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BRAIN ACTIVATION AND CONNECTIVITY IN NON-DISABLED MULTIPLE SCLEROSIS PATIENTS

Rene A. Colorado

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BRAIN ACTIVATION AND CONNECTIVITY IN
NON-DISABLED MULTIPLE SCLEROSIS PATIENTS

A
DISSERTATION

Presented to the Faculty of The University of Texas Health Science Center at Houston
and The University of Texas M. D. Anderson Cancer Center

Graduate School of Biomedical Sciences

in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

by

René Alfonso Colorado, B.S.

Houston, Texas

August, 2011

DEDICATION

I dedicate this thesis to my sister Dr. Claudia P. Colorado and my brother Rodrigo J.

Colorado who have not stopped caring for me since the day I was born.

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BRAIN ACTIVATION AND CONNECTIVITY IN
NON-DISABLED MULTIPLE SCLEROSIS PATIENTS

Publication _____

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Multiple sclerosis (MS) is the most common demyelinating disease affecting the central nervous system. There is no cure for MS and current therapies have limited efficacy. While the majority of individuals with MS develop significant clinical disability, a subset experiences a disease course with minimal impairment even in the presence of significant apparent tissue damage on magnetic resonance imaging (MRI). The current studies combined functional MRI and diffusion tensor imaging (DTI) to elucidate brain mechanisms associated with lack of clinical disability in patients with MS. Recent evidence has implicated cortical reorganization as a mechanism to limit the clinical manifestation of the disease. Functional MRI was used to test the hypothesis that non-disabled MS patients (Expanded Disability Status Scale ≤ 1.5) show increased recruitment of cognitive control regions (dorsolateral prefrontal and anterior cingulate cortex) while performing sensory, motor and cognitive tasks. Compared to matched healthy controls, patients increased activation of cognitive control brain regions when performing non-dominant hand movements and the 2-back working memory task. Using dynamic causal modeling, we tested whether increased cognitive control recruitment is associated with alterations in connectivity in the working memory functional network.

Patients exhibited similar network connectivity to that of control subjects when performing working memory tasks. We subsequently investigated the integrity of major white matter tracts to assess structural connectivity and its relation to activation and functional integration of the cognitive control system. Patients showed substantial alterations in callosal, inferior and posterior white matter tracts and less pronounced involvement of the corticospinal tracts and superior longitudinal fasciculi (SLF). Decreased structural integrity within the right SLF in patients was associated with decreased performance, and decreased activation and connectivity of the cognitive control system when performing working memory tasks. These studies suggest that patient with MS without clinical disability increase cognitive control system recruitment across functional domains and rely on preserved functional and structural connectivity of brain regions associated with this network. Moreover, the current studies show the usefulness of combining brain activation data from functional MRI and structural connectivity data from DTI to improve our understanding of brain adaptation mechanisms to neurological disease.

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LIST OF ABBREVIATIONS

25TW	25-foot timed walk
AAL	Automatic Anatomic Labeling
AD	axial diffusivity
ACC	anterior cingulate cortex
BOLD	blood-oxygen level dependent
BA	Brodmann area
BMS	Bayesian model selection
CIS	clinically isolated syndrome suggestive of the first clinical manifestation of multiple sclerosis
CNS	central nervous system
CS	control subjects
CST	corticospinal tract
DCM	dynamic causal modeling
DLPFC	dorsolateral prefrontal cortex
DTI	diffusion tensor imaging
DTT	DTI-based fiber tractography
DWI	diffusion weighted image
EPI	echo planar imaging
FACT	Fiber Assignment by Continuous Tracking
FLAIR	fluid-attenuated inversion recovery
fMRI	functional MRI
FA	fractional anisotropy

FMaj	forceps major of the corpus callosum
FMin	forceps minor of the corpus callosum
FSL	FMRIB's Software Library
FWE	family wise error
ILF	inferior longitudinal fasciculus
IFOF	inferior fronto-occipital fasciculus
MD	mean diffusivity
mm	millimeter
MNI	Montreal Neurological Institute
MPRAGE	magnetization prepared rapid acquisition of gradient echo
MRI	magnetic resonance imaging
MS	multiple sclerosis
ms	millisecond
MSFC	Multiple Sclerosis Functional Composite
NHPT	Nine Hole Peg Test
PASAT	Paced Auditory Serial Addition Task
PFC	prefrontal cortex
PPC	posterior parietal cortex
PPMS	primary progressive MS
RD	radial diffusivity
ROI	region of interest
RRMS	relapsing remitting MS
rTMS	repetitive transcranial magnetic stimulation

SD	standard deviation
sec	second
SLF	superior longitudinal fasciculus
SMA	supplementary motor area
SPMS	secondary progressive MS
SPM8	Statistical Parametric Mapping 8
TBSS	tract-based spatial statistics
TE	echo time
TI	inversion time
TR	repetition time
VOI	volume of interest
WM	white matter

CHAPTER 1

INTRODUCTION

Multiple Sclerosis

Multiple Sclerosis (MS) is an inflammatory and chronic neurodegenerative disorder affecting approximately 400,000 individuals in the U.S. and 2.5 million worldwide. It is the most prevalent demyelinating disease affecting the central nervous system (CNS) and a leading non-traumatic cause of irreversible disability in young adults. Most MS patients are diagnosed between their second and fifth decade of age and median age of onset is 28 years (Wingerchuck et al. 2001). MS is characterized by a complex and heterogeneous clinical course that can result in a wide range of neurological deficits including motor impairment, sensory disturbances, visual loss, and cognitive dysfunction. Patients with MS follow different disease courses (Lublin et. al 1996): a) relapsing remitting (RRMS), b) secondary progressive (SPMS), c) primary progressive (PPMS), and d) progressive relapsing (PRMS). The majority of patients at diagnosis (85%) begin with a RRMS disease course characterized by clearly distinct neurologic attacks, or relapses, with complete or partial recovery (remissions). Most of these patients (50% after 10 years and approximately 90% after 25 years) ultimately enter the SPMS course defined by continuous disease progression with or without occasional attacks and minor remissions. Approximately 10% of patients at diagnosis start with PPMS disease course characterized by disease progression from disease onset with occasional plateaus and temporary minor improvements but no discrete relapses or remissions. PRMS, affecting approximately 5% of patients, begins with a progressive disease course with clear relapses, with full or partial recovery and continuing progression between relapses.

Magnetic Resonance Imaging in MS

Magnetic resonance imaging (MRI) has emerged as an important tool in the diagnosis and management of MS (McDonald et al. 2001, Polman et al. 2011) and it has become an important complementary component to clinical outcomes in MS clinical trials (Li et al. 2006). For instance, in patients in whom clinical evidence of MS is not satisfactory for diagnosis, detection of MS lesions using MRI can supplement and be sufficient to diagnose MS allowing earlier therapeutic intervention (McDonald et al. 2001, Polman et al. 2011). Nonetheless, the use of MRI as surrogate marker(s) of disease activity is complicated by the modest correlation between clinical disability and radiological measures of tissue injury, or the so-called clinical-radiological paradox (Barkhof 2002). For instance, patients with extensive tissue injury, as seen by MRI, may have mild disability while others with low lesion load may be quite impaired. Several factors have been suggested to explain this paradox. These factors include the limitations of current clinical disability measures, the fact that most studies rely on a single MRI measure (e.g. T2 lesion volume, gadolinium enhancement), and ignoring repair mechanisms of the nervous system such as remyelination and clearance of inflammatory agents. Moreover, some lesions may be clinically silent while others may be more clinically apparent such as those involving the brain stem or the spinal cord. Finally, underreporting of symptoms and under recognition of signs may also contribute to the clinical-radiological paradox.

Over the last decade, accumulating evidence shows that patients with MS demonstrate altered patterns of cortical activity that may serve as a compensatory mechanism and prevent the clinical manifestations of neurological damage (Pantano et al., 2006; Pelletier et al., 2009; Reddy et al. 2000b). Reorganization of function in areas

disrupted by the disease to alternate areas may help patients to preserve function and largely explain the clinical-radiological mismatch between tissue injury and functional status. Evidence for cortical reorganization comes from multiple motor, visual and cognitive studies using functional MRI (fMRI), the dominant technique for non-invasively mapping neuronal activity. By detecting changes in blood oxygen levels, fMRI provides information about the neural regions activated during the performance of a task while the subject is being scanned. MS patients generally show increased and/or additional cortical activation relative to healthy control subjects.

Cortical Reorganization in MS

Altered patterns of cortical activity, or cortical reorganization, in MS was first described by Rombouts *et al.* in a group of patients with RRMS diagnosed with optic neuritis (Rombouts et al. 1998). They reported reduced visual cortex activation when both the affected and unaffected eyes were visually stimulated. Interestingly, they observed a trend of greater activation in recovered patients upon stimulation of either the affected or unaffected eye. Werring et al.(2000) investigated patients that completely recovered from optic neuritis and found that when stimulating the affected eye, extensive activation was seen in areas including the insula, claustrum, thalamus, and the lateral aspect of the temporal and posterior parietal cortices, in addition to activation of the visual cortex (Werring et al. 2000). On the other hand, when the unaffected eye was stimulated, only the visual cortex and the insula and claustrum were activated. Interestingly, these authors found a strong association between activated extraoccipital cortex volume and visual evoked potential latency, which is a measure of optic nerve abnormalities. These authors concluded that the

functional reorganization to visual stimulus following optic neuritis may represent a compensatory response to an abnormal input.

Cortical reorganization in MS has been well characterized while patients performed motor tasks. In an fMRI study of non-disabled RRMS patients performing hand flexion and extension, increased activation was observed in the motor cortices including the contralateral somatomotor cortex and supplementary motor areas bilaterally compared to healthy subjects (Rocca et al. 2002a). MS can present, prior to confirmed diagnosis, as a clinically isolated syndrome (CIS) involving one single site of neurological deficit such as the optic nerve. fMRI studies have shown that cortical reorganization occurs even this early in the disease course. CIS patients who underwent fMRI during a simple motor task were found to activate larger number of regions than healthy controls, which included the premotor cortex in the contralateral and ipsilateral hemispheres (Pantano et al. 2002a). Altered motor cortical activation occurs in the progressive stages of the disease and has been observed in patients with both SPMS (Rocca et al. 2003) and PPMS (Roca et al. 2005). These studies show that in addition to activating motor areas, patients in the progressive courses recruit multiple non-motor cortical networks during the performance of simple motor tasks.

Several authors have also reported cortical reorganization in MS while performing complex cognitive tasks involving attention and working memory but results are variable. Some studies found greater areas of activation in response to a given task (Audoin et al., 2003, 2005; Staffen et al., 2002; Sweet et al., 2004), others described no differences (Meyn et al., 2010), and still others reported decreased activation (Cader et al., 2006; Wishart et al., 2004). The difficulty in controlling for performance differences and disability levels may

have contributed to this variability in findings. For example, Mainiero et al. (2004), in a group of 22 RRMS patients, using a recall task and the Paced Auditory Serial Addition Task (PASAT) have reported increased activation. These authors found that during both tasks, patients had greater cortical activation in several prefrontal regions compared to healthy control subjects. Interestingly, after dividing patients by cognitive function based on task performance, they found that cortical activation was greater in those with better performance. In another study using three tasks of attention of increasing complexity, 14 MS patients were classified based on neuropsychological testing as having mild or severe cognitive impairment (Penner et al. 2003). The mildly impaired patients exhibited an increased and additional recruitment of brain areas primarily within the frontal and posterior parietal cortex. Those with severe impairment did not exhibit increased recruitment of the prefrontal cortex and there were no differences in activation of the premotor cortex compared to healthy subjects. These studies suggest that cognitive compensation in MS patients is dependent upon the capacity to recruit additional brain regions and that exhaustion of this mechanism may give rise to severe cognitive dysfunction.

The current knowledge gained from fMRI studies in MS is summarized by the recently proposed MS disease progression hypothesis by Schoonheim et al. (2010), which suggests that initial structural damage causes brain hyperactivation, which results in low disability and cognitive preservation, but after this hyperactivation peaks, progressive cognitive impairment and disability ensue (Fig. 1.1).

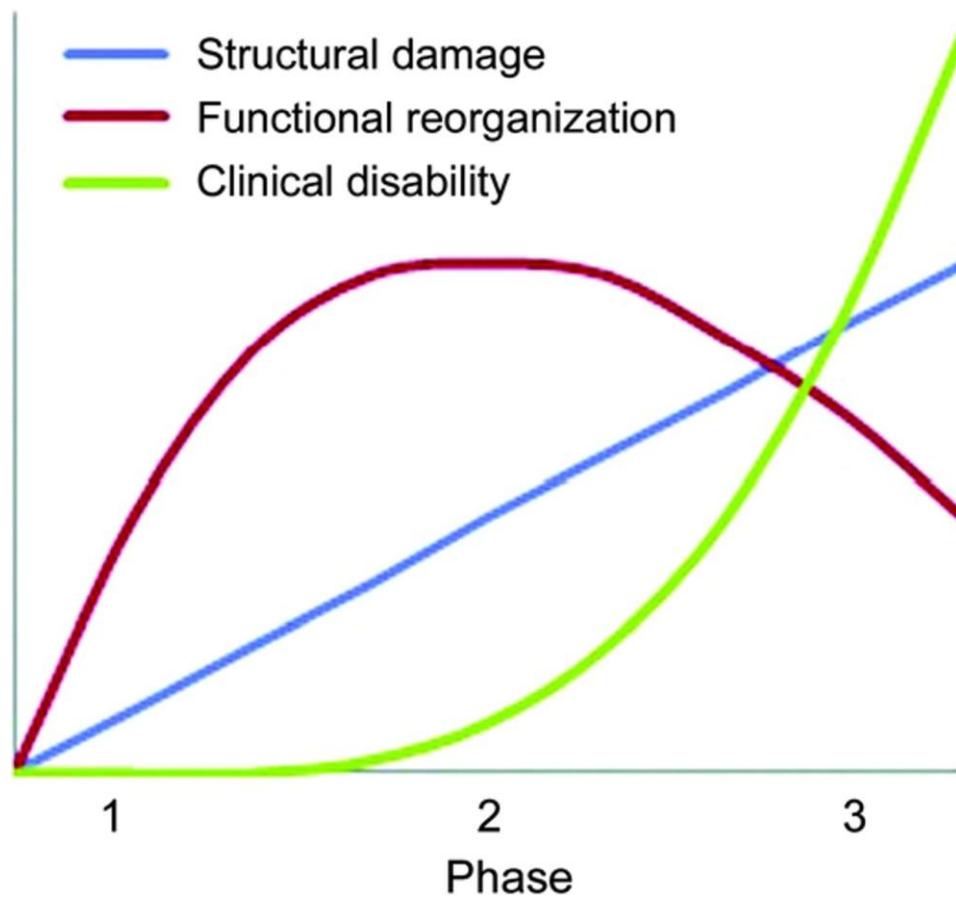


Figure 1.1 Relationship of clinical disability, structural damage and functional reorganization in MS as proposed by Schoonheim et al. (2010). Initially, very little structural damage causes a strong response in functional reorganization and hyperactivation in the brain, resulting in low disability and cognitive preservation in phase 1. After functional reorganization reaches its peak in phase 2 and decreases thereafter, cognitive impairment and disability progressively develop throughout phase 3. Reproduced with permission from: Schoonheim et al. 2010.

To date, most functional imaging studies in MS patients have investigated brain activation in a single functional neurological system (e.g. motor or working memory system). Increased functional recruitment in MS patients has been interpreted as representing reorganization of cortical regions as they pertain to the functional system investigated (e.g. motor cortical reorganization). However, there is limited knowledge about putative brain activation mechanisms that may not only be compensatory during the performance of specific tasks but may occur across different functional domains. Recent evidence from fMRI studies of working memory suggests that the specific recruitment of cognitive control regions within the prefrontal cortex (dorsolateral prefrontal cortex and anterior cingulate cortex) may be a general mechanism allowing patients to cope with increasing cognitive demands and accommodate disease-related neural dysfunction (Au Duong et al., 2005; Audoin et al., 2008; Hillary et al., 2006, 2008,).

Activation studies in MS have been essential to identify the brain's ability to elicit altered cortical activation patterns during the performance of tasks. However, behavioral responses result from the interaction of brain regions forming networks and not simply the result of isolated regional activity. Simple processes such as moving a finger involve the interaction of multiple motor regions. Therefore, determining activation levels of isolated regions when patients perform a task does not provide a complete understanding of the brain mechanisms that patients utilize to compensate for neural disruption. Changes in the interactions, or connectivity, between brain networks may also be involved in cortical reorganization in MS.

Brain connectivity can be characterized in two broad categories; one relating to the functional interaction among regions when they are active (effective connectivity) and one

to the structural components that allow regions to interact (structural connectivity). Effective connectivity is determined by the influence that one region exerts over another when performing a task and can only be determined by activating brain regions. Application of dynamic causal modeling (DCM) (Friston et al. 2003) to fMRI data provides information about effective connectivity between brain regions when a subject performs a task and determine the direct influence that one brain region exerts over another (Friston et al. 2003, Friston 2009b).

