Abnormal Functional Connectivity and Cortical Integrity Influence Dominant Hand Motor Disability in Multiple Sclerosis: A Multimodal Analysis

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Abstract: Functional reorganization and structural damage occur in the brains of people with multiple sclerosis (MS) throughout the disease course. However, the relationship between resting-state functional connectivity (FC) reorganization in the sensorimotor network and motor disability in MS is not well understood. This study used resting-state fMRI, T1-weighted and T2-weighted, and magnetization transfer (MT) imaging to investigate the relationship between abnormal FC in the sensorimotor network and upper limb motor disability in people with MS, as well as the impact of disease-related structural abnormalities within this network. Specifically, the differences in FC of the left hemisphere hand motor region between MS participants with preserved ($n = 17$) and impaired ($n = 26$) right hand function, compared with healthy controls ($n = 20$) was investigated. Differences in brain atrophy and MT ratio measured at the global and regional levels were also investigated between the three groups. Motor *preserved* MS participants had stronger FC in structurally intact visual information processing regions relative to motor *impaired* MS participants. Motor *impaired* MS participants showed weaker FC in the sensorimotor and somatosensory association cortices and more severe structural damage throughout the brain compared with the other groups. Logistic regression analysis showed that regional MTR predicted motor disability beyond the impact of global atrophy whereas regional grey matter volume did not. More importantly, as the first multimodal analysis combining resting-state fMRI,

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Key words: grey matter volume; magnetization transfer ratio; motor disability; logistic regression; multiple sclerosis; resting state fMRI; functional connectivity

INTRODUCTION

Multiple sclerosis is a chronic demyelinating and neurodegenerative disease associated with motor impairment and reduced coordination [Matthews, 1991]. Upper limb disability is a common progressive symptom present in more than 50% of people with multiple sclerosis (MS) [Holper et al., 2010; Johansson et al., 2007; Kister et al., 2013], which interferes with independent performance of activities such as bathing, dressing and writing [Kierkegaard et al., 2012; Yozbatiran et al., 2006]. The dominant upper limb is typically engaged in daily activities, therefore the maintenance of its proper function is important for quality of life [Yozbatiran et al., 2006]. The importance of addressing dominant upper limb function in people with MS is increasingly recognised (for review see [Lamers and Feys, 2014]).

Conventional magnetic resonance imaging (MRI) methods that permit quantification of MS lesion load have advanced the diagnosis of MS. Lesion load is a useful measure because it can be obtained with conventional MRI and is associated with disability as measured by the Expanded Disability Status Scale (EDSS) scores [Crespy et al., 2011; Traboulsee et al., 2003]. However, its rather modest correlation with clinical outcome measures has driven the search for more sensitive neuroimaging markers of MS pathology, including grey matter volume and magnetization transfer ratio (MTR). Grey matter volume is affected by neuroaxonal loss in MS [Siffrin et al., 2010]. Although MTR may be reflected by axonal loss and inflammation, it is primarily affected by myelin content not only in white matter [Dousset et al., 1992; Schmierer et al., 2004, 2007] but also in grey matter [Chen et al., 2013; Schmierer et al., 2010]. It has proved sensitive to both white [Chen et al., 2007, 2008; Pike et al., 2000] and grey matter damage [Fisniku et al., 2009; Jure et al., 2010], even in early MS [Crespy et al., 2011; Ranjeva et al., 2005]. Both grey matter volume and MTR have been associated with clinical disability [Fisher et al., 2008; Fisniku et al., 2008; Hayton et al., 2009] and provide complementary information relevant to the pathology of MS [Khaleeli et al., 2007; Mallik et al., 2015].

Alternative neuroimaging techniques that provide functional information may shed further light on the physiological processes related to motor disability in MS. Functional MRI (fMRI) studies involving performance of finger flexion-extension with the right (dominant) hand suggest that neural functional reorganization occurs in MS throughout the disease course [Reddy et al., 2000, 2002;

Rocca et al., 2002; Valsasina et al., 2011], potentially explaining the low correlation between lesion load and disability [Reddy et al., 2002; Rocca et al., 2002; Valsasina et al., 2011]. Specifically, recruitment of multimodal integration regions beyond the conventional sensorimotor areas may act to compensate for structural damage, thereby mitigating the clinical impact of MS on the dominant upper limb [Rocca et al., 2002; Valsasina et al., 2011]. Resting-state fMRI has also been widely used to study differences in functional connectivity in MS compared with healthy controls (for reviews see [Filippi et al., 2013; Sacco et al., 2013]). Resting-state functional connectivity (FC) analysis measures the temporal correlations of intrinsic blood oxygen level dependent (BOLD) activity across brain regions; the highly connected brain regions so identified form specific functional networks [Van den Heuvel and Pol, 2010]. Multiple functional networks have been identified reliably using this technique, including a network of regions involved in sensorimotor function that is of particular interest for understanding the control of upper limb motor function [De Luca et al., 2005; Xiong et al., 1999]. In healthy adults, this network includes bilateral primary motor cortices, premotor cortices, somatomotor cortices, somatosensory cortices, cingulate motor areas, supplementary motor area (SMA) and the insula [Habas et al., 2009; Jolles et al., 2011; Larson-Prior et al., 2009; Xiong et al., 1999]. We refer to these regions hereafter as comprising the “conventional” motor network.

