitive fatigue in our patient group, which is a significant limitation. Cognitive fatigue is also one of the main complaints and is linked to altered brain activation in MS and other syndromes (DeLuca et al., 2008; Genova et al., 2013; Cook et al., 2007). Further, fatigue related to interferon-beta-1a treatment, which several

patients in the study were receiving, has also been shown to influence fronto-thalamic circuitry recruitment (Rocca et al., 2002). Perhaps cognitive fatigue and the related changes in brain recruitment is one of the reasons of the lower COR in patients compared to controls, and taking that into account in future analyses would yield valuable information.

Lesion load or atrophy is another key characteristic of MS that can alter brain activation and functional connectivity (Rocca et al., 2010c; 2012). Regarding the research objectives of measuring fMRI reliability, we did not measure either of those in the present study. However, we did control for lesion load and leasion load changes by acquiring FLAIR images once from controls and twice from patients with MS. Nevertheless, assessing structural abnormalities and incorporating them into the analyses will be essential in long-term studies.

Finally, we limited ourselves to examine the DMN. While the DMN is the best choice to measure reliability, less robust RS networks affected in MS and perhaps better correlated with cognitive performance (e.g. executive network) should be also concidered in the future.

4.2. General Conclusions and Future Directions

There is emerging evidence of the sensitivity of the fMRI to detect functional reorganization of the brain due to disease activity and functional recovery following treatment. Previous studies suggest the potential role of fMRI in monitoring patients with established MS. Reliability of fMRI is one of the major issues preventing the broader use of this imaging technique in clinical settings. Accordingly, in the current work, we addressed the reliability of task-driven and task-free fMRI in established MS. Our overall group reliability along with single-subject reliability scores for controls were comparable to reliability scores noted previously, which is notable as our control group consisted of individuals with a fair representation of the general population seeking medical care. Data of many experimental imaging studies arise from young and healthy university students typically showing less intra- and inter-subject variability, hence cleaner data, while our control group had an arguably greater extent of intra and inter-subject variability due to ageing. Ageing of the adult brain has been associated with variety of

anatomical tissue changes in GM, WM, and more importantly from the fMRI point of view changes in the vasculature. For that reason, demonstrating a fairly high reliability in a middle-aged group is encouraging regarding the role of fMRI in clinical monitoring in the future.

The current work focused on short-term test-rest COR. As reliability tends to decrease with increasing interval, assessing medium (six months) and long-term (one year) COR in controls would be central to establish the general stability of these fMRI tasks.

We also demonstrated overall higher COR for controls compared to patients for all of our tasks. As many external factors, such as practice, the time of the scanning, and patient status (e.g. medication, symptoms, and relapse), were kept constant as possible, the overall lower COR in patients indicates that patients exhibited unstable neuronal recruitment even when obvious signs or symptoms of the disease or any changes in medication status were not present. Instability of neuronal recruitment corresponds with the nature of the disorder, regarding cognitive function as well as other neurological or neuropsychiatric signs and symptoms. Therefore, measuring the fluctuation of neuronal recruitment (i.e. using COR scores) may be a better predictor of the degree of brain pathology or recovery in MS. Further, instability of neuronal activation is perhaps more reflective of transient or repairable neuronal injury, which is the major focus of MS treatment, than changes seen over time (e.g. after one year follow-up). Low COR may indicate constant neuronal re-organization of areas taking over new functions from damaged regions. Thus, based on COR values and cognitive performance patients can receive targeted therapy to reinforce new functions and connections of certain brain areas thereby increasing efficiency of cognitive processes and alleviating cognitive fatigue. Following this line of reasoning, COR should increase subsequent to well-targeted rehabilitation.

To investigate whether neuronal instability is inherent to MS, first reliability of a very short-term interval (same day) should be evaluated. It would be also interesting to know how functional score/disease status effects stability of neuronal recruitment (i.e. COR) in a very short term. Our view is to see high same–day COR in controls and lower COR

values in patients. In addition, patients' reliability scores should correspond with their cognitive test results and functional scores.

With reference to the association between COR and cognitive performance, the relationship between COR values of RS-fMRI and indices of information processing speed demonstrated here is congruent with previous findings. This is an informative finding as measuring RS networks may allow predicting cognitive impairment in patients with confounding disabilities, such pain or weakness in the upper limb or hand digits. In the future, it would be also interesting to see, and it may be a useful information concerning rehabilitation, how an improving performance on these cognitive tasks impact COR of RS-fMRI.