Structural connectivity is task-independent and refers to the physical components (i.e. axons) that allow cortical regions to interact among each other when active. Measuring this type of connectivity is essential to understand brain function because compromised structural connectivity may result in diminished interaction between brain regions and overall CNS dysfunction. Diffusion tensor imaging is a MRI technique that uses diffusion of water molecules within tissue to infer structural integrity. Isotropic diffusion is the unrestricted motion of water molecules. Conversely, restriction of diffusion by myelin and axonal membranes within white matter result in directional water diffusion, or anisotropic diffusion, along the orientation of axons. Disruption to myelin or axonal membranes result in changes in diffusion that can be quantified by DTI parameters including fractional anisotropy (FA), mean (MD), axial (AD) and radial diffusivities (RD). FA quantifies directionality of water diffusion while MD provides a measure of overall diffusion and both are use as indicators of overall white matter integrity (Wozniak and Lim 2006). Diffusion anisotropy can be parcellated into components that reflect the degree of diffusion that is axial (AD) or radial (RD) to the main white matter tract and can provide more specific

information about tissue integrity than MD or FA (Herrera et al. 2008; Klawitter et al. 2011; Narayana et al. 2010; Song et al. 2005; Xu et al. 2008).

Central Hypothesis and Specific Aims

Given the limited knowledge of cortical reorganization patterns that may occur in multiple functional domains and the structural and effective connectivity that may accompany these changes, an investigation combining fMRI and DTI was undertaken to better understand the underlying mechanisms of cortical reorganization in MS. Patients with MS and without clinical disability were recruited for the current studies since cortical reorganization is highly expressed in these patients (Schoonheim et al 2010). As detailed in the following chapters, multiple MRI modalities and analyses were used to uncover the functional and connectivity changes in the brain networks of these patients by testing the following hypothesis.

CENTRAL HYPOTHESIS

MS patients without clinical disability show increased activation of common brain networks across functional systems that are mediated by changes in functional and structural connectivity.

SPECIFIC AIMS

1. Determine cortical recruitment in patients with MS without clinical disability across multiple functional systems. The hypothesis that non-disabled MS patients show increased recruitment of cognitive control regions within the prefrontal cortex across sensory, motor and cognitive tasks is tested. Blood-oxygen level dependent (BOLD) signal changes were

measured using fMRI in patients with RRMS with no detectable clinical disability as assessed by the Expanded Disability Status Scale (EDSS) while they observed flashing checkerboards, performed right and left hand movements, or executed the N-back working memory task.

2. Investigate if alterations in cortical activation in MS patients without clinical disability are mediated by changes in effective connectivity. The hypothesis that MS patients show altered functional integration of cognitive control regions during the performance of the N-back task is tested. DCM is used to model functional interactions of the working memory system network comprising prefrontal and posterior parietal cortical regions.

3. Investigate brain structural connectivity and its relation to prefrontal cortex function in MS patients without clinical disability. Microstructural integrity of major white matter tracts is investigated in non-disabled MS patients. Using DTI fiber tractography and tract-based spatial statistics (TBSS) diffusion measures associated with tissue structural integrity are obtained in major white matter tracts and correlated to measures of cortical activation and effective connectivity during the performance of the N-back task.

Significance

A better understanding of cortical reorganization in MS patients and the functional and connectivity changes that may accompany it may allow us to exploit these plasticity mechanisms of the mature CNS with behavioral, pharmacological and nonconventional

interventions, and ultimately improve clinical recovery. Second, identifying these changes may provide us with a better monitoring tool for the evaluation of efficacy of therapies that, for instance, are targeted at augmenting intrinsic neuroplasticity. These findings may allow customized patient treatment. Third, this knowledge would advance our understanding of the heterogeneity of disease progression in MS and may clarify, for instance, why some patients with RRMS progress to SPMS after 10 years while others do so after 25 years. This ultimately would empower physicians to provide a more accurate disease prognosis. Finally, the implications of identifying the processes that lead to cortical reorganization would have great impact not only for patients with MS but other neurological diseases because cortical reorganization appears to be a common adaptive mechanism of the CNS.

MATERIALS AND METHODS

Subjects

Twenty two patients with clinically definite MS (Polman et al., 2011) were recruited from the MS Clinic of The University of Texas Medical School at Houston. Inclusion criteria were: relapsing-remitting course of the disease, absence of neurological abnormal signs in the upper limbs, and EDSS score of ≤ 1.5 . The EDSS is the most widely used clinical instrument in MS and it quantifies disability in eight functional neurologic systems; a score of 0 implies no disability while a score of 10 means death due to MS. The functional systems are: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral, and other. A detailed clinical neurological examination and EDSS scoring was performed by an experienced neurologist within one month prior to imaging, except for one patient who was scanned two months later. All patients were in remission from the time of clinical

examination until the imaging session. The Multiple Sclerosis Functional Composite (MSFC) was administered to the patients on the day of scanning (Cutter et al., 1999). This battery involves assessment of upper extremity function with the Nine Hole Peg Test (NHPT), lower extremity function with the 25-foot timed walk (25FTW) and sustained attention and information processing speed with the three-second PASAT. During this task, subjects must add verbally presented single digits to the one preceding it every three seconds. A total of 60 trials were conducted. The Edinburgh Handedness Inventory (Oldfield, 1971) was administered to control for hand dominance during motor tasks. The control group consisted of 23 age and gender matched healthy volunteers with no history of psychiatric or neurological disorders and normal MRI. Both groups had at least 13 years of education. The study was approved by our Institutional Review Board and all subjects provided a written informed consent. Demographic, clinical characteristics and behavioral performance of the subjects are presented in Table 1.1.

MRI data acquisition

Subjects underwent one MRI session lasting approximately 60 minutes on a 3.0 T Philips Intera MRI scanner with a Quasar gradient system (maximum gradient amplitude 80 mT/m, slew rate 200 T/m/s) and an 8-channel head coil (Philips Medical Systems, Best, Netherlands). The structural MRIs included a 3D high-resolution T1-weighted magnetization prepared rapid acquisition of gradient echo (MPRAGE) sequence (echo time (TE) 3.7 ms, repetition time (TR) 8.1 ms, 1.0 mm isotropic resolution and FOV of 256 mm × 256 mm × 170 mm), a 3D T2-weighted sequence (TE 362.9 ms, TR 2500.0 ms), a fluid-attenuated inversion recovery (FLAIR) sequence (TE 337.2 ms, TR 8000.0 ms, inversion

time (TI) 2400.0 ms) and a diffusion-weighted sequence (DWI; TE 55.0 ms, TR 8000.0 ms, 3.0 mm \times 44 slices, matrix 128², FOV 256² mm², 32 diffusion gradient directions, b = 1000 s/mm²). The image geometry for both T2-weighted and FLAIR is identical to that of the MPRAGE. The fMRI data was collected using a gradient echo echo planar imaging (EPI) sequence with the following parameters: TE 30.0 ms, TR 2015.0 ms, 3.0 mm \times 33 slices, matrix 80 \times 80, FOV of 220 mm \times 220 mm, 90° flip angle.

Data Preprocessing and Analysis

Preprocessing and statistical analysis of data, as described in detail in each chapter, was conducted using the following software: Statistical Parametric Mapping 8 (SPM8) (Wellcome Trust Centre for Neuroimaging, University College London, UK) implemented in Matlab (Mathworks, Natick, Massachusetts), FMRIB's Software Library (FSL) version 4.1 (Smith et al., 2004), DTI Studio (Johns Hopkins University, Baltimore, Maryland) (Mori et al. 2002), and Stata version 10.0 (Stata Corporation, College Station, Texas).

Table 1.1 Demographics, clinical data and task performance.

	Patients	Controls	p*
Total (females/males)	18/3	21/7	
Age, mean +/- sd	41.4 +/- 10.2	38.1 +/- 12.5	0.38
Duration since diagnosis, mean +/- SD	7.4 +/- 6.68	n/a	
Duration since first symptoms, mean +/- SD	10.2 +/- 7.35	n/a	
EDSS, median (range)	0 (0-1.5)	n/a	
PASAT score, mean +/- sd	49.82 +/- 7.8	n/a	
25-foot timed walk			
Trial 1, mean time +/- SD (sec)	4.6 +/- 1.2	n/a	
Trial 2, mean time +/- SD (sec)	4.5 +/- 0.9	n/a	
Time to complete Nine Hole Peg Test			
Mean time right hand +/- SD (sec)	18.8 +/- 2	17.9 +/- 1.6	0.14
Mean time left hand +/- SD (sec)	19.1 +/- 2	18.8 +/- 2.5	0.68
N-back 0-back condition performance			
Mean accuracy +/- SD (% correct)	95.8 +/- 2	94.6 +/- 6	0.42
Mean reaction time +/- SD (sec)	0.55 +/- 0.07	0.55 +/- 0.07	0.94
N-back 2-back condition performance			
Mean accuracy +/- SD (% correct)	87.3 +/- 4	87 +/- 6	0.86
Mean reaction time +/- SD (sec)	0.77 +/- 0.1	0.77 +/- 0.2	0.88

* *P* values are for an unpaired two-sample student's *t*-test.

Note: data is shown for the group of subjects used for the fMRI motor studies.

CHAPTER 2

MULTI-TASK fMRI IN NON-DISABLED MS PATIENTS

INTRODUCTION

Multiple sclerosis commonly results in visual and motor dysfunction as well as cognitive impairment in 40 to 70% of patients (Chiaravalloti and DeLuca, 2008). While the majority of individuals afflicted with MS accumulate clinical disability throughout their lifetime, approximately one-quarter of them experiences a course with minimal or no detectable disability (Ramsaransing et al., 2001) even in the presence of significant lesion load on MRI (Strasser-Fuchs et al., 2008). The brain mechanisms that may allow these patients to escape developing clinical disability are poorly understood.

As previously described, fMRI studies provided some insight to this question. As summarized by Schoonheim et al. (2010), initial structural damage causes brain hyperactivation, which results in low disability and cognitive preservation, but after this hyperactivation peaks, progressive cognitive impairment and disability ensue. While this hypothesis elegantly describes the temporal pattern of altered brain activation observed in MS patients, it also illustrates the lack of specific mechanisms that may prevent development of disability in patients, especially in patients who follow a milder course.

A recent meta-analysis of 33 functional imaging studies suggests that altered patterns of brain activation during working memory tasks may represent increased use of the cognitive control system (Hillary, 2008). Across several clinical populations including MS, there was a consistent recruitment of cognitive control regions, including the dorsolateral prefrontal cortex (DLPFC) and, to a lesser extent, the ventrolateral prefrontal cortex and anterior cingulate cortex (ACC) (Audoin et al., 2003; Bobholz et al., 2006; McAllister et al., 1999; Perlstein et al., 2003; Sweet et al., 2004). It has been proposed that increased use of the cognitive control system may be a mechanism to allow patients to cope with increasing

cognitive demands and compensate disease-related neural dysfunction (Au Duong et al., 2005; Audoin et al., 2008; Hillary et al., 2006, 2008,).

Given this evidence, we investigated whether increases in activation of the cognitive control system are also observed in MS patients with no clinical disability as assessed by EDSS. Specifically, we hypothesized that these patients would show increased recruitment of cognitive control regions across sensory, motor and cognitive tasks. To verify this hypothesis, we measured BOLD signal changes using fMRI in patients with RRMS with no detectable clinical disability. The fMRI paradigms included a visual stimulus (flashing checkerboards), right and left hand movements, and the N-back working memory task.

MATERIALS AND METHODS

Data acquisition

Functional MRI involved a total of six sessions of gradient echo EPI while subjects performed during two sessions either the motor, visual or cognitive task. Presentation of the tasks was based on a block design in which periods of an activating condition alternated with periods of a control condition with sessions lasting between 4 to 6 min. To improve the spatial coverage of the occipital cortex, TR and the number of slices for the visual sessions were increased to 2177 ms and 36 slices, respectively.

fMRI Stimuli and Design

Stimuli for each task were programmed using the E-Prime software (Psychology Software Tools, Pennsylvania, PA) and presented using the Eloquence functional imaging system (Invivo Corporation, Gainesville, FL) through an LCD screen inserted into the head

coil. Responses to the tasks were recorded using a keypad. Subjects were trained beforehand for accurate performance using the mock scanner that is a part of the MRI suite. The paradigms were presented in the following order: cognitive, visual, and motor. The visual task consisted of control periods of minimal visual stimulation with a black screen and a flashing red crosshair alternating with activating periods of a full field radial checkerboard flashing at 8 Hz (Drobyshevsky et al., 2006; Schneider et al., 1993) (Fig. 2.1). In order to confirm that subjects were looking at the screen during the task, they were instructed to press a key at the beginning of the flashing checkerboards. Two sessions of 12 blocks (6 control, 6 activation) were included and 96 EPI volumes were acquired per session.

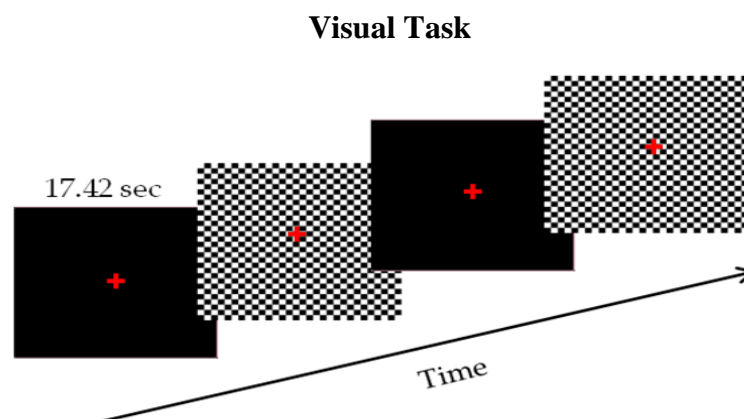


Figure 2.1 Schematic representation of black screen (rest) and flashing checkerboard during the visual fMRI task.

The motor task consisted of blocks of rest alternated with blocks of flexion and extension of the last four fingers of the right or left hand and was based on prior fMRI motor studies in MS (Pantano et al., 2002b; Rocca et al., 2002a). Subjects were visually cued with the words “REST”, “RIGHT” or “LEFT” (Fig 2.2). Hand side was alternated after each rest period. Two sessions of 21 blocks (11 control, 10 activation) were included and 168 EPI volumes were acquired per session. Subjects were trained to self-pace movements at 1 Hz and correct execution of the task and mirror movements were monitored via video cameras.

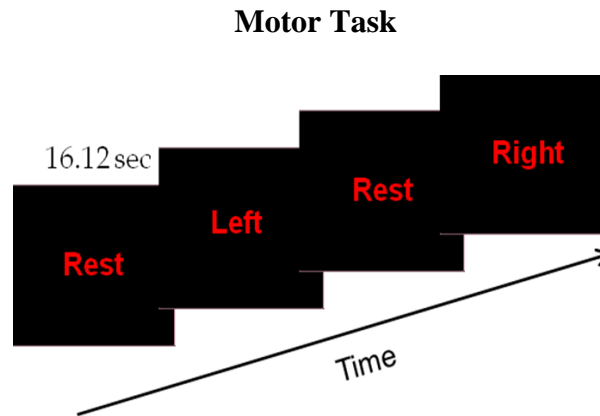


Figure 2.2 Schematic representation of hand movement conditions and resting condition during the fMRI motor task.

To identify cortical areas involved in the working memory, the widely used N-back task was implemented (Drobyshevsky et al., 2006; Owen et al., 2005) (Fig 2.3). This paradigm was chosen over other common ones such as PASAT because it allowed precise automated acquisition of response reaction time. During the control condition (0-back), subjects were instructed to respond with their index finger (positive response) to a sequence of 10 red letters shown one at a time if presented with the letter “X”, and with their middle finger (negative response) otherwise. For the activating condition (2-back), subjects were shown a series of 10 yellow letters one at a time and were instructed to provide a positive response if the current letter was the same as that presented two letters previously and a negative response otherwise. Each letter was displayed for 1 sec and the inter-stimulus interval lasted for 2 sec. Stimuli to distracter ratio was 1:5. Two sessions of 15 blocks (8control, 7 activation) were included and 150 EPI volumes acquired per session. Reaction time and percent correct responses (accuracy) were recorded for both conditions. Subjects with an accuracy of < 50% for the positive trials during the 2-back condition were excluded from the analysis.