In some FC studies of MS patients, regions of the sensorimotor network have shown decreased functional connectivity [Janssen et al., 2013; Lowe et al., 2002; Rocca et al., 2012], which correlated with lesion load and disability [Janssen et al., 2013]. In contrast, investigations restricted to patients early in the disease course showed *increased* sensorimotor connectivity compared with healthy controls, which was interpreted as reflecting functional compensation for tissue damage [Basile et al., 2013; Faivre et al., 2012; Roosendaal et al., 2010]. The direction of changes in functional connectivity is hypothesized to depend on shifting balances between neuronal damage and compensatory mechanisms as MS disability progresses [Basile et al., 2013]. Demonstration of preserved motor ability is required for changes in connectivity to be interpreted as compensatory; otherwise such changes may be merely epiphenomenal to neuronal dysfunction and damage.

In summary, both structural and functional abnormalities are associated with physical disability in MS (for

reviews see [Filippi and Agosta, 2010; Sacco et al., 2013]). However, little is known about how the combination of these factors contributes to preservation or loss of motor abilities in MS. We propose that progress in understanding the functional mechanisms that intervene between MS pathology and disability will benefit from a focused analysis of the structural, functional, and behavioural changes associated with a specific, well-characterized brain network. Thus, the current study employed multimodal neuroimaging approaches to directly investigate the relationships between disease-associated structural abnormalities, abnormal resting-state functional connectivity in the sensorimotor network, and dominant hand motor disability in MS.

First, we examined the association between FC of the dominant hand sensorimotor network and dominant hand motor disability as indexed by the 9-Hole Peg Test (9HPT). Based on previous findings from studies using fMRI [Rocca et al., 2002, 2012], we predicted that poorer 9HPT performance among MS patients would be associated with weaker FC between the hand motor area and regions in the conventional motor network, whereas MS participants with normal manual dexterity would show stronger FC with the hand motor area in diverse regions outside the conventional motor network. Second, we investigated the relationship between abnormal connectivity to the hand knob area of the motor cortex and structural integrity measures including volume and MTR within the regions of interest. The structural measures included whole-brain measures of lesion load, grey matter, and normal-appearing white matter, as well as network-specific measures within the cortical regions that we identified as showing group differences in their functional connections to the dominant hand motor region. Finally, we explored whether these structural measures within the regions of interest predicted 9HPT performance. We hypothesized that damage within the conventional motor network might be a prerequisite for functional reorganization that incorporates non-conventional regions into the motor network. Thus, we predicted lower grey matter volumes and MTR in some regions of the conventional motor network even among MS participants with preserved motor function. However, the greatest structural abnormalities were expected in those with impaired motor function.

MATERIALS AND METHODS

Participants

A total of 43 participants with MS (mean age $\pm$ SD: 49.23 ± 10.87 years, 24 females) were recruited: 27 with a relapsing-remitting course, 4 with primary progressive MS and 12 with secondary progressive MS. To reduce variability associated with differences in hemispheric dominance, participants who self-reported as non-right-handed (≤ 0.5

on the Edinburgh Handedness Inventory [Oldfield, 1971]) were excluded. Exclusion criteria also included: claustrophobia, typical contraindications to MRI (e.g., pregnancy, pacemaker, ferromagnetic metal in body), history of mental illness or serious health conditions other than MS, and clinical relapses within 3 months prior to the study. Time since diagnosis, medications for MS treatment and most recent EDSS score were collected from retrospective clinical chart review. A control group of 20 age- (mean $\pm$ SD: 44.22 ± 13.28 years) and sex-matched (12 females) healthy controls (HC) was also recruited.

Ethics Committee Approval

The Research Ethics Board of the Montreal Neurological Institute and Hospital approved this study and written informed consent was obtained from all participants.

Behavioural Assessments

Participants performed the 9HPT to assess upper limb function as part of the Multiple Sclerosis Functional Composite (MSFC) [Cutter et al., 1999] in the context of a larger battery of performance-based tasks and questionnaires. This study focused on performance of the dominant hand in right-handed individuals. Participants with MS whose 9HPT z-scores were within two standard deviations (SD) of published norms [Oxford Grice et al., 2003] were assigned to the motor-preserved (MP) group. Those who were slower than two SDs from the normal mean were considered motor impaired (MI). All HC participants scored within two SDs of the normative controls. Correlational analyses used raw 9HPT completion times and included age and sex as covariates in the model.

MRI Acquisition

MRI was performed in a separate session (mean interval $\pm$ SD: 5.27 ± 6.60 days) from the behavioural assessment on a 3T Siemens Magnetom Trio Tim scanner using a 32-channel phased-array head coil at the McConnell Brain Imaging Centre of the Montreal Neurological Institute. The MRI protocol, which has been optimized by members of our group for use in MS clinical trials [Archambault-Wallenburg et al., 2013] included:

1. T1-weighted (T1w) Fast Low Angle Shot (FLASH) sequence: 20/5 ms [repetition time/echo time (TR/TE)], 27° flip angle, 192 contiguous slices, 1 mm slice thickness, 256×256 mm² field of view (FOV), 1×1 mm² in plane resolution.
2. Proton density-weighted (PDw) FLASH sequence pair: 33/3.81 ms [TR/TE], 10° flip angle, performed with and without a magnetization transfer weighting pulse, with the same geometry as the T1w sequence.