We limited ourselves to measure COR only of the DMN. Although the DMN is the most robust, a few other RS networks exist that could provide helpful information about cognitive and other neurological processes relevant to MS, including the executive control, the sensory/motor, and the visual network.

In case of the SDMT task, our result indicated that low COR values are region specific. While patients had a slight difference in COR of GM, COR of WM was strikingly lower compared to controls. This outcome raises the issue of separating WM and GM more accurately to assess functional along with structural changes of WM in this patient population. The increasing number of higher field clinical scanners (i.e. 3 T) and application of suitable imaging sequences should allow better detection of the BOLD signal in WM. As previously demonstrated, field strength of the MRI (Mazerolle et al., 2013a) and using asymmetric spin echo sequence (Brewer et al., 2008; Gawryluk et al., 2009) can improve WM fMRI activation. While there is evidence of detecting the BOLD signal in larger WM tracts, advancing resolution of the fMRI to measure hemodynamic changes in smaller WM fibres without the contamination of the GM signal and thus to better characterize tissue specific pathology would be an important step in the future. Further, as described, structural imaging techniques sensitive to microscopic neuropathology are also available, such as Myelin Water Imaging (MacKay et al., 1994) and Diffusion Tensor Imaging (Basser et al., 1994). These advanced imaging techniques can provide information on subtle and microscopic injuries impacting functional changes and compensatory mechanisms present in MS. The combination of these techniques

would likely improve our understanding of disease activity at neuronal level, and would help to provide prompt and effective intervention to preserve brain tissue and prevent permanent, gross neuropathology.

It is interesting that correlations of EDSS with COR in GM and in WM are similar, which would indicate that tissue injury in both areas contribute equally to symptomology of MS. Based on our current knowledge of underlying neuropathology of RRMS, WM instability should show higher correlation with the functional scores (EDSS) compared to GM instability. A more precise separation of WM and GM fMRI may remedy this controversy. On the other hand, it is possible that using a clinical tool that better correlates with cognitive function than the EDSS does, such as the Multiple Sclerosis Functional Composite, would have produced more tissue specific association between disease/ cognitive status and neuronal instability.

As dysfunction of specific areas, such as the anterior cingulate cortex during RS-fMRI has been proposed to be a characteristic of MS, one might wonder whether measuring COR of a specific area as opposed to examining the whole brain increases the power of the study and therefore, it is a better choice. We have chosen to examine the whole brain as neuronal reorganization and compensatory mechanisms can be at large scale and are highly patient specific. Prior determination of the ROI may have led to overlooking important other brain areas contributing to individual symptomology.

Among the three examined paradigms, the results of the SDMT are probably the most promising. In part, because it has resulted in the most straightforward and most congruent findings with our expectations, and in part, because it is already validated and commonly used in MS. Therefore, perhaps the SDMT should be the major focus of future fMRI reliability studies. Nevertheless, the role of the Poffenberger Task should be further examined in the disorder, along with the testing of the DMN in different conditions, such as how fatigue, medication, or pain effects activation and functional connectivity.

There are several factors influencing reliability that cannot be changed, but should be monitored in the future, including cognitive fatigue, pain, or spasticity. Effect of periodic treatment on COR, such as a monthly infusion, is also unknown. On the other hand, there are factors that can be adjusted to our advantage, such as practice of the cognitive tasks, using breaks between long scans, as well as careful sequence and paradigm planning

based on the patient needs and ability. One advantage of single-subject fMRI over group fMRI is that it allows certain variability in task administration (e.g. order or the length of the paradigms).

Reliability of fMRI has been the focus of interest, as fMRI provides abundant possibilities for in vivo assessment of the human brain. However, the acceptable threshold for fMRI reliability has not yet been established. As the BOLD signal is affected by numerous factors, such as the complexity of data analysis, and because the neurological basis of the hemodynamic response is not yet fully understood, establishing globally accepted and standardized criteria for fMRI reliability entails a tremendous task in the future. Our results suggest that apart from measuring fMRI reliability, COR may be a marker for neuronal instability in MS, and therefore further investigations regarding its role should be considered at high priory. If COR truly reflects neuronal instability, it may play an important role in well-targeted therapy and rehabilitation of cognitive impairment in MS. Rehabilitation can be designed to help new brain regions to take over and promote new functions and stabilize neuronal networks, and thus enhance cognitive efficiency and potentially alleviate cognitive fatigue in established MS. Accordingly, using COR as a means of assessing neuropathology through functional instability in MS is perhaps a more realistic choice for the immediate future.

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