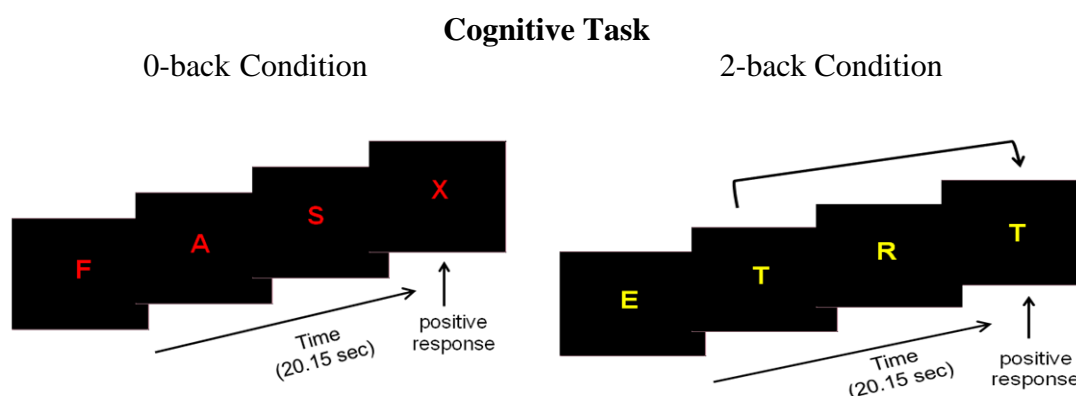


Figure 2.3 Schematic representation of control condition and working memory and attention condition during the fMRI cognitive task.

fMRI processing and analysis

Preprocessing and analysis of the fMRI data were performed using SPM8 software (Wellcome Trust Centre for Neuroimaging, University College London, UK) implemented in Matlab (Mathworks, Massachusetts, USA). Volumes with significant artifacts were identified using the ArtRepair toolbox (<http://cibsr.stanford.edu/tools/ArtRepair/ArtRepair.htm>) based on scan-to-scan motion (>1 mm/TR) and outliers relative to the global mean signal (> 5 SD from global mean). Images with artifacts were repaired by interpolation from the nearest unaffected volumes. Images then underwent intra-subject linear motion correction to reduce head motion effects. Subjects with head motion greater than 3.0 mm in translation or 3.0° in rotation were eliminated from the analysis of the corresponding task. Functional-structural coregistration of fMRI and MPRAGE data was performed to improve spatial localization of activity. Each subject's MPRAGE was then normalized to the coordinates of the Montreal Neurological Institute (MNI) template (Collins et al., 1995; Mazziotta et al., 2001) and the resulting transformation was applied to the fMRI data. Subsequently, fMRI data underwent resampling to a 2 mm isotropic resolution, spatial smoothing with an 8 mm full-width-at-half-maximum isotropic Gaussian kernel and temporal filtering with a high pass filter ($t = 128$ sec).

Statistical analysis was performed using a two-level stage random-effect analysis. At the first level, significant signal changes due to the effect of interest (i.e. flashing checkerboards, hand flexion-extension, 2-back condition) versus baseline condition for each subject were assessed with t statistical parametric maps (t maps). Individual t maps were then used in the second-level analysis to assess differences in brain activation at the group

level between MS patients and control subjects. A cluster-defining threshold of $p = 0.01$ and cluster P values after correction for multiple comparisons using Random Field Theory (Adler, 1981) to control for family wise error (FWE) rate of less than 0.05 (Friston et al., 1996) were used in all analyses. Anatomical labeling of regions of activation was accomplished with the Automatic Anatomic Labeling software package (AAL) (Tzourio-Mazoyer et al. 2002).

RESULTS

Quality Control Measures: Handedness, Head Motion and Task Performance

Two patients were excluded from all the analyses due to severe head motion and poor performance during the 2-back paradigm. Two additional left-handed patients (Olfield, 1971) were excluded from the analysis of the motor paradigm. Two controls were excluded from all analyses due to motion artifacts. Two additional controls did not perform the visual task and two others were excluded from the analysis of the 2-back paradigm due to poor performance. The final analysis consisted of 20 patients and 19 controls for the visual and 2-back paradigm and 21 controls and 18 patients for the motor paradigm.

Groups did not differ in mean age, or time to complete the Nine-Hole Peg test when using their right or left hand (Table 1.1). No mirror movements were noted during hand movements while performing the motor tasks. Performance on the 2-back paradigm, measured as accuracy and reaction time, did not differ between the patient group and healthy controls in either the control trial or the working memory trials (Table 1.1).

fMRI within-group analysis

Comparison of the observation of flashing checkerboards to a black screen in a within-group analysis resulted in extensive activation of the occipital cortex in both groups (Fig 2.4a). Analyses of right and left flexion-extension hand movements compared to rest resulted in extensive activation of contralateral primary motor regions and minimal activation of ipsilateral motor regions in both groups (Fig 2.4b, 2.4c). During the performance of the 2-back condition compared to the 0-back condition, both groups activated a network of frontoparietal and midline structures associated with working memory tasks (Fig 2.4d) (Owen et al., 2005).

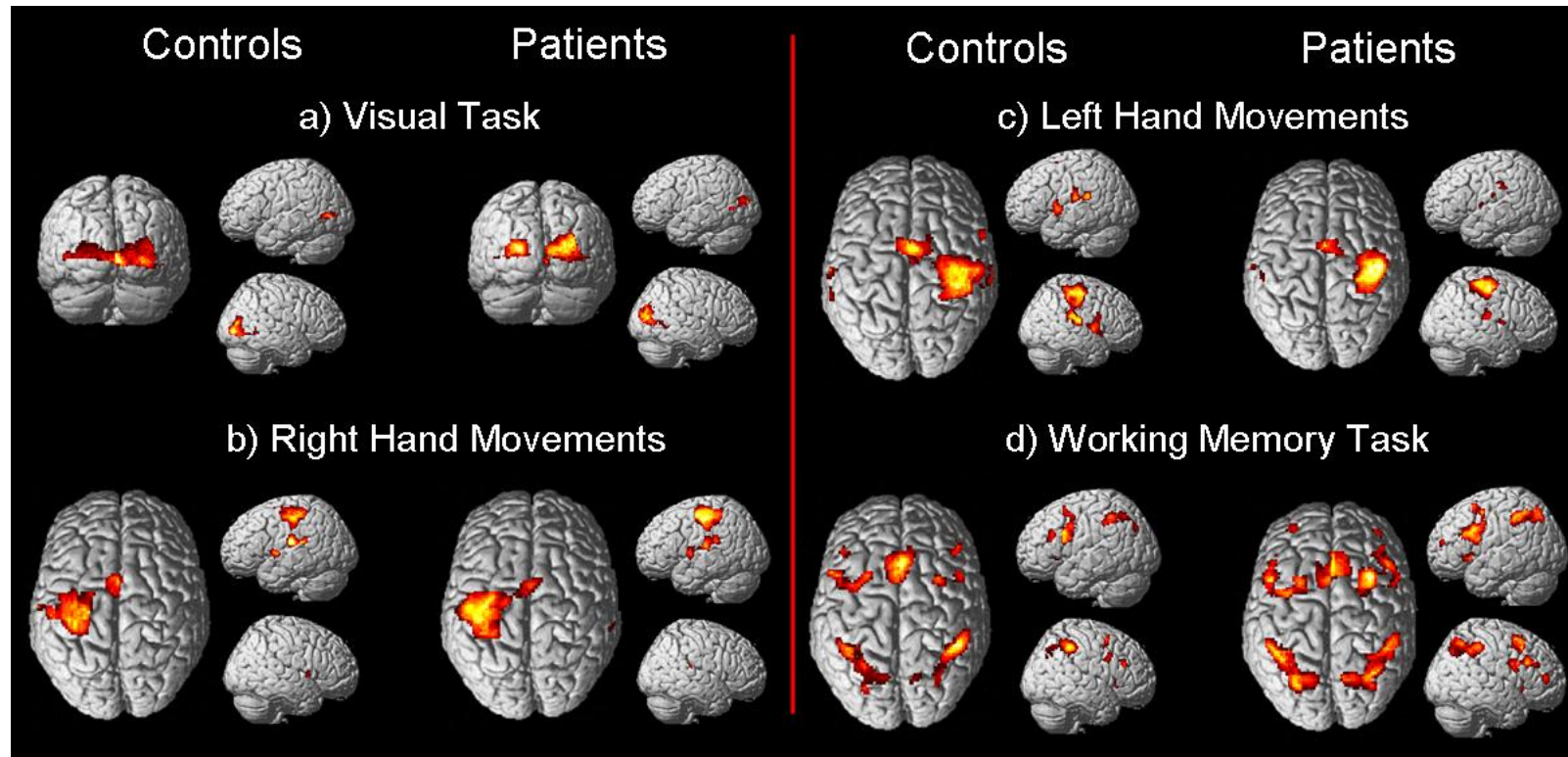


Figure 2.4 Cortical activation patterns in MS patients with no disability and control subjects during the (a) visual task, (b) right hand movements, (c) left hand movements and (d) performance of the 2-back task (one sample t-test, $p < 0.05$ FWE-corrected at voxel and cluster level). Images are in neurological convention (left is left). Red-orange color scale represents the alpha level ($1 - p$).

fMRI between-group analysis

A two-sample comparison of patients versus controls identified clusters of greater activation in patients during the right hand, left hand and 2-back conditions while none were identified during the visual task. Controls failed to show greater activation compared to patients during any of the conditions. The coordinates of the maximum voxel t value, its approximate anatomical location, number of voxels, percent whole brain BOLD, p-value, and center of mass are shown for each significant cluster in Table 2.1. The following sections provide a more complete description of the anatomical location of each cluster.

Right Hand Movements

Group comparison of subjects while performing right hand movements identified one cluster of significantly greater activation in patients (Fig. 5a). Regions in this cluster involved right precentral gyrus (BA4) and postcentral gyrus (BA5), left superior parietal gyrus (BA7), bilateral supplementary motor areas (SMA) (BA6), middle cingulate cortex (BA31), and precuneus (BA5 and 7).

Left Hand Movements

For the left hand condition, two clusters were found in which patients showed greater activation than controls (Fig. 5b). The main regions inside these clusters included right superior and middle frontal gyri (BA9 and 10) within the DLPFC (BA 9 and 46), right insula (BA 13), and bilateral middle and anterior cingulate cortices (BA 24 and 32). Other regions included right inferior frontal gyrus pars triangularis and pars opercularis, SMA, putamen, caudate and superior temporal gyrus.

Working Memory Task

During the performance of the 2-back condition compared to the 0-back condition, patients showed greater activation than controls in one cluster (Fig. 5c). Regions in this cluster primarily included right superior and middle frontal gyri (BA 9, 10 and 46), and middle and anterior cingulate cortices (BA32). Other regions included right inferior frontal gyrus pars orbitalis (BA11), opercularis, and triangularis.

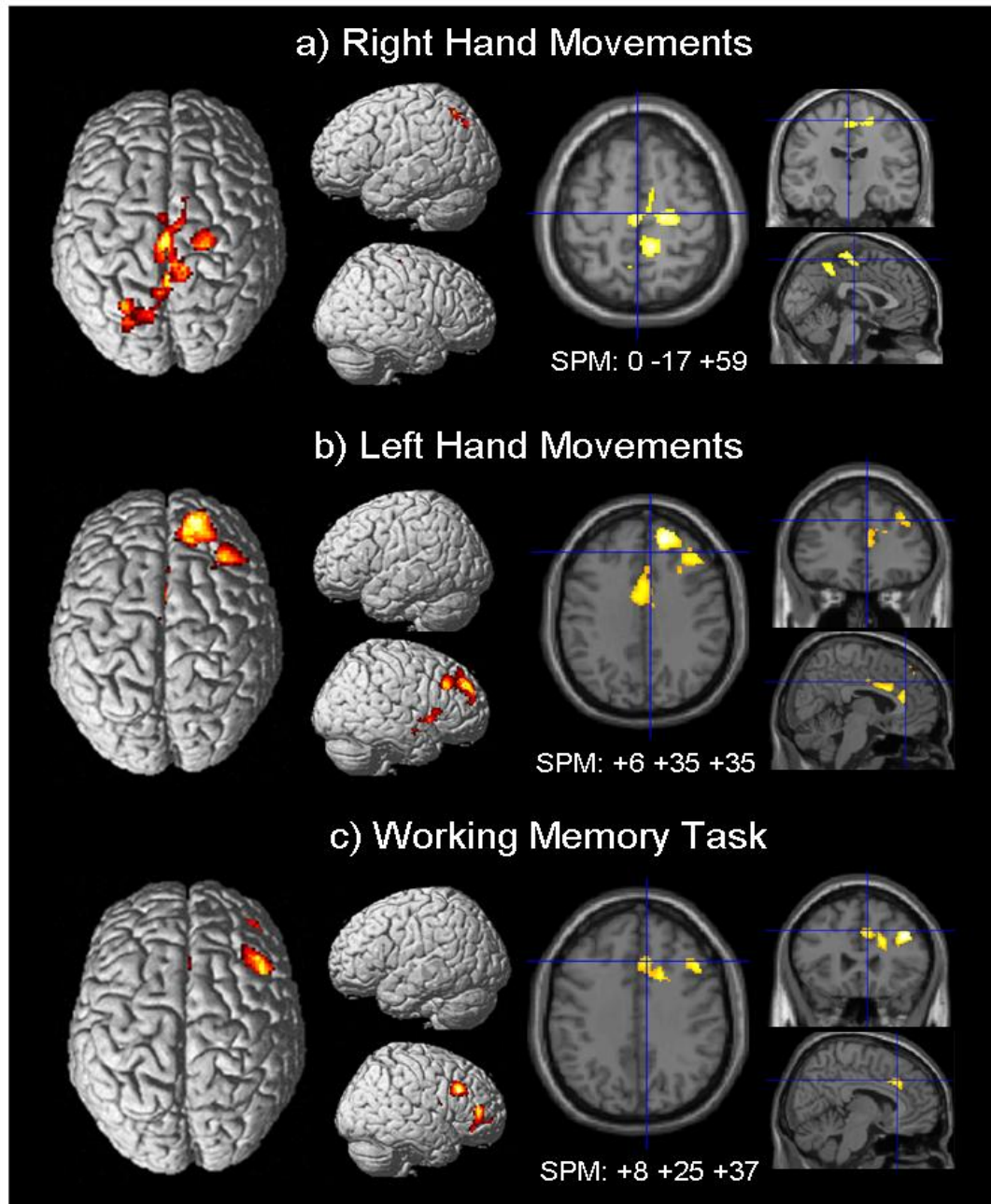


Figure 2.5 Areas of increased brain activation in MS patients with no clinical disability relative to controls during (a) right hand movements, (b) left hand movements and (c) performance of the 2-back task (two sample t-test, $p < 0.05$ FWE-corrected at cluster level). Red-orange color scale represents the alpha level ($1 - p$).

Table 2.1 Random Effects comparison of activations during the visual paradigm, right and left hand movements and 2-back task between MS patients (MS) and control subjects (CS).

Condition	Comparison	Cluster label (center of mass)	Corrected cluster p	Mean difference across all voxels in cluster (\pm 90% CI) [% whole brain BOLD]	Voxels in cluster	Maximal voxel T	X Y Z	Location
VISUAL	MS > CS		< 0.05					No significant clusters
	CS > MS		< 0.05					No significant clusters
RIGHT	MS > CS	1 (3,-38,54)	0.01	0.531 (0.242)	1616	3.76	8 -41 57	R precuneus (BA5)
						3.67	18 -21 59	R precentral g (BA4)
						3.33	-4 -27 67	L supplementary motor area (BA6)
LEFT	CS > MS							No significant clusters
	MS > CS	1 (9,28,32)	0.004	0.400 (0.165)	1687	4.69	16 47 33	R superior frontal g (BA9)
						3.79	20 55 19	R superior frontal g (BA10)
						3.69	2 11 29	R middle cingulate g (BA24)
		2 (34,18,10)	0.022	0.446 (0.192)	1248	4.69	28 17 19	R insula (BA13)
						3.89	44 -5 -17	R superior temporal g
						3.83	24 21 13	R caudate
	CS > MS		< 0.05					No significant clusters
2-BACK	MS > CS	1 (28, 30, 18)	0.002	0.453 (0.133)	1893	4.61	40 23 31	R middle frontal g (BA9)
						4.53	20 43 11	R anterior cingulate g (BA32)
						4.32	36 45 7	R middle frontal g (BA10)
	CS > MS		< 0.05					No significant clusters

P values are corrected with FWE adjustment for multiple comparisons at cluster level and for two-tailed tests. Within each significant cluster, the three maximal voxel t values that are greater than 8 mm apart and their approximate anatomical location are shown. Coordinates are in MNI space (mm). BA = Approximate Brodmann area, g = gyrus, L = left. R = right.

DISCUSSION

The current studies provide the first fMRI characterization of recruitment of cognitive control regions across multiple tasks in the subset of MS patients who have lesions but do not have clinical disability. The current results in these patients demonstrate increased activation of the right DLPFC and ACC during the performance of a demanding working memory task. Moreover, this pattern of functional recruitment also was observed during the performance of non-dominant hand movements. These results support the mounting evidence for increased functional recruitment of cognitive control brain regions in the working memory system of MS patients with low disability (Au Duong et al., 2005; Audoin et al., 2008; Hillary et al., 2006; Sweet et al., 2004, 2008) and provide new evidence for its role in the motor system.

The approach taken in these studies addresses many of the issues contributing to the inconsistent findings observed in fMRI studies in MS. Brain activation was investigated in a homogeneous MS patient group based on disability because as prior studies have shown, functional recruitment patterns in MS patients vary by disability (Rocca et al., 2005). Patients with accuracy and reaction times equivalent similar to those of healthy controls were used to limit between-group differences in behavioral performance. This is important because it is challenging to interpret increased brain activation as adaptive in patients whose performance is not rather comparable to that of controls. While performance measures were not controlled in early fMRI studies (Hillary et al., 2003; Penner et al., 2003; Staffen et al., 2002), others have only control for equivalent accuracy but not reaction time (Forn et al., 2006; Sweet et al., 2006) which results in the comparison of patient and control groups that may differ in information processing speed. This is nontrivial since decreased processing

speed is the most common cognitive deficit and the primary cause of information processing impairments in MS patients (DeLuca et al., 2004). Finally, the same cohort of patients and controls was used across the different studies. This reduces the variability from interindividual differences in brain activation and allows probing for brain mechanisms that may be common across functional domains.