3. PDw and T2-weighted (T2w) dual turbo spin echo sequences: 2.1 s/17 ms/76 ms [TR/TE_{PDw}/TE_{T2w}], 120° flip angle, 60 contiguous slices, 3 mm slice thickness, 256 × 256 mm² FOV, 1 × 1 mm² in plane resolution.
4. T2w 3D Fluid Attenuated Inversion Recovery (FLAIR) sequence: 6 s/355 ms [TR/TE], 192 contiguous slices, 1 mm slice thickness, 256 × 256 mm² FOV, 1 × 1 mm² in plane resolution.
5. Resting state echo planar images covering the whole brain: 2.26 s/30 ms [TR/TE], 90° flip angle, 38 contiguous slices (interleaved acquisition), 4 mm slice thickness, 256 × 256 mm² FOV, 4 × 4 mm² in plane resolution, no parallel imaging technique used. This spatial resolution is within the range of other movement related fMRI studies [Kristo et al., 2014; Valsasina et al., 2011; Wegner et al., 2008] and represents the optimization of resolution opposed to signal to noise ratio. For this scan, participants were instructed to rest with their eyes closed without falling asleep, and to think of nothing in particular. Each functional scan contained 133 image volumes after the first three volumes were discarded to allow the magnetization to reach equilibrium.

MRI Processing and Analysis

Magnetization transfer ratio (MTR) image processing: MTR, expressed as percent units (pu), was calculated for each voxel as follows: $MTR = (M_0 - M_{mt})/M_0 \times 100$ (pu), where M_0 and M_{mt} were the images obtained without and with MT saturation pulse, respectively. MTR maps were coregistered onto their corresponding T1w images.

Image segmentation and lesion delineation: T1w images were entered into FreeSurfer (version 5.1.0, <http://surfer.nmr.mgh.harvard.edu>) for white and grey matter segmentation [Fischl et al., 2002]. During segmentation, to fix the topological errors that may have occurred due to image quality and lesion presence, the first author performed minimal manual editing to remove non-brain tissues and fill the holes in the white matter that were lesions. White matter lesions were delineated by a trained researcher (JCN) on the T2w images using a semi-automated method [Francis, 2004] supported by information from T1w, FLAIR and PDw images. These lesion masks were subtracted from the white matter to obtain normal appearing white matter (NAWM). Volumetric measures for NAWM, whole-brain grey matter and T2w lesion load (T2-LL) were normalized for subject head size using the VSCALING parameter, based on skull-constrained registration to MNI152 standard space, provided by SIENAX [Smith et al., 2002], part of FSL (<http://fsl.fmrib.ox.ac.uk/fsl>). To minimize the impact of partial volume effects in the NAWM and grey matter, we excluded any voxels with an MTR value of less than 10 pu that may be due to CSF. Individual T1 brain

images were registered to the MNI template with ANTS non-linear registration [Avants et al., 2008] for group analysis.

Pre-processing of resting-state fMRI data: Pre-processing was done in FSL and included slice timing based on interleaved acquisition, motion correction with reference to the mean volume, skull stripping, band-pass filtering (0.01–0.08 Hz) to remove magnetic field drifts of the scanner [Foerster et al. 2005], and regressing out nuisance parameters including motion correction parameters (reflecting residual motion), and ventricular and white matter signals (estimating subject respiration and cardiac effects) [Windischberger et al., 2002]. Mean and maximum absolute root mean square motion estimates were computed [Jenkinson, 1999; Jenkinson et al., 2002] to quantify the subject head movement. No subject showed excessive head motion (maximum framewise displacement among all the subjects was 1.17 mm). The ventricular signal was calculated as the mean signal over the whole ventricular region and the white matter signal was the mean signal over the white matter region, where both ventricle and white matter were segmented from T1 images in the T1 preprocessing steps and transformed to the fMRI space based on the transformation using boundary-based registration [Greve and Fischl, 2009]. To allow group level analysis, the individual fMRI data were then transformed to the template space by combining the transformation to their corresponding T1 image space using boundary-based registration [Greve and Fischl, 2009] with the transformation from the T1w image to MNI space. Finally, the fMRI data were spatially smoothed using a 6 mm full-width-at-half-maximum (FWHM) Gaussian filter.

Functional connectivity with the seed region: The left hemisphere hand knob representing hand motor function (LM1) was identified and segmented based on the detailed description provided by Yousry et al. 1997, who demonstrated high inter-rater reliability of 97.9% in the axial plane when tested with a sample of 198 hemispheres including brains of both healthy individuals and people affected by different pathologies [Yousry et al., 1997]. To keep the LM1 definition consistent across subjects, only the portion of Yousry et al.'s LM1 that lies within the grey matter region on the MNI template (Fig. 1, Row 1, Blue region) was included in our analysis. In practice, we delineated it in the hand knob shape in the MNI152 template (resolution: 1 × 1 × 1 mm³) with 23 slices in the superior–inferior orientation, 19 slices in the left–right orientation, and 17 slices in the anterior–posterior orientation. With all the functional data in the MNI template space and the LM1 as the seed region, the average BOLD time-course from this seed region was cross-correlated with the time-courses from all other brain voxels, and the correlation coefficients were transformed to Fisher's z-scores [Zar, 2010] to improve the normality of the correlation coefficients. This whole-brain functional correlation map generated for each subject represents the strength of correlated

Figure 1.

Left panel: functional connectivity maps of the LM1 seed region for HC, MP and MI groups. Right panel: the corresponding map for each group projected to the hemispheric surfaces using BrainNet Viewer (<http://www.nitrc.org/projects/bnv/>) for the purpose of visualization. LM1 seed region is shown in the first

row in blue. Voxel wise analyses were corrected for cluster extension ($t > 5.8$, $P_v < 0.05$, FWE corrected, $P_c < 0.05$, FWE corrected, $k > 16 \text{ mm}^3$), with age and sex controlled. Volume-based maps were displayed in the radiological convention. Note: R, right hemisphere; L, left hemisphere.

resting-state BOLD signal fluctuations with the seed. The LM1 seed region in the template space was transformed back into the individual T1 space for individual LM1 volume calculation.

Statistical Analysis

Chi-squared tests were used to compare groups on categorical variables including sex, phenotypes of MS and medication usage. One-way ANOVAs were used to compare groups (HC, MP and MI) on variables including age, time since diagnosis, MSFC total score, 9HPT z-scores and head motion parameters. To control for the potential impact of age and sex on outcomes, ANCOVA tests were used with age and sex as covariates for the normally distributed variables including 9HPT completion times, volumes and MTR of NAWM, whole-brain GM and the LM1 seed region. Both one-way ANOVA and ANCOVAs were followed by post-hoc comparison with Tukey's HSD tests when appropriate. Comparisons on non-normally distributed variables (EDSS and T2-LT) between MP and MI

groups were done using the Mann–Whitney–Wilcoxon Test.

Resting-state fMRI analysis was performed using SPM12 (<http://www.fil.ion.ucl.ac.uk/spm/>). One-way ANCOVA analysis was performed to examine and compare FC patterns of each group (HC, MP, and MI) with age and sex as nuisance covariates. The main effect of each group was calculated with a voxel level of $P (P_v) < 0.05$, FWE corrected. The F-contrast was masked by the composite of the main effects of the three groups ($P_v < 0.05$). Then, between-group contrasts were masked by the F-contrast result ($P_v < 0.05$, Bonferroni corrected by number of comparisons). All voxel-wise analyses were corrected for cluster extension at $P (P_c) < 0.05$. The voxel and cluster levels of p values were chosen to obtain reasonable spatial specificity and cluster extent of fMRI findings as recommended previously by Woo et al., [2014]. The correlations between the mean FC values in the regions showing significant between-group FC differences and the 9HPT completion time were explored separately for the MS and the HC participants with age and sex as covariates ($P < 0.05$,

TABLE I. Demographic, clinical and structural imaging and functional imaging motion characteristics of healthy controls (HC), MS participants with preserved motor ability (MP) and MS participants with impaired motor ability (MI)

Characteristics	HC	MP	MI	P-value	Post-hoc
Number of participants	20	26	17	–	–
Age, years: mean ± SD	44.22 ± 13.28	49.93 ± 12.25	48.15 ± 8.57	0.26 ^a	–
Sex: female/male (F/M ratio)	12/8 (1.5)	15/11 (1.36)	9/8 (1.13)	0.91 ^b	–
Phenotype: RRMS/SPMS/PPMS	–	19/4/3	8/8/1	0.08 ^c	–
Time since diagnosis, years: mean ± SD ^d	–	12.80 ± 9.81	7.41 ± 5.01	0.06 ^a	–
Medication treatment: none/yes	–	15/11	8/9	0.49 ^f	–
EDSS: median (range)	–	2 (0–6)	3.5 (0–6.5)	0.051 ^e	–
MSFC, z-score: mean ± SD	0.61 ± 0.33	0.33 ± 0.52	–0.61 ± 0.50	<0.001 ^a	HC>MI***, MP>MI***
RH_9HPT, time(s): mean ± SD	17.99 ± 2.19	19.91 ± 2.22	27.43 ± 3.31	<0.001 ^g	HC>MI***, MP>MI***
LH_9HPT, time(s): mean ± SD ^h	19.67 ± 2.34	20.57 ± 2.50	29.24 ± 7.78	<0.001 ^g	HC>MI***, MP>MI***
RH_9HPT, z-score: mean ± SD	0.13 ± 0.74	0.57 ± 0.73	4.18 ± 1.48	<0.001 ^a	HC>MI***, MP>MI***
LH_9HPT, z-score: mean ± SD ^h	0.45 ± 1.06	0.44 ± 0.76	3.95 ± 2.60	<0.001 ^a	HC>MI***, MP>MI***
T2-LL, ×10 ³ mm ³ : median (range)	–	3.36 (0.21–49.50)	5.54 (2.53–47.48)	0.78 ^e	–
NAWMV, ×10 ⁵ mm ³ : mean ± SD	6.74 ± 0.27	6.11 ± 0.69	6.10 ± 0.54	<0.001 ^g	HC>MP**, HC>MI**
WBG MV, ×10 ⁵ mm ³ : mean ± SD	8.74 ± 0.50	8.24 ± 0.54	8.05 ± 0.64	0.003 ^g	HC>MP*, HC>MI**
LM1 volume, ×10 ³ mm ³ : mean ± SD	1.19 ± 0.31	1.12 ± 0.26	0.99 ± 0.32	0.14 ^g	–
NAWM MTR: mean ± SD	44.00 ± 0.54	43.88 ± 1.05	42.86 ± 1.71	0.008 ^g	HC>MI*, MP>MI*
WBG MTR: mean ± SD	36.45 ± 0.57	36.49 ± 0.92	35.74 ± 1.23	0.03 ^g	MP>MI*
LM1 MTR: mean ± SD	35.34 ± 1.03	35.50 ± 1.38	34.45 ± 2.14	0.09 ^g	–
Mean motion, mm: mean ± SD	0.20 ± 0.15	0.24 ± 0.14	0.25 ± 0.16	0.49 ^a	–
Max motion, mm: mean ± SD	0.40 ± 0.45	0.50 ± 0.23	0.27 ± 0.23	0.48 ^a	–

Note: RRMS, relapse–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis; EDSS, Expanded Disability Status Scale; RH_9HPT, right hand 9-Hole Peg Test performance; LH_9HPT, left hand 9-Hole Peg Test performance; T2-LL, T2 white matter lesion load (normalized volume); NAWMV, normal appearing white matter normalized volume; WBG MV, whole-brain grey matter normalized volume; LM1, left hemisphere hand motor region; For post-hoc statistics, * represents $P < 0.05$, ** represents $P < 0.01$ and *** represents $P < 0.001$.