Motor Tasks

Patterns of activation consistent with motor execution and planning were observed in our patients and controls during the performance of right and left hand movements (Loubinoux et al., 2001). Group comparison during right hand movements showed that patients increased activation of the ipsilateral primary motor cortex (BA4) as well as bilateral activation of regions associated with the sensorimotor network (BA5-7, 31). Consistent with our findings, similar fMRI motor studies in MS patients with low disability have reported increased activation of bilateral sensorimotor regions during right hand movements (Giorgio et al., 2010; Pantano et al., 2002a, Rocca et al. 2005). An increase in activation of regions outside of the classical motor network has also been described during right hand movements, which was not observed in this study. However, the patients in these studies had greater disability (Wang et al., 2007) or were in the progressive stage of the disease (Rocca et al., 2002b).

Interestingly, when patients performed the same motor task with the non-dominant hand, additional areas not typically activated in simple motor tasks, including the bilateral ACC and the right DLPFC, were recruited. These findings may be related to the increased cognitive effort that performing non-dominant hand movements requires. While most motor

fMRI studies in MS have involved right hand movements, a recent fMRI study by Rico et al. (2010) examined bilateral movements in patients with clinically isolated syndrome suggestive of the first clinical manifestation of MS (CIS) with low disability and devoid of corticospinal dysfunction. Consistent with our findings, these authors found increased activation of the ACC when patients performed non-dominant hand movements but not with dominant hand movements. These findings suggest that non-dominant hand movements result in recruitment of brain networks involved in cognitive control in patients with MS and minimal or no disability.

Cognitive Task

Performance of the 2-back condition in both groups activated brain regions associated with working memory tasks (Owen et al., 2005). However, between-group comparison identified increased activation in patients mainly involving the right DLPFC and right ACC. Increased activation of these regions in MS patients with low disability has been described across different fMRI working memory studies (Audoin et al., 2008; Bobholz et al., 2006; Sweet et al., 2004), and consistent with our findings, several investigators have noted increased DLPFC activation primarily involving the right hemisphere (Hillary et al., 2006). For instance, a recent longitudinal fMRI study of a group of patients with CIS and low disability identified increased levels of activation in the right DLPFC and ACC in patients who improved their scores in the PASAT over 1 year relative to patients who did not (Audoin et al., 2008). These authors concluded that recruitment of adaptive cognitive control processes may limit the cognitive dysfunction associated with MS.

Posterior parietal regions which are involved with storage of visual short term memory (Todd and Marois, 2004; Vogel and Machizawa, 2004; Xu and Chun, 2006), have also been reported to show increased activation in MS patients performing working memory tasks (Penner et al., 2003; Wishart et al., 2004). Group differences in these regions were not observed in this study or that by Sweet et al. (2004) who investigated brain activation in patients with MS and low disability during 2-back task performance. This finding may be related to preservation of short term memory storage. Importantly, the patients studied by Sweet et al. (2004), similar to the ones in the current study, had normal information processing speeds as reflected by their normal reaction times.

Visual Task

As expected, the performance of the visual task resulted in extensive activation of the occipital cortex in both groups, which is consistent with previous fMRI studies in healthy subjects using similar paradigms (Drobyshevsky et al., 2006; Schneider et al., 1993). However, between-group comparison yielded no group differences in our studies. This is somewhat different from the altered patterns of cortical activity reported by Rombouts et al. (1998) in a group of RRMS patients with unilateral optic neuritis. These authors reported reduced visual cortex activation upon monocular stimulation of the affected and unaffected eyes. They observed a trend of greater activation in recovered patients upon stimulation of both the affected and unaffected eyes. Similar findings have been reported by other investigators (Werring et al., 2000). A possible explanation for these discrepant results is that none of our patients had a recent episode of optic neuritis.

Increased Cognitive Control in MS

The aim of the current investigation was to test if non-disabled MS patients show increased recruitment of cognitive control regions across visual, motor and working memory tasks. The current findings support in part our hypothesis and indicate that these patients recruit the right DLPFC and ACC while performing working memory tasks and non-dominant hand movements. These two regions are key elements of the neural architecture of the cognitive control system, which involves maintaining mental states representing goals and the means to accomplish them (Miller and Cohen, 2001) and require processes such as planning, attention and working memory. The DLPFC is involved in multiple executive processes including monitoring, manipulation and integration of multiple pieces of information (Petrides, 2000; Rypma and D'Esposito, 1999; Tanji and Hoshi, 2008). While the ACC has also been implicated in multiple high-order cognitive processes, it has gained particular attention for its involvement in conflict monitoring (Botnivik et al., 1999; Egner and Hirsch, 2005; Kerns et al., 2004). This evidence led to an influential model of cognitive control in which the role of the ACC is to identify the occurrence of conflictive information and to signal the DLPFC to resolve such conflict (Botvinick et al., 2001; Carter et al., 2007). Other investigators propose that the primary role of the ACC is not conflict monitoring but rather a function in response selection, estimation of reward uncertainty and direct implementation of actions needed to resolve conflict (Mansouri et al., 2009; Roelofs et al., 2006). Moreover, recent evidence suggests that the identification of conflict is recognized by the DLPFC, with a subsequent behavioral adjustment accomplished by the interaction of the DLPFC and ACC (Morishima et al., 2010).

Although we lack an accepted model for the dynamic interaction of the DLPFC and ACC to allow individuals with intact neural networks cope with conflicting and demanding cognitive situations, it is clear that they are essential for cognitive control. In cases involving damaged neural pathways such as seen in patients with MS, higher levels of cognitive control, and therefore DLPFC and ACC recruitment, may be required at lower cognitive load thresholds (Hillary et al., 2006). If increased cognitive control allows patients to cope with increasing cognitive demands, usage of this brain mechanism should not only be limited to the working memory system but should function across any situation that demands greater cognitive effort. Current findings support this idea and provide novel evidence for increased recruitment of cognitive control when patients perform mildly demanding motor tasks.

Another important finding of the current study is the lateralization of functional recruitment to the right DLPFC during both the working memory and motor tasks. Interestingly, preferential recruitment of the right DLPFC has also been described in fMRI working memory studies in MS, as previously mentioned, as well as other clinical populations, including victims of traumatic brain injury (McAllister et al., 1999; Perlstein et al., 2003) and patients with major depression (Fitzgerald et al., 2008). Moreover, several investigators have observed a relationship of increasing right DLPFC recruitment with increasing demands while performing working memory tasks in healthy controls (D'Esposito et al., 1999; Mostofsky et al., 2003; Rypma et al., 1999, 2002). These investigations have suggested that recruitment of the right DLPFC while performing working memory tasks may involve a general response to cerebral challenge (Hillary et al., 2006). Consistent with this idea, we observed increased right DLPFC activation when

patients performed the more cognitively demanding tasks including the working memory task and non-dominant hand movements, while only minimal recruitment occurred during dominant hand movements and no involvement during the visual task.

Conclusion

In conclusion, these findings support the growing evidence that increased activation of cognitive control brain regions, particularly in the right hemisphere, may be an important mechanism allowing patients with MS to accommodate to the neural disruption caused by this disease. Moreover, the current study shows the usefulness of testing the same cohort of patients during multiple tasks to identify adaptive brain mechanisms that may be sustained across different functional domains.

Limitations

While this study is an important step towards better understanding the role of cognitive control recruitment in non-disabled MS patients, it is not without its limitations. First, as with most previous fMRI studies in MS, the cross-sectional nature of this investigation and the small number of subjects limit the generalization of these results. Nonetheless, the homogeneity of the patients included in this study makes this group reasonably representative. Longitudinal studies with larger homogenous patient groups testing multiple functional domains should provide better understanding of brain mechanisms that may be protective for the developing neurological disability in MS.

CHAPTER 3

EFFECTIVE CONNECTIVITY IN NON-DISABLED MS PATIENTS

INTRODUCTION

A complete understanding of the underlying mechanisms giving rise to increased cognitive control recruitment in MS patients is essential if these mechanisms are to be used as targets for diagnostic or therapeutic intervention. The results of the current fMRI studies and previous investigations (Au Duong et al., 2005; Audoin et al., 2008; Hillary et al., 2006; Sweet et al., 2004, 2008) suggest that MS patients with low disability increase cortical recruitment of the prefrontal cortex when performing motor and working memory tasks relative to control subjects. Examining activation of isolated brain regions without considering their mutual interaction allows only a partial understanding of the mechanisms underlying a particular behavior. The aim of the current study was to investigate the functional interaction of cognitive control brain regions during the performance of a demanding cognitive task in patients with MS and no clinical disability.

There are multiple approaches available to quantify connectivity of brain networks based on fMRI (Rowe et al. 2010), which does not measure brain electrical activity directly but uses changes in the blood oxygen levels resulting from neural activation. The current study used DCM (Friston et al. 2003) to quantify effective connectivity, which focuses on the direct influence of one brain region over another (Friston et al. 2003, Friston 2009b). Compared to other models for probing effective connectivity such as those based on econometrics including Granger causal modeling (Goebel et al 2003), DCM uses a generative model of how the observed fMRI data are generated based on underlying neuronal states rather than on models based on temporal interactions among the observed hemodynamic data themselves (Friston 2009a). A recent study using an animal model of epilepsy simultaneously conducted fMRI and intracranial electroencephalography and

compared how Granger causal modeling and DCM modeled neuronal signal propagation (David et al. 2008). Accurate modeling of the source of neuronal discharges was obtained only with DCM. Another advantage of DCM is that it allows evaluation of the direct modulation of effective connectivity by experimental manipulations (Friston et al. 2003, Friston 2009b).

In the present study, functional integration of cognitive control regions during the performance of the N-back task was investigated in MS patients without clinical disability. Given the increased DLPFC and ACC recruitment when patients performed this task, it was hypothesized that patients show altered connectivity among prefrontal cortical regions compared to healthy control subjects. This hypothesis was tested by using DCM to model functional interactions within a working memory network comprising the DLPFC, ACC and posterior parietal regions using fMRI data obtained during the performance of the N-back task.

MATERIALS AND METHODS

Dynamic causal modeling

Preprocessed statistical parametric maps utilized for the current study were obtained from the N-back fMRI experiment described in Chapter 2. While increased activation of cognitive control regions was also observed in patients when performing left hand movements during the motor fMRI study, methodological constraints limit the application of DCM to the motor task to study connectivity of prefrontal cortex regions. This is because in order to study changes in functional brain connectivity using DCM in clinical populations, only commonly activated regions in both controls and patients can be analyzed (Seghier et

al. 2010). Activation of the prefrontal cortex in both groups was observed only during the performance of the N-back task. Moreover, because of the central role of cognitive control regions in working memory processes, the N-back task is well suited to probe the connectivity of the prefrontal cortex.

DCM analysis was conducted using the SPM8 software (Wellcome Trust Centre for Neuroimaging, University College London, UK). The goal of DCM is to draw inferences about the strengths of connections among neuronal populations and the changes resulting from context-dependent manipulations (Friston et al., 2003). The approach involves constructing realistic models of interacting cortical regions and determining how their interactions are influenced by experimental conditions (e.g. task conditions). The components of a DCM include: (a) intrinsic connections, which describe the coupling or effective connectivity of one region to another one, (b) modulatory inputs, which assess the change in effective connectivity between regions induced by an experimental condition, and (c) driving inputs, which describe the direct influence that an experimental manipulation has on the state of a specific region (Friston et al., 2003; Stephan et al., 2007). The units for these parameters are Hertz. A positive effective connectivity value indicates that activity in one region results in an increase in the rate of change of another region while a negative value indicates a decrease rate of change. Because there can often be several hypothesized functional networks, an approach using a Bayesian statistics was used to select the ideal model among a group of competing models (Penny et al., 2004). Bayesian model selection (BMS) identifies the optimal DCM using a weighing of how good the model explains the patterns of changes observed in the fMRI data during a task and the model's relative complexity (e.g. number of nodes).

Model Definition

Finding the best model from which to obtain connectivity parameters is crucial for DCM studies. While testing a few models may bias selection towards one particular type of model, testing too many models may decrease the probability of finding the best one. The principles described by Seghier et al. (2010) of compatibility, size, and plausibility were employed in designing the DCMs in this study. The brain areas defining the model space in the DCMs involved core regions of the working memory system. Regions chosen showed high levels of activation during the N-back task in the within-group fMRI analyses and were consistently described as being active in previous fMRI studies of the N-back task (Owen et al. 2005). The DCMs consisted of 5 volumes of interest (VOIs) including bilateral DLPFC (BA9) within the inferior frontal gyrus, dorsal ACC (BA 32) and bilateral inferior parietal lobules within posterior parietal cortices (PPC) (BA40). To create the models, the time course series of fMRI signal changes during the N-back task was extracted from a spherical volume (10 mm radius) from the first-level fMRI analysis for every subject in each of the 5 VOIs. Using this data, the principal eigenvariate was calculated, adjusted for effects of interest, and used in the final estimation of the DCMs. To ensure that models were comparable across subjects, the specific coordinates from where data was extracted in each subject was limited to a distance of 15 mm from the group coordinates within the aforementioned regions, which are shown in Table 3.1.

Table 3.1. MNI group coordinates from the main effect 2-task > 0-back task in random-effects within-group analysis ($P < 0.05$ FWE-corrected) used for VOI time series extraction.

Region of Activation	Group	MNI Coordinates			T value	BA
		<i>x</i>	<i>y</i>	<i>z</i>		
Right DLPFC	CS	42	-41	43	12.30	40
	MS	36	-51	45	9.01	40
Left DLPFC	CS	-44	9	31	8.85	9
	MS	-46	9	31	10.23	9
ACC	CS	-6	19	45	10.24	32
	MS	-4	19	45	8.34	32
Right DLPFC	CS	44	11	37	8.19	9
	MS	44	9	29	9.20	9
Left PPC	CS	-42	-47	51	10.96	40
	MS	-36	-49	47	12.62	40

BA = Brodmann area, DLPFC = dorsolateral prefrontal cortex, ACC = anterior cingulate cortex, PPC = posterior parietal cortex.
FEW = Family wise error

Model Connectivity

A basic DCM was initially built with bidirectional intrinsic connections between all regions except the DLPFC and its contralateral PPC (Fig. 3.1). This model was subsequently used to produce 24 DCMs that were classified into 3 families of models based on their driving input, which was either the right DLPFC (family 1), the left DLPFC (family 2) or bilateral DLPFC (family 3). Eight DCMs within each family were produced by alternating the effective connectivity that the 2-back condition modulated within prefrontal cortex regions (Table 3.2).

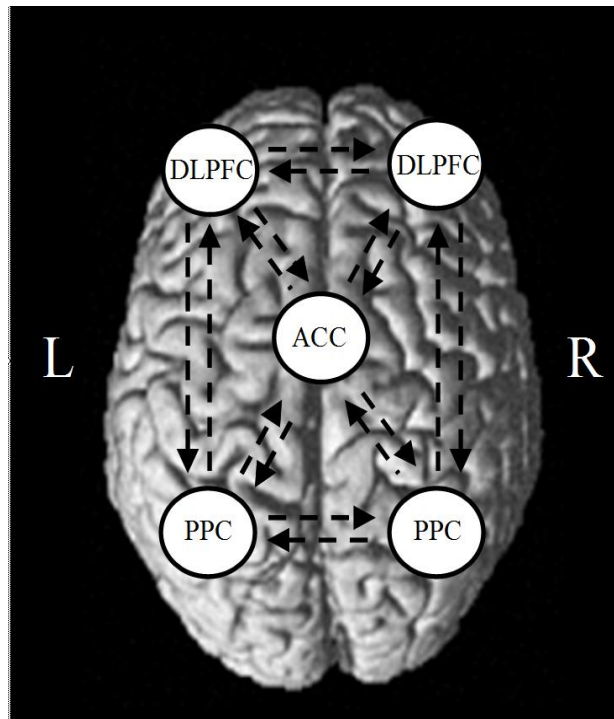


FIGURE 3.1 Network of intrinsic connections among the five regions of interest. Arrows represent the direction of the connection modulated by the 2-back condition. L = left, R = right, DLPFC = dorsolateral prefrontal cortex, ACC = anterior cingulate cortex, PPC = posterior parietal cortex.

TABLE 3.2. Outline of the eight DCMs composing family 2. The other 16 DCMs for family 1 and family 3 were produced by alternating the driving input to the right DLPFC or bilateral DLPFC, respectively.