^aOne-way ANOVA test.

^b3X2 Chi-square test.

^c2X2 Chi-square test with relapse–remitting MS and progressive MS in MP and MI groups

^dData on time since diagnosis were not available from 2 MP participants and 2 MI participants.

^eMWW: Mann–Whitney–Wilcoxon Test.

^f2X2 Chi-square test.

^gANCOVA test (controlled for age and sex).

^hThere was one missing data point on 9HPT measurement for the left hand from MI participants due to the severity of the disease.

FDR corrected). The regions showing significant FC differences between groups in the template space were transformed back into the individual T1 space for grey matter MTR and normalized grey matter volume calculation. Furthermore, one-way ANCOVA was used for between-group comparisons of MTR and volumes for the regions showing significant group difference in FC, controlling for age and sex. Finally, to assess whether grey matter volume and/or MTR could predict whether a MS participant would be in the motor preserved or impaired group, total grey matter volume and mean MTR value from the regions with significant group difference in FC were calculated and tested in separate age- and sex-adjusted binary logistic regression models. Whole-brain GMV was also included as a covariate to evaluate whether the predictors were regionally specific or simply a reflection of global severity of disease. Odds ratios with 95% confidence intervals (CI) and their

corresponding P -values were calculated for each structural measure.

RESULTS

Characterization of the Groups

Demographic, clinical and structural imaging characteristics of the HC, MP and MI groups are shown in Table I. MS-related medications in the MS sample included Interferon beta-1a (18.60%), Fingolimod (13.95%), Ceralifimod (2.33%), Glatiramer Acetate (6.98%) and Siponimod (4.65%). All groups were matched on age and sex. The two MS groups had comparable EDSS scores, proportions of participants on MS-related medication, time since diagnosis, and T2-LL. Participants with a progressive course of MS were slightly more represented in the MI group than

TABLE II. Correlation between dominant hand 9HPT completion times and each demographic, clinical and structural neuroimaging measure in the whole MS sample (N = 43)

Characteristics	<i>r</i>	<i>P</i> -value
Age	0.02	0.88
Sex	-0.25	0.10
Time since diagnosis ^a	-0.23	0.18
EDSS ^b	0.43	0.005**
T2-LL ^b	0.10	0.52
NAWMV	-0.14	0.39
WBG MV	-0.09	0.58
LM1 volume	-0.17	0.27
NAWM MTR	-0.34	0.03*
WBG MTR	-0.32	0.04*
LM1 MTR	-0.23	0.15

Note: *: $P < 0.05$; **: $P < 0.01$; Correlations were controlled with age and sex for items other than "Age" and "Sex"; EDSS, Expanded Disability Status Scale; T2-LL, T2 white matter lesion load (normalized volume); NAWMV, normal appearing white matter normalized volume; WBG MV, whole-brain grey matter normalized volume; LM1, left hemisphere hand motor region.

^aData on time since diagnosis were not available from 2 MP participants and 2 MI participants.

^bSpearman correlation was applied due to the non-normal distribution of EDSS and T2-LL.

in the MP group, although this difference was not statistically significant (Chi-square $P > 0.05$). As intended, the MI group had worse dominant hand 9HPT performance ($P < 0.001$) than the MP and HC groups, which did not differ ($P > 0.05$); the same was true for performance with the non-dominant hand and the MSFC total score. Age- and sex-controlled volumes of NAWM and whole-brain GMV were smaller in both MS groups when compared with the HC group. The MP group showed significantly higher mean MTR within both NAWM and whole-brain GM than the MI group, but comparable values with the HC group. No significant group differences were found for either grey matter volume or MTR within LM1. No significant differences were observed between groups for head motion.

Table II shows the results of correlation analyses between age- and sex-adjusted 9HPT completion times of the right hand and each clinical and structural neuroimaging measure. 9HPT time correlated positively with EDSS score, and negatively with MTR in the whole-brain grey matter and normal appearing white matter. No significant relationship was observed between 9HPT completion time and time since diagnosis, or volumes of NAWM and whole-brain grey matter. 9HPT completion time was not significantly associated with grey matter volume or MTR in the LM1 region of interest. Higher T2-LL was associated with longer time since diagnosis ($r = 0.33$, $P < 0.05$), and with lower volumes of whole-brain grey matter ($r = -0.69$, $P < 0.001$) and normal-appearing white matter ($r = -0.54$, $P < 0.01$), but not with 9HPT completion time.

Functional Connectivity of Each Group

The LM1 related networks for the three groups are shown in Figure 1. Conventional motor regions, such as bilateral primary motor (BA4), premotor and supplementary motor area (SMA, BA6) and somatosensory cortices (BAs 1/2/3/5/7), as well as bilateral insula (BA43) were present in all three groups. The LM1 related network also covered posterior visual areas including bilateral cuneus and lingual/fusiform gyri (BAs 17/18/19), and lateral occipital (BAs 18/19) regions in the MP group, but not in the other two groups.

Comparisons of the LMI Related Network between Groups

The F-test showed that there were FC differences between groups in the bilateral sensorimotor and somatosensory association cortices (precentral gyrus/postcentral gyrus/superior parietal lobule, BAs 1/2/3/4/5/7), cuneus, lingual/fusiform and lateral occipital cortex (BAs 17/18/19) (Fig. 2, Panel A; Table III). FC in bilateral sensorimotor and somatosensory association cortices (postcentral gyrus/superior parietal lobule, BAs 1/2/3/5/7) was significantly stronger in the MP and HC groups than in the MI group. The MI group also showed significantly weaker FC in bilateral cuneus and lingual/fusiform (BAs 17/18/19), and lateral occipital cortex (BAs 18/19) when compared with the MP group (Fig. 2, Panel B; Table IV).

FC Correlations with 9HPT

When assessing all MS participants as one group, FC of all the regions showing significant group differences in the omnibus F-test had a negative correlation with 9HPT completion time, which reached or approached significance ($P_s \leq 0.05$) (Table III, far right column). In the HC group, functional connectivity of the LM1 did not correlate significantly with performance on the motor task ($P_s > 0.1$).

Structural Comparisons between Groups within the Functionally Defined ROIs

Among the regions with group differences in functional connectivity, the volumes of grey matter in the right lingual/fusiform gyrus and bilateral sensorimotor and somatosensory region were smaller in the MI group than in the HC group. Of these regions, right lingual/fusiform gyrus and left sensorimotor and somatosensory regions were also smaller in the MP group than in the HC group. Bilateral cuneus and bilateral lingual/fusiform showed significantly lower MTR in the MI participants than in the HC and MP participants (Fig. 3, Supporting Information Tables SI and SII). MTR values and grey matter volume in the LM1 seed region did not differ between groups. For all of the regions investigated, a trend of lower MTR and

Figure 2.

Panel (A) shows 3-group comparisons of functional connectivity of the LMI seed region with ANCOVA F-contrast. Voxel wise analyses were corrected for cluster extension ($F > 3.16$, $P_v < 0.05$, $P_c < 0.05$, $k > 544 \text{ mm}^3$), with age and sex controlled, and masked by the composite of the main effects of the three groups. Panel (B) shows two-group comparisons of functional

connectivity of the LMI seed region. Voxel wise analyses were corrected for cluster extension ($t > 2.47$, $P_v < 0.05$, Bonferroni correction for number of comparisons, $P_c < 0.05$, $k > 296 \text{ mm}^3$), with age and sex controlled, and masked by F-contrast. Maps were displayed in the radiological convention.

TABLE III. Three-group comparisons of functional connectivity of the left-hemisphere hand motor seed region from ANCOVA F-contrast

Regions	BA	Peak F value	Peak z value	Peak MNI Coordinates (x y z)	Size (mm^3)	Correlation r with 9HPT
R-SM/SPL	1/2/3/4/5/7	10.16	3.59	26 -50 58	9752	-0.41*
L-SM/SPL	1/2/3/4/5/7	7.13	2.93	-46 -36 60	2,976	-0.36*
R-lin/fusi	17/18/19	8.39	3.23	8 -50 0	2,328	-0.31 [§]
L-lin/fusi	18/19	6.98	2.89	-8 -54 -4	640	-0.40*
R-LO	18/19	10.44	3.64	54 -66 0	1,752	-0.58***
L-LO	18/19	6.88	2.86	-48 -72 4	576	-0.33*
B-cuneus	17/18/19	9.96	3.55	-6 -90 28	4,000	-0.47**

Note: All regions passed the thresholds of $P_v < 0.05$ and $P_c < 0.05$, $k > 544 \text{ mm}^3$, while controlling for age and sex; Last column showed correlation of the mean connectivity in each ROI with 9HPT completion time, while controlling for age and sex ($§$: $P = 0.05$, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, FDR corrected). BA, Brodmann area; R, right; L, left; B, bilateral; SPL, superior parietal lobule; LO, lateral occipital; SM, sensorimotor area (including both precentral and postcentral gyri); lin, lingual gyrus; fusi, fusiform gyrus.

TABLE IV. Between-group comparisons of functional connectivity of the left-hemisphere hand motor seed region

	Regions	BA	Peak t value	Peak z value	Peak MNI Coordinates (x y z)	Size (mm ³)
HC > MI	R-SM/SPL	1/2/3/4/5/7	4.37	4.05	28 -40 68	6,752
	L-SM/SPL	1/2/3/4/5/7	3.42	3.25	-34 -30 68	1,336
MP > MI	R-PostCG/SPL	1/2/3/5/7	4.08	3.81	26 -50 58	4,624
	R-PostCG	1/2/3	3.01	2.89	48 -16 56	304
	R-Sup. PostCG	1/2/3/5	3.26	3.11	16 -32 78	624
	L-PostCG	1/2/3	3.74	3.53	-46 -36 60	776
	L-PreCG	3/4	3.39	3.22	-42 -18 62	608
	R-lin/fusi	17/18/19	4.00	3.74	18 -66 -8	1,856
	L-lin/fusi	18/19	3.30	3.14	-8 -56 -4	368
	R-LO	18/19	4.48	4.13	54 -66 0	1,744
	L-LO	18/19	3.66	3.46	-48 -72 4	552
	B-cuneus	17/18/19	4.32	4.00	-6 -90 28	3,912

Note: Regions shown passed the thresholds of $P_v < 0.05$, Bonferroni correction for number of comparisons and $P_c < 0.05$, $k > 296 \text{ mm}^3$, while controlling for age, sex, and masked by F-contrast ($P_v < 0.05$, $P_c < 0.05$, $k > 544 \text{ mm}^3$); BA, Brodmann area; R, right; L, left; B, bilateral; SPL, superior parietal lobule; LO, lateral occipital; PostCG, postcentral gyrus; PreCG, precentral gyrus; Sup., superior; SM, sensorimotor area (including PostCG and PreCG); lin, lingual gyrus; fusi, fusiform gyrus.

grey matter volume was observed among MI participants compared with MP and HC participants, although not all observations were statistically significant.