<u>Family 2</u>			
DCM	Input	Modulation	
		From	To
1	L-DLPFC	R-DLPFC →	R-PPC
2	L-DLPFC	R-DLPFC →	ACC
3	L-DLPFC	R-DLPFC →	R-DLPFC
4	L-DLPFC	L-DLPFC →	R-DLPFC
5	L-DLPFC	ACC →	R-DLPFC
6	L-DLPFC	ACC →	L-DLPFC
7	L-DLPFC	L-DLPFC →	L-PPC
8	L-DLPFC	L-DLPFC →	ACC

L = left, R = right, DLPFC = dorsolateral prefrontal cortex, ACC = anterior cingulate cortex, PPC = posterior parietal cortex. Arrows symbolize the direction of the intrinsic connection modulated by the 2-back condition.

Model Comparison by Bayesian Model Selection

After constructing the 24 models for each subject, the DCMs were supplemented with a forward model of how the neuronal activity was transformed into the measured fMRI signals (Friston et al., 2003). Subsequently, BMS was used to compare the modelled fMRI signal of each model to that of the observed fMRI data (Penny et al. 2004) in order to identify the optimal model. Model comparison was performed within each group using a random effects approach at the family and individual model level. A random-effects approach, compared to fixed-effects, was used because it allows for differences in favored models at the individual level (i.e. not all subjects may have identical functional networks) and is optimal for DCM studies using clinical populations (Stephan et al. 2009). Because different functional networks may also be different between controls and patients, DCMs were tested independently for each group. Given the large number of DCMs tested, the family inference extension of BMS which identifies a favored group of models was used (Penny et al. 2010). After identifying the favored family of DCMs, inference at the model level was used to select the best DCM within the favored family for patients and controls. Selection of the optimal family and individual model was based on the exceedance probability. This measure provides the probability of belief that a particular family or model has a better tradeoff based on model fitting and model complexity compared to the alternative families or models tested (Stephan et al. 2009).

Group Comparisons and Correlations

DCM parameters (effective connectivity, driving inputs and modulations) were extracted for each subject from the optimal model and a one-sample t-test was used to

determine if parameters were significantly different from zero at the within-group level. Between-group comparison of parameters was achieved with an unpaired two-tailed *t*-test for parametric data and the Mann-Whitney U-test for nonparametric data. Normality of data was tested with the Saphiro-Wilk test. The resulting *p* values from these analyses were corrected for multiple comparisons using the Bonferroni procedure.

Regression analysis in the patient group using Spearman's rank correlation coefficient was performed to examine the correlation between DCM parameters and cognitive performance during the 2-back task (accuracy and reaction time) performed during fMRI and the PASAT (total score) performed outside the scanner. These analyses were conducted using Stata version 10.0.

RESULTS

Subjects

Extraction of time-series for all the regions was not possible for two control subjects because the closest region of activation was more than 15 mm away from the group coordinate. These subjects were therefore excluded from the analysis. The final groups included 20 patients and 17 controls.

Model Selection by BMS

Random-effects analysis using BMS at the family inference level showed that model family 2 (left DLPFC as driving input) was substantially favored compared to the other two families in both groups. The exceedance probability (probability of belief that this family is more likely than the other two) was 0.97 in the control group and 0.92 in the patient group

(Fig. 3.2a, 3.2b). Subsequent BMS of the eight models of family 2 favored model 8, in which the 2-back task modulated the intrinsic connection of the left DLPFC towards the ACC (Fig. 3.3). The exceedance probability (probability of belief that this model is more likely than the other eight) for this model was 0.66 for the control group and 0.98 for the patient group (Fig. 3.2 c and d). A second model was modestly favored in the control group in which the forward connection of the left DLPFC to the left PPC was modulated. Given the substantial support for family 2 model 8 (Fig. 3.3) as the optimal DCM, the effective connectivity, modulatory and driving input values were extracted from this model to perform within and between group analyses.

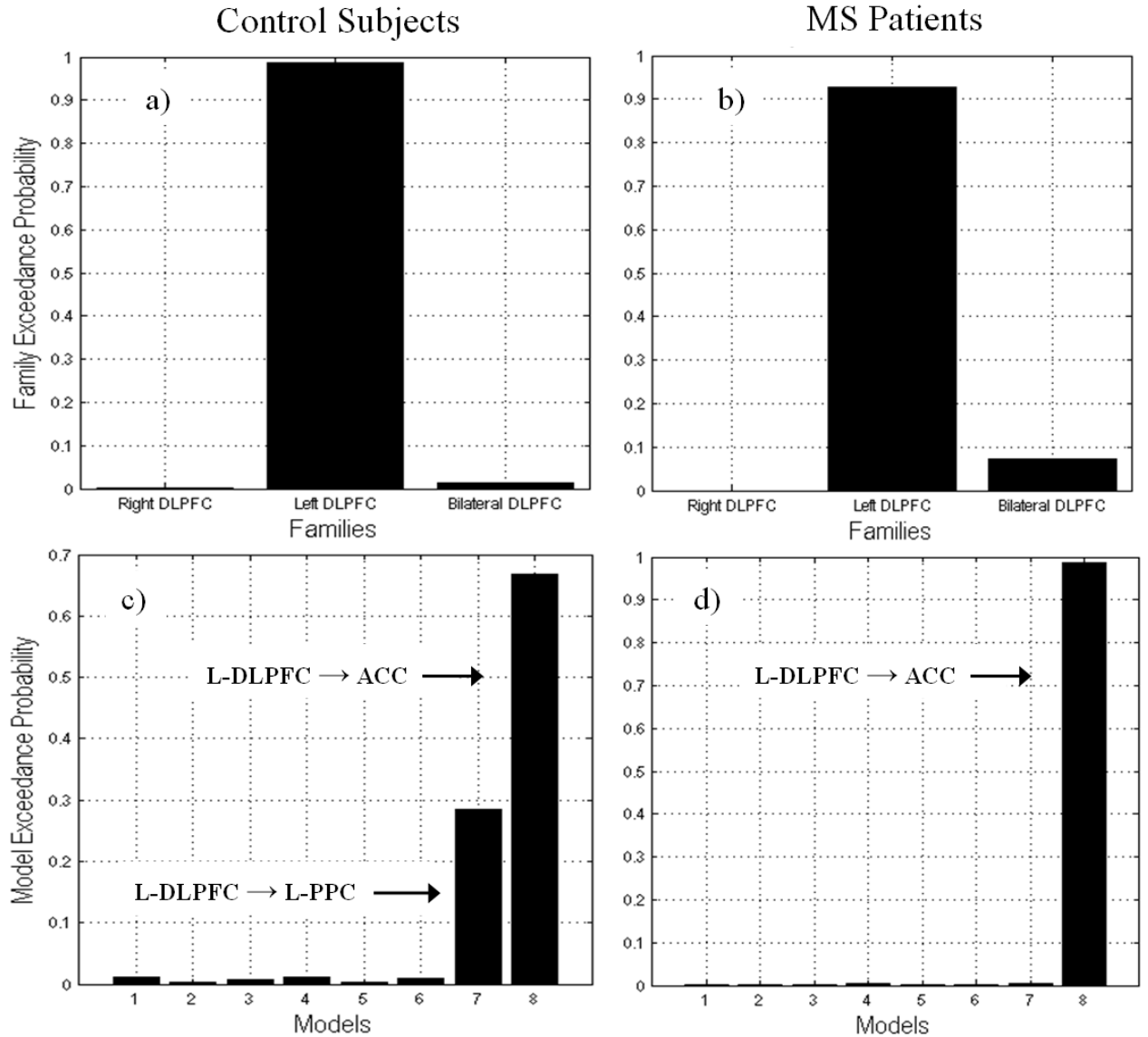


FIGURE 3.2 Results of the random-effects BMS analysis at the family and individual model level. Exceedance probabilities for the three model families for control subjects (a) and (b) MS patients are shown in the top row. Exceedance probabilities for the 8 DCMs of the model family having the left DLPFC as driving input for control subjects (c) and MS patients (d) are shown in the bottom row. X-axis = family in (a) and (b) and model number in (c) and (d), Y-axis = exceedance probability, L = left, DLPFC = dorsolateral prefrontal cortex, ACC = anterior cingulate cortex, PPC = posterior parietal cortex.

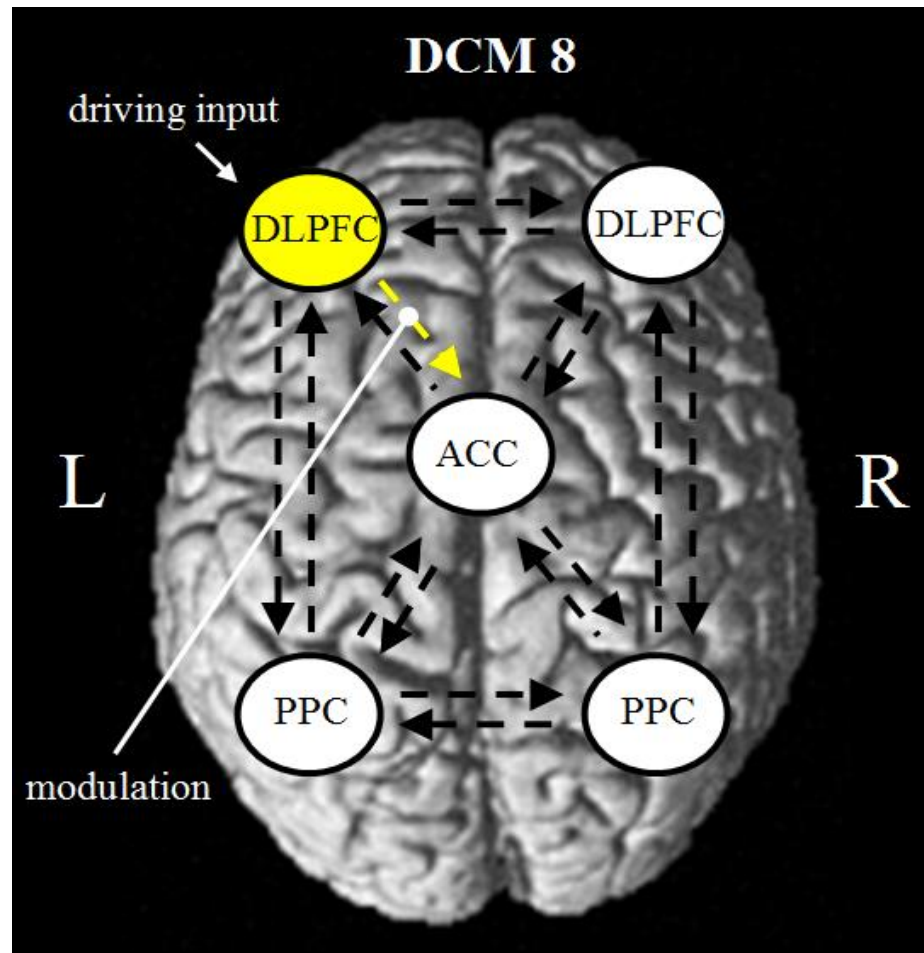


FIGURE 3.3 Dynamic causal model (DCM) chosen for within and between group analyses of effective connectivity, modulatory parameters and driving input during the performance of the 2-back task in MS patients and control subjects. L = left, DLPFC = dorsolateral prefrontal cortex, ACC = anterior cingulate cortex, PPC = posterior parietal cortex.

Individual group analysis

Effective connectivity for all intrinsic connections was significantly greater than zero except those directed towards the left DLPFC in both groups ($p < 0.05$) (Table 3.3). Input and modulation parameters were also significantly greater than zero in both groups.

Group Differences

Comparison of group means for effective connectivity or modulation parameters revealed no significant differences after Bonferroni correction. Prior to Bonferroni correction, MS patients showed a decreased effective connectivity from the left DLPFC to the left PPC compared to controls ($p = 0.02$). Driving input within the left DLPFC was significantly greater in MS patients ($p = 0.03$).

Correlation of DCM Parameters and Behavioral Performance

Regression analysis between DCM parameters and performance of the 2-back task in the patient group did not identify any significant correlations. A significant association ($r = 0.51$, $p = 0.037$) of increasing effective connectivity from the ACC towards the left DLPFC and increasing accuracy in the PASAT was observed.

TABLE 3.3 Mean and standard deviations (SD) of the DCM parameter estimates of the optimal DCM, including effective connectivity, driving inputs and modulation of intrinsic connections in healthy controls subjects and MS patients. *P* values are shown for between-group comparisons.

	Control Subjects		MS Patients		<i>p</i>
	Mean	SD	Mean	SD	
<u>Effective Connectivity</u>					
R-DLPFC → ACC	0.09*	0.04	0.09*	0.06	n.s.
R-DLPFC → L-DLPFC	0.03	0.09	0.01	0.09	n.s.
R-DLPFC → R-PPC	0.20*	0.07	0.20*	0.07	n.s.
L-DLPFC → ACC	0.29*	0.05	0.28*	0.08	n.s.
L-DLPFC → L-PPC	0.38*	0.07	0.32*	0.06	n.s. †
L-DLPFC → R-DLPFC	0.26*	0.10	0.29*	0.09	n.s.
ACC → L-DLPFC	0.05	0.11	0.01	0.11	n.s.
ACC → L-PPC	0.23*	0.08	0.18*	0.07	n.s.
ACC → R-PPC	0.31*	0.07	0.31*	0.10	n.s.
ACC → R-DLPFC	0.17*	0.06	0.15*	0.06	n.s.
R-PPC → ACC	0.07*	0.07	0.07	0.09	n.s.
R-PPC → L-PPC	0.14*	0.08	0.11*	0.05	n.s.
R-PPC → R-DLPFC	0.11*	0.09	0.09*	0.07	n.s.
L-PPC → ACC	0.13*	0.05	0.10*	0.07	n.s.
L-PPC → L-DLPFC	0.06	0.12	0.00	0.08	n.s.
L-PPC → R-PPC	0.28*	0.10	0.23*	0.07	n.s.
<u>Driving Input</u>					
L-IFG	0.16*	0.09	0.25*	0.15	0.03 ††
<u>Modulations</u>					
L-IFG → ACC	0.23*	0.10	0.25*	0.09	n.s.

†† Indicates between-group differences and * indicates significantly greater than zero within-group ($p < 0.05$ corrected for multiple comparisons). ‡ Indicates between-group differences at $p < 0.05$ uncorrected for multiple comparisons.

DISCUSSION

The current study provides the first investigation of effective connectivity in the working memory system in MS patients without clinical disability. Current results suggest that these patients engage similar functional networks as those used by healthy control subjects when performing the 2-back task. In addition, conducting this task resulted in no alterations in the functional interaction of prefrontal and parietal cortical regions. The only group difference identified was the greater influence on the rate of change in the activity of the left DLPFC in response to the 2-back task in patients compared to control.

DCM was recently developed as an approach to understanding functional brain interactions and aimed to overcome the limitations of previous similar approaches (Friston et al. 2009a). Its application to the study of brain function in clinical populations has increased rapidly (Friston et al. 2009a; Seghier et al. 2010) along with improvements to optimize its appropriate implementation. The approach taken in the current study was aimed at overcoming the limitations of previous clinical studies using DCM such as using an arbitrarily defined network model or choosing the optimal model for patients based on data from the control group (Seghier et al. 2010). To avoid relying on a predefined working memory model, multiple DCMs were systematically constructed to find an optimal model that can best explain the observed fMRI data. Because patients and controls may use different brain networks when performing cognitive tasks, BMS was performed in each group separately. This approach provides additional valuable information because it allows inferences at a systems level of whether patients may not only have altered connectivity within a functional network but whether they use a different network altogether. The nodes

used in the DCMs were carefully chosen to limit within and between group variability and avoid comparing functionally different brain areas.

Model Selection

The systematic comparison of the constructed DCMs favored in both groups the model in which the 2-back task directly influences activation of left DLPFC and modulated the forward connection from this region to ACC. The evidence supporting this model was substantially greater for MS patients than for control subjects, which also favored an alternative model involving frontoparietal modulation. These results can be interpreted as indicating that the functional neural architecture when performing working memory task is similar between non-disabled MS patients and healthy controls. However, a neurofunctional network in which the 2-back task modulates the prefrontal-cingulate interaction is favored in patients more than in controls. These results also highlight the heterogeneity of functional networks in healthy populations.

Additionally, the current BMS results provide insight into the roles of the DLPFC and ACC within the architecture of the cognitive control system. As discussed in chapter 2, the specific roles of the DLPFC and ACC in cognitive control, particularly in conflict monitoring, are controversial (Botvinick et al., 2001; Carter et al., 2007, Mansouri et al., 2009, Morishima et al., 2010). Earlier models suggested that ACC recognizes conflictive information and signal the DLPFC to resolve such conflict (Botvinick et al., 2001; Carter et al., 2007). Recent studies favored a contrasting model in which conflict is recognized by the DLPFC and behavioral adjustment results from the interaction between both regions (Morishima et al., 2010). While the N-back task is not a paradigm designed for testing

conflict monitoring, its requirements for high level of attention, identification of changing information and behavioral adjustment are similar to the demands of tasks for conflict monitoring. Under this assumption, the model favored in this study involving the 2-back task-dependent modulation of the connection from left DLPFC to ACC favors the model proposed by Morishima et al. (2010) because this model suggests that performance of this task results in a greater influence of DLPFC over ACC, rather than the other way round.