Structural Neuroimaging Predictors of Motor Disability

Logistic regression analysis of the data from MS participants was performed to determine whether structural changes in the LM1 related network predict membership in the MP or MI group independently of whole-brain GMV. The regions from which regional GMV and MTR were calculated are shown in Table III. The results of the regression analysis are shown in Table V. In the regions with significantly different functional connectivity between groups, mean MTR, but not regional GMV, was significantly predictive of motor disability beyond the contribution of global atrophy. Holding age, sex and global GMV constant, we observed that for every 1-pu decrease of MTR, the odds of having preserved motor ability (versus having impaired motor ability) decrease by a factor of 1.91.

Due to the fact that global MTR values of NAWM and whole-brain grey matter were found significantly correlated with 9HPT performance (Table II), we performed additional logistic regressions of these measures separately to assess their ability to predict motor disability, controlling for age, sex, and whole-brain GMV. Neither of the two measures significantly predicted motor disability beyond the impact of global atrophy ($P_s > 0.05$).

DISCUSSION

In this study, we identified patterns of LM1 related FC that were associated with impairment and preservation of dominant hand motor function among people with MS.

Where group differences in the pattern of FC were observed, we also identified structural differences in grey matter volume or MTR between groups. Finally, we found that within the regions with abnormal dominant hand motor region related FC, MTR predicted motor disability whereas grey matter volume did not.

Correlates of Motor Impairment in MS

In all groups, the LM1 region was functionally connected with sensory and motor related regions (BAs 1–6, 43) in a pattern consistent with that previously reported for FC motor networks in healthy people [Habas et al., 2009; Jolles et al., 2011; Larson-Prior et al., 2009; Xiong et al., 1999]. Nevertheless, MS participants with motor impairment differed from those with preserved motor function and healthy controls, in that they had weaker FC in sensorimotor and somatosensory association regions. Reductions in FC in a patient population could reflect structural damage to underlying tissue [Lowe et al., 2002]. Consistent with this hypothesis, we found that participants in the motor impaired group showed a trend toward smaller grey matter volumes and lower MTR values, both globally and regionally. These results are consistent with previous observations of lower FC in people with MS compared with HC [Lowe et al., 2002; Rocca et al., 2012], but we extend these findings by demonstrating their association with motor task performance. Considering our findings on structural abnormalities, we suggest that the weaker FC in motor related networks may be the result of loss of structural connectivity due to accumulated demyelination and neuroaxonal loss [Compston and Coles, 2008], leading to the loss of functional cofluctuations [Lowe et al., 2002] and abnormalities of network efficiency in

Figure 3.

Normalized volume (upper panel) and mean MTR (lower panel) within brain regions of interest for HC, MP and MI participants were shown with mean and the standard error (SE), adjusted for age and sex. One-way ANCOVA analysis was done for each measure and each region across the MP, MI participants and HC while controlling for age and sex (with their *P*-values shown in Supporting Information Tables SI and SII). Significant post hoc comparisons were indicated with “*” after applying Tukey HSD test. Note: **P* < 0.05; ***P* < 0.01; NAWM, normal-appearing white matter; WBGM, whole-brain grey matter; LM1, left hemisphere hand motor seed region; R, right; L, left; SM, sensorimotor area (including precentral and postcentral gyri); SPL, superior parietal lobule; LO, lateral occipital.

people with MS, and ultimately to motor impairment [Rocca et al., 2012].

Correlates of Preserved Motor Function in MS

The inclusion of a healthy control group allowed us to test whether increased FC in the motor network might support preservation of hand function among people with MS, as had been suggested by others [Basile et al., 2013; Faivre et al., 2012; Roosendaal et al., 2010]. In this study, visual-related regions including bilateral cuneus, lateral occipital and lingual/fusiform were seen in the LM1 related motor network of the motor-preserved MS participants, but not in the motor-impaired MS participants or the HC group. FC in these regions was significantly stronger than that observed

in the MI group, and correlated with better motor performance among the MS participants (Table III). Compared with the healthy control group, the MP group showed stronger FC in the right lingual/fusiform and left cuneus. However, these differences did not survive correction for statistical significance, which limits our ability to draw conclusions about compensatory reorganization of function. Regional MTR values were preserved in the MP group, which may explain why FC in these regions was preserved even though global and regional structural volumes were below those of healthy controls and whole-brain lesion loads were comparable to those of the MI group.

To understand the atypical involvement of visually associated brain regions in the network related to motor function in the MP group, we turn to previous studies using task-related functional imaging. Increased activation in the occipital lobe was seen during the performance of simple motor tasks in people with primary-progressive MS compared with HC [Ceccarelli et al., 2010; Rocca et al., 2002]. Visual cortex is capable of integrating visual input with other somatosensory stimuli [Price, 2000]. This visual-sensory interaction is activated in normal people when performing complex motor tasks [Jenkins et al., 1994] and provides feedback to guide and shape the movement online [Glickstein 2000; Glover, 2004]. When a movement is not performed as intended, whether due to reduced motor control, or impaired sensory input (e.g., due to optic neuritis), increased communication between sensory and motor regions may be required to complete an action, ultimately resulting in enhanced FCs between these regions. Our result suggests that stronger functional connections with MTR-intact visual-related areas, may help to preserve motor behaviour that could otherwise be impaired as result of disease-related damage to the brain.

Previous investigators proposed that reorganization of FC precedes the onset of clinical manifestations of motor dysfunction in MS [Harirchian et al., 2010], and may serve a compensatory role to limit motor disability at earlier stages of disease progression when the lesion burden is presumably lighter [Basile et al., 2013; Roosendaal et al., 2010]. Although there were more people with secondary-progressive MS in our MI group than in the MP group, lesion loads and global brain atrophy were comparable in the two groups, and did not correlate with motor

TABLE V. Structural measures of the regions with functional connectivity differences between groups contributing to prediction of motor disability

Measure	Odds ratio (%)	(95% CI) (%)	<i>P</i> -value
Mean MTR	191.28	(100.84–362.83)	0.047 ^a
Total GMV	100.57	(99.85–101.30)	0.12

Note: GMV, normalized grey matter volume; CI, confidence interval.