Group Differences

Analysis of effective connectivity values within each group is consistent with the notion that patients and controls have similar neurofunctional architecture when performing the 2-back task. Positive effective connectivity was observed in similar regions in both groups. The greatest increases in effective connectivity in both groups were observed in the forward connections of the left DLPFC and the ACC, with largest value involving the connection from the left DLPFC to left PPC in both groups. Interestingly, this same connection was the only one with a lower value in patients compared to controls, without Bonferroni correction. Absence of alterations in prefrontal effective connectivity when performing the 2-back task could be seen as unexpected given the increase right DLPFC and ACC activation that patients showed when performing this task. However, it is possible that increase activity within those regions may be what allows patients to maintain normal levels of effective connectivity within this network and normal performance of the task.

Our results disagree with those of a recent DCM study of brain networks involved in the Stroop attention task in MS patients with low disability (Rocca et al. 2009). These authors found increased effective connectivity of sensorimotor regions to right prefrontal

regions and decreased connectivity to ACC during Stroop performance. These discrepant results may relate to several factors including the differences in the cognitive processes involved in each task since the Stroop task has a greater attention and lower working memory demands than the 2-back task. Additionally, the network modeled in this study included sensorimotor and cerebellar regions that were not included in our models. Finally, the patients participating in that study had greater disability and longer disease duration than our patients, which may contribute to the observed altered connectivity not seen in the current patient group.

The only DCM parameter showing significant between-group differences in this study was that the 2-back task resulted in greater driving input of the left DLPFC in patients compared to controls. This finding signifies that patients undergo a greater rate of change in the activity of the left DLPFC when performing this task, which may contribute to the normal effective connectivity observed within the network. This greater rate of change of the left DLPFC may be what drives the increase recruitment of the right DLPFC and ACC observed during fMRI activation studies during the performance of this task. Overall, this finding is consistent with the idea that increase functional recruitment of the cognitive control system is an important compensatory mechanism in MS patients.

Regression Analysis

Effective connectivity from the ACC to the left DLPFC correlated positively with PASAT accuracy. This finding is interesting but difficult to interpret because the effective connectivity values relate to 2-back task and not the PASAT, which was performed prior to imaging. However, it suggests that increase in the functional interaction in these regions

may be important when performing other cognitively demanding tasks and again highlights the importance of prefrontal-cingulate interaction when patients perform cognitively demanding tasks.

Conclusions

In conclusion, the current studies suggest that patients with MS without clinical disability use similar functional networks and have similar interactions within the brain regions forming these networks when performing working memory tasks as those of healthy control subjects.

Limitations

The current study had several limitations. First, the N-back paradigm used in this study involved only one level of complexity. Testing patients during greater cognitive demands may reveal connectivity changes otherwise not detected with the 2-back task. In order to maintain a medium level of complexity, only 5 core regions of the working memory system were included in the DCMs tested. The possibility that more complex models may more accurately reflect the underlying neuronal events during this task cannot be excluded. Another limitation in the models tested was that only linear influences of the 2-back condition on single intrinsic connections were tested. The performance of 2-back task may have simultaneous influence in the connectivity of more than one intrinsic connection and may occur in a nonlinear fashion.

CHAPTER 4

STRUCTURAL CONNECTIVITY IN NON-DISABLED MULTIPLE SCLEROSIS PATIENTS

INTRODUCTION

While cortical reorganization appears to be a mechanism limiting disability in MS patients, several studies show that damage to the brain white matter (WM) beyond that seen in conventional MRI is an important contributor to clinical disability in MS (Fox 2008; Hasan et al. 2005; Raz et al. 2010). Specifically, several studies using DTI suggest that cognitive dysfunction in MS results from a disconnection syndrome caused by injury to functionally relevant WM tracts (Calabrese and Penner 2007, Dineen et al. 2009). DTI is an MRI technique sensitive to microstructural alterations of the WM and therefore provides information about structural connectivity between cortical regions.

The findings from the current fMRI studies and several published reports demonstrated increased activation in the prefrontal cortex (PFC) in MS patients compared to controls when performing working memory tasks (Audoin et al., 2003, 2005; Audoin et al., 2008; Bobholz et al., 2006; Forn et al. 2007 Staffen et al., 2002; Sweet et al., 2004). However, whether these alterations in cortical activation in MS patients are purely compensatory continues to be debated. While some studies show that increased PFC recruitment by patients results in equivalent task performance to that of controls (Forn et al. 2007, Sweet et al. 2004), others observed decreased performance despite increased PFC recruitment (Hillary et al. 2003, Chiaravalloti et. al 2005, Penner et al. 2003). In patients increased cortical recruitment during a given task may not result in optimal performance if axons transmitting those neural signals are severely impaired. Therefore, indentifying the structural connectivity of regions showing increased functional recruitment may determine their compensatory value and whether their recruitment indeed results in improved behavioral performance.

Against this background, we investigated the integrity of major WM tracts in the current group of MS patients. The major goals of this study were twofold: 1) to assess the integrity of major WM tracts in these patients and 2) to determine the relation between integrity of functionally-relevant fiber tracts and functional activation and effective of cognitive control regions. Given the findings of increased cognitive control recruitment and normal performance and effective connectivity during the 2-back task observed in the current patients, it was hypothesized that they would have preserved structural connectivity within the superior longitudinal fasciculus (SLF), which connects regions facilitating working memory function (Karlsgodt et al. 2008).

DTI parameters including FA, MD, AD and RD within major association, commissural and projection WM tracts were obtained. FA quantifies anisotropy, or the directionality of water diffusion, and reflects the degree of alignment and integrity of fiber tracts and can range from 0 to 1 (0 = isotropic or no preferential diffusion, 1 = unidirectional). MD, which is independent of diffusion direction, reflects overall diffusion within a structure reflecting integrity of cellular components. Diffusion perpendicular (RD) and parallel (AD) to the main fiber tract, can provide greater pathological specificity than FA or MD (Hasan and Narayana 2006; Song et al. 2005). There is some evidence that demyelination preferentially increases RD while reduced AD is more related to acute axonal injury (Herrera et al. 2008; Klawitter et al. 2011; Narayana et al. 2010; Song et al. 2005; Xu et al. 2008). These parameters were obtained within the aforementioned tracts using a DTI-based fiber tractography (DTT) approach. To confirm and complement the results from the DTT studies, a non-hypothesis driven whole brain analysis of FA across major WM tracts was performed using TBSS (Smith et al. 2006).

MATERIALS AND METHODS

Data preprocessing

The current studies were conducted using the diffusion weighted images (DWI) acquired as described in chapter 1. Preprocessing of DWI data was performed using FSL version 4.1 (Smith et al., 2004). Data were first visually inspected and volumes with large artifacts such as severe subject motion were removed from analysis. Images were then corrected for eddy current distortions and simple head motion by affine registration to a volume without diffusion gradient using FSL Diffusion Toolbox. Subsequently, volumes were stripped of extrameningeal tissues using FSL Brain Extraction Tool (Smith et al., 2002) with an extraction factor of 0.2 to 0.3.

DTI Tractography Analysis

Preprocessed DWI data were used to identify major WM tracts using DTT. Tractography methods utilize DTI information regarding the directionality of diffusion to estimate the most likely fiber orientation within each voxel in the brain. Comparing the fiber orientation from voxel to voxel, algorithms are used to infer continuity of fibers and reconstruct WM pathways. The tractography procedure in this study was performed using DTI Studio software (Johns Hopkins University, Baltimore, Maryland) based on the Fiber Assignment by Continuous Tracking (FACT) algorithm (Mori et al. 2002; Jiang et al. 2006). The tracking procedure was initiated from all voxels in the brain (brute-force) and was interrupted in areas with values of FA lower than 0.2, which reflect areas of low directionality and unlikely to contain organized WM fiber bundles. The procedure was also

stopped when the algorithm took bends between two voxels lower than 60° , which reflect unlikely sharp fiber turns or a jump to an unrelated fiber tract.

Once fibers have been tracked from all the voxels in the brain, a rater drew a region-of-interest (ROI) in specific brain regions that allows isolating only the reconstructed fibers passing through that ROI. In the current study, we followed a protocol developed to isolate major WM tracts using a multiple ROI approach that has been validated for reproducibility at the inter-rater and inter-institutional level (Wakana et al. 2007). An example using this approach to segment the CST tract is described in figure 4.1. Tractography of the tracts of interest was performed by two raters who were blind to the subject's group and identity. Inter-rater reliability was evaluated using Bland-Altman bias analyses.

The protocol used provides specific locations to draw ROIs to facilitate isolating specific tracts and reduce the inter-rater variability in ROI placement. However, this protocol does not specify the sizes for the ROIs, which can introduce rater bias. To address this issue, one of the raters conducted preliminary reconstruction of all the tracts of interest in several subjects and optimized the maximum and minimum size of ROI1 and ROI2 that resulted in consistent reconstruction of each tract of interest.

The following WM tracts were reconstructed. Commissural fibers included those connecting the occipital lobes via the splenium of the corpus callosum, or forceps major (FMaj) and those connecting the frontal lobes via the genu of the corpus callosum, or forceps minor (FMin). Association tracts included bilateral inferior longitudinal fasciculi (ILF), bilateral inferior fronto-occipital fasciculi (IFOF), and bilateral SLF. Projection tracts included bilateral corticospinal tracts (CST). Tractography was performed in the subject's native space to minimize the effects of template misalignment from registration procedures.

The mean values of all the DTI parameters (FA, MD, RD and AD) were calculated from all the voxels within the volume of the segmented tracts. In cases where the reconstructed fiber tracts yielded anatomically incorrect pathways, the values from that tract were excluded from further analysis. Data was evaluated for normal distribution with the Saphiro-Wilk test. Comparison of group means for the diffusion parameters was accomplished by unpaired two-tailed *t*-tests for parametric data and the Mann-Whitney U-test for nonparametric data. Resulting *p* values were corrected for multiple comparisons using Bonferroni correction.

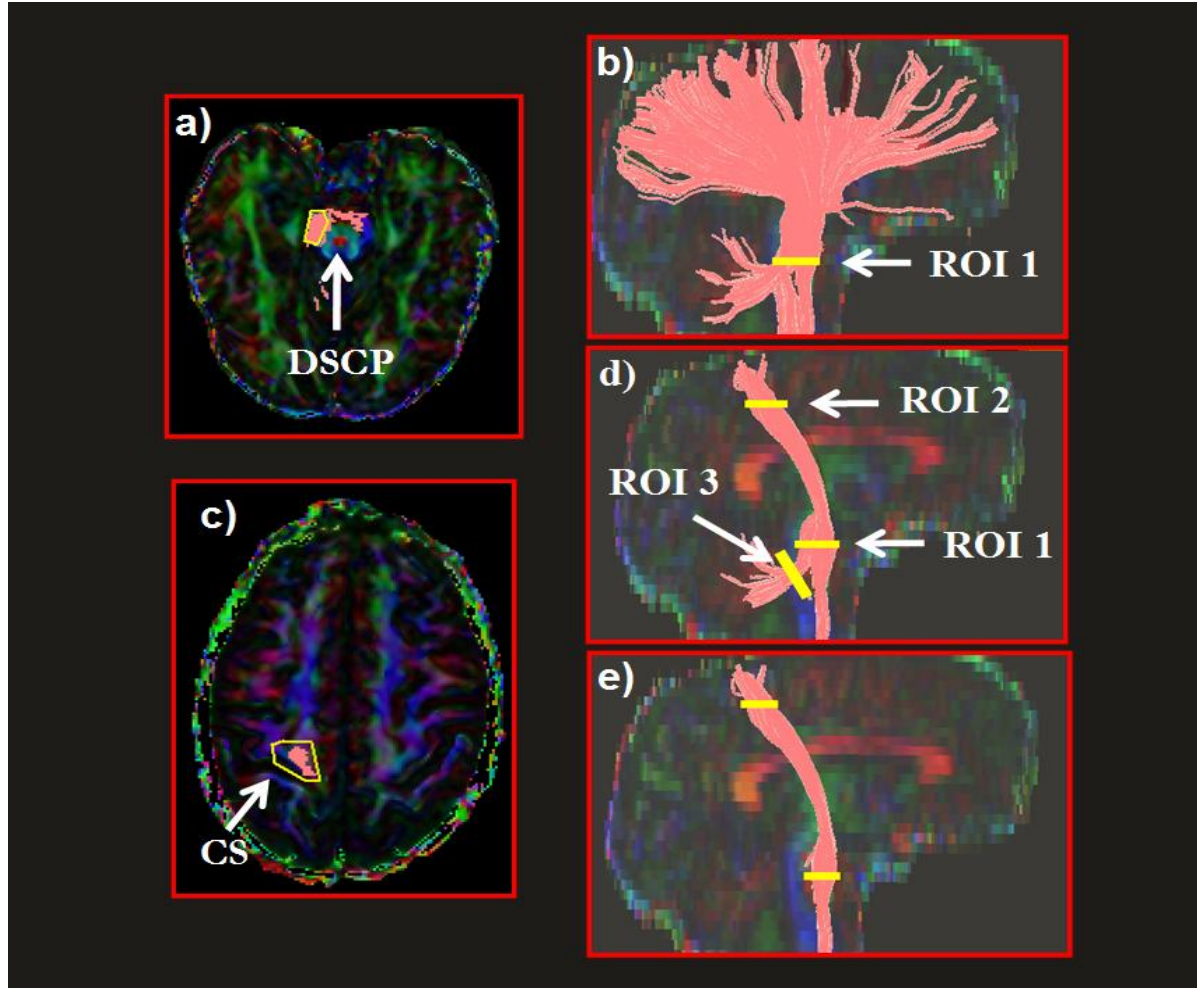


Figure 4.1 Illustrative figure of the segmentation of the corticospinal tract (CST) using DTI-based fiber tractography and an established protocol (Wakana et al. 2007). The first region of interest (ROI) is drawn on an axial color-coded FA map in the cerebral peduncle at the level of the decussation of the superior cerebellar peduncle (DSCP) (a and b). The central sulcus (CS) and the fiber projections to the motor cortex are identified in a superior FA map. A second ROI is drawn in the axial slice after the bifurcation of the motor and sensory cortex to isolate the CST (c and d). A third ROI can be drawn to exclude fibers that may not appear to belong to the CST such as cerebellar peduncle fibers (d). Final segmented tract is shown in (e). Red, green, and blue in the color-coded FA maps, represent fibers running along the right-left, anterior-posterior, and superior-inferior orientations, respectively.

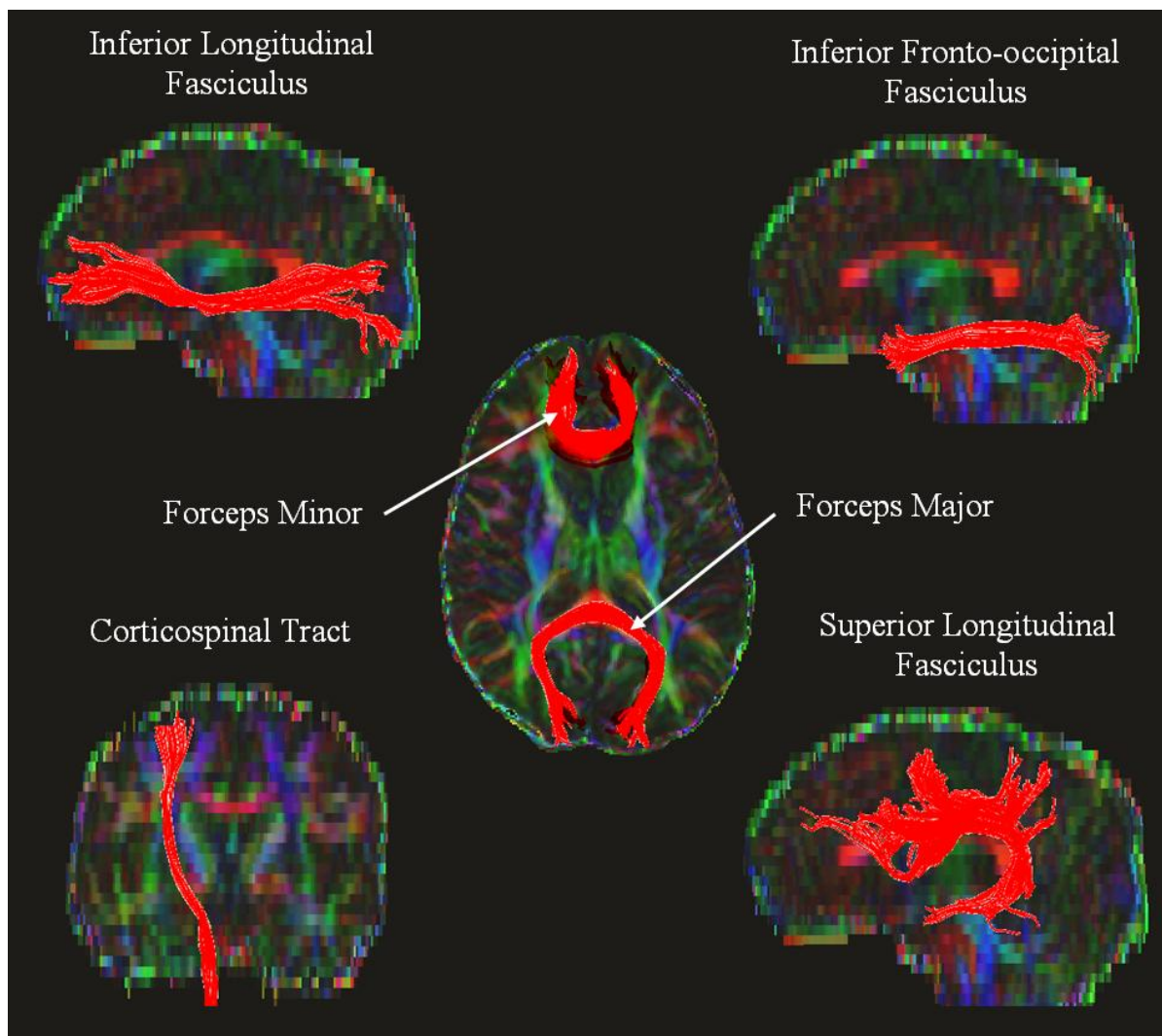


Figure 4.2 Representative figures of segmented fiber tracts. Tracts are shown in red and are overlaid on color-coded FA brain maps. Red, green, and blue in the FA maps, represent fibers running along the right-left, anterior-posterior, and superior-inferior orientations, respectively.

Tract-Based Spatial Statistics Analysis

TBSS (Smith et al., 2006) is a recently developed technique that allows non-hypothesis driven and rater-independent voxel-wise group analysis of DTI data. The approach involves an optimized registration procedure of subjects FA maps to a template with a subsequent projection of these FA maps to a mean “skeleton” group FA map which guides between-group statistical analysis of FA values at each voxel (voxel-wise) within the skeleton.

This analysis was conducted using TBSS version 1.2 (Smith et al., 2006) and various modules within FSL. The first step involved generating FA maps from the preprocessed DWI data using the DTIFIT module. Subsequently, FA maps for each subject were aligned to the Montreal Neurological Institute (MNI) template using nonlinear registration (Rueckert et al., 1999) to allow for between-group comparison. Next, a mean FA image was calculated (Fig 4.3a) and then thinned to create a mean FA “skeleton” which represented the centers of all tracts common to all subjects (Fig. 4.3b). A threshold of $FA > 0.3$ was used to exclude non-skeleton voxels because lower thresholds resulted in the inclusion of voxels that appear outside WM regions. Each subject's aligned FA map was then projected onto this skeleton and the resulting data was subjected to a voxel-wise between-group statistical analysis. Regions of group differences in FA within skeleton voxels were obtained by two sample t-test thresholded using threshold-free cluster enhancement (Smith, 2009) at $p < 0.05$, after correction for multiple comparisons controlling for family-wise error (FWE) rate. Anatomical labeling was subsequently accomplished by overlaying the Johns Hopkins University International Consortium for Brain Mapping (JHU ICBM)-DTI-81 WM atlas

(Mori et al., 2008) on the FWE-corrected statistical map of significant between-group differences.

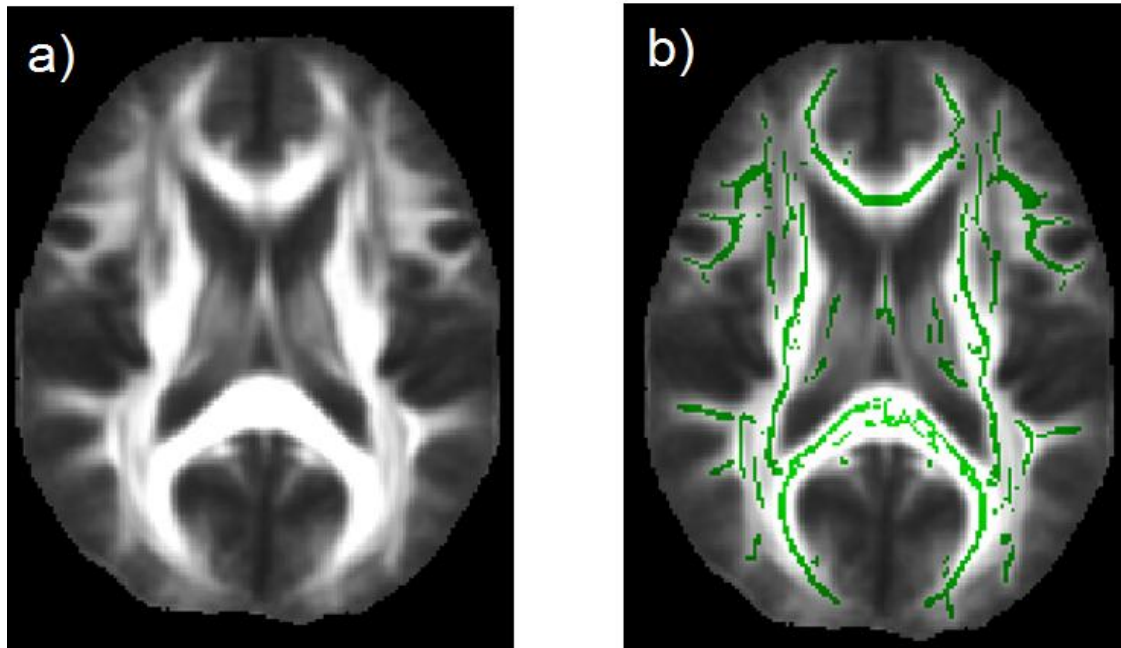


Figure 4.3 Mean FA map (a) and FA skeleton (b) use to conduct TBSS analysis between MS and controls subjects. Areas included in the skeleton are shown in green and are overlaid on the mean FA. Brighter areas in (a) represent regions of high anisotropy due to high directionality in water diffusion.

Correlation between DTI, DCM, fMRI and behavioral measures.

Regression analysis using Spearman's rank correlation coefficient was performed for the patient group to examine associations between the measures of brain activation obtained from the N-back fMRI study described in chapter 2, brain functional integration from the DCM study described in chapter 3, measures of cognitive performance and WM integrity parameters within the SLF. The measures utilized for these analyses included MD, RD, AD, and FA within the SLFs, effective connectivity values within prefrontal regions during the performance of the 2-back task, accuracy and reaction time during the 2-back task and accuracy during the PASAT, and activation levels (% whole BOLD signal) in regions of increased recruitment within the right dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) during the performance of the 2-back task. These analyses were conducted using Stata 10.0. Because of the multiple correlations performed, only coefficients > 0.5 ($p < 0.02$) are reported.

RESULTS

Subjects

One control subject was excluded from all the analyses due to severe motion artifacts and three others because they underwent a different DWI sequence. The final analyses consisted of 20 patients and 19 controls. DTI parameters were excluded from analyses for one control subject for the bilateral ILF and left IFO and for one patient for the left IFO because the segmented tracts were anatomically incorrect.

DTI-based Tractography Analysis

Inter-rater reproducibility for diffusion parameters obtained from fiber tracking was excellent as determined by Bland-Altman bias analyses (Fig. 4.4). Group comparison of diffusion parameters within major tracts obtained through DTT yielded differences in several WM tracts (Table 4.1 and 4.2). A significantly decreased FA in patients compared to controls was observed in the forceps minor ($p = 0.022$) and the left ILF ($p = 0.015$). Patients also showed increased RD in the forceps minor ($p = 0.021$), right ($p = 0.029$) and left ($p = 0.018$) ILF and left IFOF ($p = 0.010$). MD was higher in patients than controls in the right ILF ($p = 0.03$) and left IFO ($p = 0.021$). AD was not significantly different between patients and controls in any of the tracts. The following trends ($p < 0.07$) were observed; increased MD in the F-Maj, right IFO and right CST, decreased FA in the left IFO and increased AD in F-Maj in the patient group.

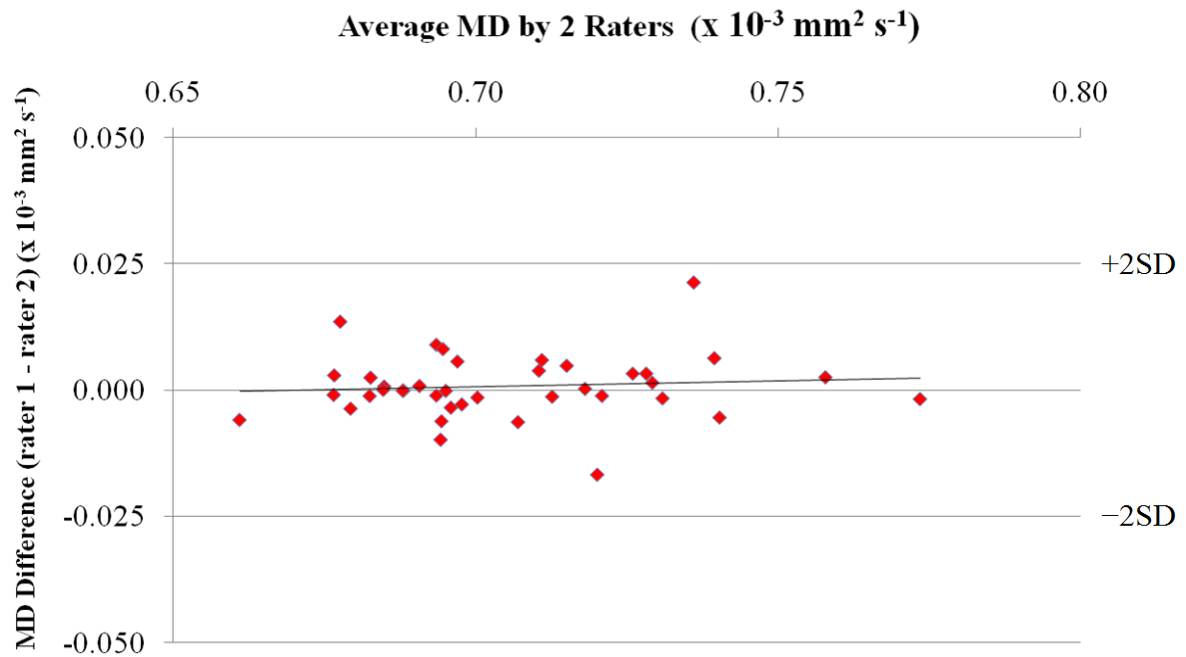


FIGURE 4.4 Bland-Altman bias plot of MD values from the 39 subjects within the right corticospinal tract. The X-axis represents the mean of the two raters, considered to be the truth, and the Y-axis represents the difference (bias) between the two raters. A linear regression least-squares fit curve did not identify a trend between the two raters.

Table 4.1 Fractional anisotropy (FA), radial diffusivity (RD) ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$), axial diffusivity (AD) ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$) and mean diffusivity (MD) ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$) of commissural and projection fiber tracts in controls healthy subjects (CS) and multiple sclerosis patients (MS).

	<u>FMin</u>		<u>FMaj</u>		<u>R</u>		<u>CST</u>		<u>L</u>	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
FA										
CS	0.537	± 0.021	0.624	± 0.016	0.566	± 0.022	0.561	± 0.021		
MS	0.512*	± 0.025	0.614	± 0.020	0.571	± 0.022	0.563	± 0.023		
RD										
CS	0.491	± 0.025	0.444	± 0.023	0.439	± 0.015	0.460	± 0.014		
MS	0.522*	± 0.032	0.471	± 0.040	0.450	± 0.022	0.475	± 0.030		
AD										
CS	1.266	± 0.043	1.435	± 0.038	1.208	± 0.050	1.242	± 0.055		
MS	1.269	± 0.036	1.480	± 0.058	1.247	± 0.051	1.285	± 0.059		
MD										
CS	0.749	± 0.026	0.775	± 0.023	0.695	± 0.018	0.720	± 0.020		
MS	0.771	± 0.028	0.808	± 0.044	0.716	± 0.026	0.745	± 0.035		

R = right hemisphere, L = left hemisphere, SD = standard deviation, FMin = forceps minor of the corpus callosum, FMaj = forceps major of corpus callosum, CST = corticospinal tract, * = $p < 0.05$ (corrected for multiple comparisons).

Table 4.2 Fractional anisotropy (FA), radial diffusivity (RD) ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$), axial diffusivity (AD) ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$) and mean diffusivity (MD) ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$) of association fiber tracts in controls healthy subjects (CS) and multiple sclerosis patients (MS).

	<u>ILF</u>				<u>IFOF</u>				<u>SLF</u>			
	<u>R</u>		<u>L</u>		<u>R</u>		<u>L</u>		<u>R</u>		<u>L</u>	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
FA												
CS	0.479	± 0.024	0.492	± 0.021	0.502	± 0.024	0.508	± 0.022	0.451	± 0.024	0.461	± 0.020
MS	0.459	± 0.027	<u>0.466*</u>	± 0.024	0.489	± 0.032	<u>0.486*</u>	± 0.025	0.445	± 0.022	0.458	± 0.024
RD												
CS	0.537	± 0.035	0.556	± 0.027	0.514	± 0.027	0.529	± 0.024	0.521	± 0.020	0.541	± 0.018
MS	<u>0.578*</u>	± 0.047	<u>0.603*</u>	± 0.060	0.545	± 0.050	<u>0.573*</u>	± 0.049	0.540	± 0.035	0.557	± 0.030
AD												
CS	1.188	± 0.047	1.273	± 0.042	1.207	± 0.036	1.269	± 0.045	1.073	± 0.040	1.127	± 0.036
MS	1.228	± 0.069	1.305	± 0.086	1.246	± 0.058	1.295	± 0.058	1.092	± 0.037	1.158	± 0.051
MD												
CS	0.754	± 0.036	0.795	± 0.028	0.745	± 0.023	0.776	± 0.025	0.705	± 0.019	0.736	± 0.017
MS	<u>0.795*</u>	± 0.051	0.837	± 0.066	0.779	± 0.048	<u>0.814*</u>	± 0.049	0.724	± 0.033	0.758	± 0.033

R = right hemisphere, L = left hemisphere, SD = standard deviation, ILF = inferior longitudinal fasciculus, IFOF = inferior fronto-occipital fasciculus, SLF = superior longitudinal fasciculus, * = $p < 0.05$ (corrected for multiple comparisons).

TBSS Analysis

The voxel-wise group comparison of FA within major WM tracts identified multiple regions of decreased FA in patients compared to controls (Fig. 4.5). Decreased FA was observed extensively within the body of the corpus callosum. Involvement within the callosal fornices included the body of the forceps minor and mid-body and tails of the forceps major. Patients also showed increased FA in the bilateral IFOF and ILF with greater contribution of left posterior regions. Minor involvement of bilateral CSTs was observed around the level of the body of the corpus callosum. There was minimal involvement of the left anterior thalamic radiation and SLF. No regions were identified in which patients had higher FA than controls.

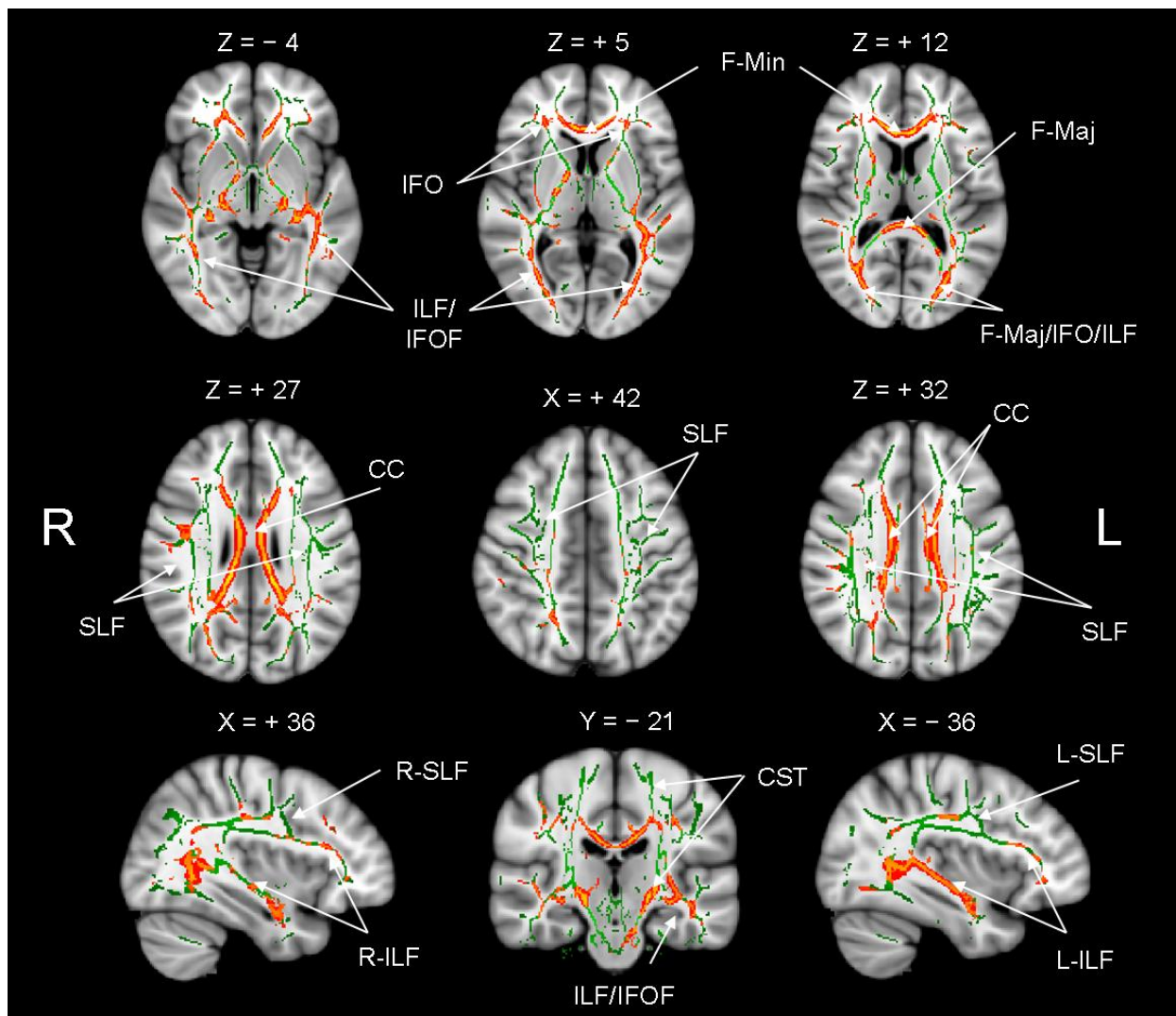


Figure 4.5 Results of the TBSS analysis between MS patients and control subjects. Areas of reduced FA in patients compared to controls ($p < 0.05$, corrected) are shown in red-orange and are overlaid on the MNI template brain and the mean FA skeleton mask (green). Red-orange color scale represents the alpha level ($1 - p$).

Multi-modal correlations

An association of increasing MD within the right SLF and increasing % whole brain BOLD within areas of increased recruitment in the right DLPFC ($r = -0.533$, $p = 0.016$) was observed. Decreasing FA within the right SLF was associated with increasing PASAT accuracy ($r = -0.522$, $p = 0.032$) and increased effective connectivity from right DLPFC ($r = -0.596$, $p = 0.006$) and ACC ($r = -0.574$, $p = 0.008$) towards the left DLPFC. Increasing MD within the left SLF was associated with decreasing activation in the right DLPFC ($r = -0.627$, $p = 0.003$).

Table 4.3 Spearman's rank correlation coefficient between mean diffusivity (MD) and fractional anisotropy (FA) in patients within the right and left superior longitudinal fasciculus (SLF) and performance during the 2-back task and the PASAT, % whole brain BOLD within regions of increased fMRI activation during the performance of the 2-back task and effective connectivity (EC) of prefrontal regions during the performance of the 2-back. Only correlations with coefficient > 0.5 and $p < 0.02$ are shown.

	Right SLF		Left SLF	
	MD	FA	MD	FA
Behavioral Tasks				
2-Back Accuracy				
2-Back Reaction Time				
PASAT		0.522		
fMRI-BOLD				
R-DLPFC	-0.533		-0.627	
ACC				
DCM-EC				
R-DLPFC → L-DLPFC		0.596		
R-DLPFC → ACC				
L-DLPFC → R-DLPFC				
L-DLPFC → ACC				
L-DLPFC → ACC				
ACC → R-DLPFC				
ACC → L-DLPFC		0.574		

R = right, L = left, DLPFC = dorsolateral prefrontal cortex, ACC = anterior cingulate cortex. Arrows symbolize the direction of the intrinsic connection modulated by the 2-back task.

DISCUSSION

During the current studies, the microstructural integrity of major WM tracts was investigated in MS patients without clinical disability. Based on their cognitive performance and pattern of cortical function during the 2-back task, we predicted that these patients will have preservation of integrity of SLF. We tested this hypothesis by using DTI-based fiber tractography and TBSS. The main findings of these studies are that these patients have substantially altered diffusion measures within the corpus callosum and fiber tracts within inferior and posterior regions of the brain including bilateral ILF and IFOF. A less severe involvement was observed within the bilateral CST and SLF. Decreased integrity of the right SLF was associated with decreased cognitive performance and decreasing functional integration within the prefrontal cortex. Decreased integrity in bilateral SLF was associated with decreased activation in areas within the right DLPFC where patients showed increased recruitment during the 2-back task.

By combining DTT and TBSS to assess diffusion properties within major WM tracts of the brain, we aimed to reduce the limitations associated with each approach. To reduce the effects of template misalignment and account for inter-individual differences in tract anatomy, tractography was performed in each subject's native image state. Moreover, because the TBSS skeleton can be very thin in certain regions, ranging from one to only a few voxels, the volumes analyzed within each tract can be smaller than their real size. To reduce rater bias associated with the ROI-based approaches, we implemented a validated protocol for the reconstruction of major WM tracts (Wakana et al. 2007). TBSS analysis has the advantage of being automated, rater-independent, non-hypothesis driven and voxel-wise. This approach allows the direct group comparison of FA in a representative skeleton of WM

tracts of the whole brain (Smith et al. 2006). Overall, we observed good agreement between the results of both methods. However, the TBSS analysis detected subtle differences as those seen in the CSTs and SLFs that were not detected with the DTT approach. This is possibly due to the averaged metrics of all the voxels of the entire fiber tract volume provided by the DTT method compared to the voxel-wise approach used in TBSS.

Chronic demyelination and axonal degeneration associated with MS result in an overall reduction in anisotropy within WM. Consistently, we also observed increased MD in patients, reflecting decreased barriers to overall diffusion, decreased FA, reflecting decreased directionality within the WM and decreased RD, reflecting increased diffusion perpendicular to the main white matter tract. Consistent with previous DTI studies in MS patients with low disability (Kern. Et al 2009, Giorgio et al. 2010), decreases in FA were associated with increases in RD, which suggests that the diffusion changes observed in these patients are more related to demyelination than acute axonal damage. Overall, the diffusion alterations observed in the current studies signify a decreased integrity of WM. The following sections address the topographical distribution of these changes.

Corpus Callosum and Inferior Tracts

Consistent with previous studies in MS patients with low disability, we found altered diffusion measures within the corpus callosum using both DTT and TBSS (Raz et al. 2009, Rocca et al. 2009). Diffusion changes were more apparent in the forceps minor than in the forceps major as reflected by the increased FA and increased RD observed in the former but not the latter. These findings may be related to lower myelination of anterior callosal tracts compared to posterior ones as suggested by prior DTI studies (Hasan et al. 2010), which

may render them more susceptible to demyelination and axonal injury associated with MS (Schmierer et al. 2007).

Severe alteration in diffusion measures was observed by both methods in bilateral ILF and IFOF. These findings are consistent with previous DTI studies in patients with MS and low disability (Ceccaralli et al. 2008, Raz et al. 2009, Rocca et al. 2009). The ILF and IFOF are bundles with extensive involvement in the transfer and processing of visual stimuli (Catani et al. 2003, Schmahmann et al. 2007). While none of the patients had severe compromise of visual function, it is possible that these abnormalities may have resulted in subtle visual changes not identifiable during a neurological examination or EDSS scoring.

Corticospinal Tracts

The CST contains primarily motor axons that are relevant to motor function. Its damage results in higher disability as measured by the EDSS, which is mainly weighted towards motor function. In fact, it has been suggested that the chronic degeneration of this tract may be the pathological substrate for the progressive stage of MS (Kremenchutzky et al. 2006). While only a trend of increased MD in the right CST was detected with DTT, TBSS analysis shows that decreased FA was limited to the cerebral peduncles bilaterally. We interpret these findings as a relatively limited involvement of the CST in these patients who had no motor function deficits as detected by the nine hole peg test (Table 1.1). In line with these findings, Pagani et al. 2005 found that CIS patients with CIS motor symptoms had greater MD within the CST than those without it. When compared to controls, patients without motor symptoms showed a limited increase in MD but a more pronounced increase was observed for those with motor symptoms.

Superior Longitudinal Fasciculi

The SLF forms an extensive network serving multiple higher cognitive functions including language, complex motor function, spatial attention, information retrieval, and working memory (Markis et al. 2005). Analysis using tractography did not detect between group differences in diffusion parameters within the SLF, and the TBSS analysis showed decreased FA in limited regions. Our findings are in partial disagreement with those of Raz et al. (2009) who studied a group of 34 CIS patients using TBSS. These authors found decreased FA in majority of WM tracts studied and while the greatest involvement was noted in the corpus callosum and CSTs, they also observed extensive involvement of the SLF. While patients were possibly in the early phase of the disease, the range of disability of patients in that study was broader than in the current study, which may explain in part the differences in our findings. In addition, all the patients in that study underwent MRI within 3 months of the clinical attack, which presumably was associated with active inflammatory lesions that may have altered diffusion metrics.

The relative preservation of integrity within the SLF in the current group of patients may be an important contributor to their overall conserved level of function. This may be related to the importance of this tract in many high level cognitive processes. Consistent with this idea, we observed an association between decreased integrity within the right SLF and decreased cognitive performance on the PASAT. In line with these findings, other authors have also observed similar associations of diffusion measures within SLF and PASAT performance in MS patients (Dineen et al. 2009, Heccke et al. 2010).

To further understand the association of structural connectivity and behavioral performance between SLF integrity and PASAT performance, we correlated fMRI signal in

regions within the right DLPFC and bilateral ACC where patients show increased activation when using their working memory. An association between compromised integrity within the right SLF and decreasing activation within the right DLPFC was observed. This finding signifies that compensatory functional recruitment within the right DLPFC when performing cognitive tasks diminishes as greater damage is incurred to tracts that transmitting its signals. This may be related to an overall decrease in information flow to the DLPFC from other regions of the brain.

We found that decreased integrity of the right SLF was not only associated with decreased right DLPFC recruitment but also with its effective connectivity to the left DLPFC. Decreased right SLF integrity was also associated with decreased effective connectivity from ACC to the left DLPFC. Interestingly, the right DLPFC and ACC were the main regions which showed increased recruitment when patients performed the working memory task. On the other hand, the left DLPFC showed greater driving input in patients when performing this same task.

While these associations should be interpreted with caution given the small sample size, the following mechanism can be suggested that could explain these relations. Compromised right SLF integrity may result in a decrease in the information flow to the right DLPFC and therefore its recruitment by patients when performing demanding cognitive tasks. Diminished recruitment of the right DLPFC may result in decrease in effective connectivity from right DLPFC to the left DLPFC. Given the crucial role of the left DLPFC as a driving region during working memory tasks, decreased right to left DLPFC coupling may in turn result in suboptimal function of the left DLPFC and diminished performance during cognitive demands.

Conclusion

The current study indicates that patients with MS without clinical disability have substantial alterations in callosal, inferior and posterior WM regions and less pronounced involvement of the CSTs and SLF. Decreased WM integrity within the right SLF in MS patients without clinical disability is associated with decrease in cognitive performance, prefrontal cortical recruitment and functional integration when performing cognitive tasks.

Limitations

While this study is an important step towards better understanding the topographical distribution of WM damage in non-disabled MS patients and the relation of SLF integrity to prefrontal cortex function, it is not without its limitations. Areas of very low FA such as those with edema or lesions may have resulted in altered or interrupted fiber tracking. This effect may have been minimal as we did not detect significant differences in number of fibers tracked between patients and controls in any of the tracts. As previously mentioned, the small sample size of the current studies limits the generalization of these results. Finally, given the homogeneity in disability of the current group of patients, the findings of the regression analyses may not be applicable to other patient groups such as those with higher disability.

CHAPTER 5
CONCLUSIONS AND FUTURE DIRECTIONS

We have learned over the last decade that an important mechanism allowing MS patients to preserved function despite MRI-defined brain damage may be related to their ability to increase cortical recruitment when performing tasks. This knowledge has come primarily from functional neuroimaging studies examining brain activation in groups of MS patients performing motor or working memory tasks. While these studies have uncovered a promising and exciting adaptive ability of the adult nervous system, they have also only provided a partial picture of the underlying brain mechanisms that allow this phenomenon to occur.

The current studies aimed to advance our understanding of cortical reorganization in MS patients. These studies were undertaken in a group of patients without clinical disability because cortical reorganization is well characterized in these patients and more importantly because they are more likely to respond to therapeutic intervention. We first investigated whether MS patients increase recruitment of common brain cortical regions when performing tasks using different functional systems. Based on the expectation that cognitive processes result from the interaction of brain regions and not isolated brain activity, we then assessed the functional interaction of brain regions of the working memory system during the performance of demanding cognitive task. Finally, we investigated the integrity of major white matter tracts in these patients, which provide the physical structures for communication with remote brain regions and allow for their functional interaction.

To answer the first question, we conducted fMRI studies to investigate the patterns of activation of MS patients without clinical disability during the performance of sensory, motor and cognitive tasks. The results from these studies demonstrated that these patients increased activation of the right DLPFC and ACC during the performance a working

memory task and during non-dominant hand movements. These results support the growing evidence for increased functional recruitment of cognitive control brain regions in the working memory system of MS patients with low disability and provided new evidence for its role in the motor system.

Using DCM, we then investigated the functional interaction of cognitive control regions in these patients during the performance of a working memory task. These results indicated that non-disabled MS patients engage similar functional networks to those used by healthy control subjects when performing the n-back task. In addition, no alterations in the effective connectivity of prefrontal and parietal cortical regions occur when they performed this task. Finally, this study suggested that working memory tasks have greater influence on the activity of the left DLPFC in patients than controls supporting the importance of cognitive control recruitment in these patients.

Finally, we investigated the structural connectivity in the brain in these patients by assessing the integrity of major white matter tracts. The structural connectivity studies indicated that patients with MS without clinical disability have substantial alterations in callosal, inferior and posterior white matter regions, but less pronounced involvement of the corticospinal and superior longitudinal tracts. Furthermore, to gain a more complete understanding of cortical reorganization in MS patients, we then correlated information gained from the three types of studies conducted in this project. Correlations from this analyses suggested that decreased white matter integrity within the right SLF in non-disabled MS patients is associated with decrease performance, and prefrontal cortical activation and functional integration when performing cognitive tasks.

In summary, these studies suggest that patient with MS without clinical disability increase the recruitment of the cognitive control system across functional domains and rely on a preserved functional and structural architecture to perform cognitive demanding tasks. In addition these studies show the potential of combining brain activation and functional integration data from fMRI and structural connectivity data from DTI to improve our understanding mechanisms of brain adaptation in neurological diseases.

While the current studies have several limitations including the small sample sizes and its cross-sectional nature, they provide future direction for studies aimed to improve therapeutic and diagnostic interventions in MS patients. Given the important role that recruitment of the cognitive control system has when patients perform various tasks, future clinical interventions may focus to increase cognitive control in MS patients. Current advances in imaging technology may allow monitoring efficiency of cognitive control recruitment while patients undergo rehabilitation and provide feedback to increase use of the cognitive control system. Evaluation of cognitive control recruitment may allow identification of patients at higher risk for developing cognitive and clinical deficits. Finally, this knowledge opens a window to the possibility of enhancing cognitive control through emerging therapeutic interventions such as repetitive transcranial magnetic stimulation (rTMS), which has provided encouraging results in MS (Koch et al., 2008) and other neurologic diseases (Padberg and George 2009; Ridding et al., 2007).

While techniques and approaches to assess the functional interaction of brain regions are still in their infancy and continue to be improved, they offer great potential to gain a more complete understanding of loss of function in MS patients. Measuring effective connectivity of brain regions, in particular within the prefrontal cortex, may complement

conventional surrogates of disease burden such as T2 lesion load. Improved knowledge about the specific mechanisms underlying adaptive brain processes in MS patients, especially in those without disability, may help guide therapeutic interventions of the future. For instance, if interventions target increasing the activation of specific cortical regions (e.g. behavioral, pharmaceutical, rTMS), identifying whether these therapies results in increased or decreased interaction between brain regions would be very valuable. This would allow determining if the interventions ultimately result in beneficial or adaptive connectivity among regions. Grefkes et al. (2010) have begun testing these ideas in victims of damage to the motor cortex from stroke. Using rTMS to inhibit the neuronal activity of selected motor regions, they have shown an improvement in motor performance in those patients who underwent this treatment. They concurrently used fMRI and DCM to study effective connectivity during this procedure. Interestingly, they found behavioral improvements correlated with effective connectivity of the stimulated regions.

Measuring structural connectivity and white matter integrity within specific functionally-relevant tracts have demonstrated their great diagnostic potential. Recent evidence has shown that RD (associated to demyelination) within transcallosal hand motor fibers was able to predict hand motor function decline over a period of one year (Kern et al. 2011). Studies based on RD in the optic nerve have also shown promising results to monitor visual recovery in MS patients suffering optic neuritis (Naismith et al. 2010). Our study provides support for the crucial role that SLF may have in the preservation of function in patients with MS. Future studies further examining this association may help determine the potential prognostic value that measures of integrity within this tract may have. In addition measuring the integrity of specific tracts may help guide interventions targeted to improve

the functional interaction of brain networks. Identifying severe disruption of fiber integrity within particular regions may help guide the selective enhancement of functional networks at different sites with better structural connectivity. Such approaches would involve customized therapeutic interventions.

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VITA

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