^a*P*-value < 0.05.

performance ($P_s > 0.05$). Given that the MP and the HC groups showed comparable global and regional MTR values, and that neuro-axonal loss and demyelination can occur independently [Khaleeli et al., 2007; Mallik et al., 2015], perhaps functional compensation is more limited by cortical demyelination (indicated by low MTR) rather than by atrophy (neuro-axonal loss). This possibility is supported by our logistic regression of measures from regions with abnormal between-group FC, where regional MTR, rather than regional GMV, significantly predicted motor disability beyond the impact of global atrophy. Notably, MTR values of NAWM and whole-brain GM did not significantly predict motor disability beyond the impact of global atrophy. This suggests that the link between low regional myelination and poor motor function is not merely an artefact of higher burden of neuropathology, but is related to the essential role of these regions in motor control.

Changes in ipsilateral motor network activity have been linked to upper limb motor function in CIS [Harirchian et al. 2010; Pantano et al., 2002] and RRMS [Reddy et al., 2000]. We tested for ipsilateral network activity by examining FC with a seed located in the right-hemisphere hand motor region (RM1). Results were similar to those obtained for LM1, except for the absence of the left lingual region (Supporting Information Tables SIII and SIV), and correlated significantly with better 9HPT performance (Supporting Information Table SIII). Indeed, the LM1 and RM1 were highly functionally connected (Fisher's $z = 1.15 \pm 0.48$ across all the subjects, where the mean z -score corresponded to an r -value of 0.82, $P < 0.001$). Thus, the highly functional synchronization of RM1 related motor network with LM1 related motor network suggests bilateral contributions to the compensatory FC for motor preservation. This is consistent with previous work suggesting that increases in both intra- and inter-hemispheric effective connectivity within the motor network contribute to the maintenance of motor function [Rocca et al., 2010] and serve to limit the functional expression of neuropathological abnormalities (for review see [Tomassini et al., 2012]).

Limitations of this study include uncertainty about the possible roles of clinical relapses, and the duration of motor impairment, in driving changes in functional connectivity. Although participants in this study continued to self-report as right-handed, motor impairment affecting the dominant hand may force a temporary or even more permanent shift in hand preference or use [Dellatolas et al., 1993]. Even a single clinical relapse involving hemiparesis can lead to changes in functional activation patterns in patients with MS [Pantano et al., 2002]. Thus, a complete understanding of individual differences in neurofunctional compensation will require a systematic investigation into the impact of clinical relapses. Secondly, our study did not include analyses of spinal cord lesions. A recent analysis of the corticospinal tract in a large sample

of MS patients highlighted the impact of structural damage to the spinal cord in determining motor function [Daams et al., 2015]. Moreover, although we excluded patients with recent onset or exacerbation of clinical symptoms we did not scan with a contrast agent to identify any sites of acute neuroinflammation. It is not yet known whether disease-related factors such as acute inflammation and lesion chronicity influence functional motor networks or their effectiveness at preserving motor function.

Image quality of MS participants may suffer from partial volume effects, especially for individuals with greater structural damage and atrophy. To minimize the contribution from partial volume voxels in MTR values, we excluded voxels with an MTR < 10 pu [Fisniku et al., 2009; Hayton et al., 2009]. However, we did not apply erosion techniques due to the small size of some of the ROIs and because voxel erosion may reduce the sensitivity to cortical demyelination in the subpial layers where a substantial proportion of cortical grey matter demyelination occurs [Kutzelnigg et al., 2005; Magliozzi et al., 2007]. Future studies examining functional connectivity within selected networks might employ higher resolution scanning protocols to allow finer grained analysis of the associations between FC and structural changes within specific regions. Finally, we used a single test—9HPT—to measure aspects of motor disability associated with manual dexterity and gross motor functions [Goodkin et al., 1988; Yozbatiran et al., 2006]. It is an open question whether the forms of FC adaptation observed here generalize to other aspects of manual motor function in MS, such as those related to grip force, sub-phases of actions [Carpinella et al., 2014], or specific hand movements. Work on these aspects of motor function could be especially informative in a disease like MS that is characterized by wide variation in both lesion location and clinical presentation.

CONCLUSION

By combining structural, functional and behavioural data in the analyses, this study shed new light on the functional mechanisms intervening between MS pathology and disability. These results demonstrate how focusing on a single well-characterized functional system can improve our understanding of changes in functional connectivity observed in various neurologic conditions. Future studies might use a similar approach to understanding impaired mobility or cognitive functioning by incorporating non-conventional structural measures, such as metrics of MTR, or possibly diffusion-weighted imaging, instead of relying solely on brain atrophy and lesion load when investigating the link between MS pathophysiology and symptom manifestations. Longitudinal studies are still needed to confirm that individual functional brain networks reorganize in response to early and subsequent structural damage in MS.

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REFERENCES

- Archambault-Wallenburg M, Arnold D, Narayanan S, Pike GB, Collins DL (2013): Cortical surface analysis of multi-contrast mr data to improve detection of cortical pathology in multiple sclerosis. *LNCS* 8159:138–149.
- Avants BB, Epstein CL, Grossman M, Gee JC (2008): Symmetric diffeomorphic image registration with cross-correlation: Evaluating automated labeling of elderly and neurodegenerative brain. *Med Image Anal* 12:26–41.
- Basile B, Castelli M, Monteleone F, Nocentini U, Caltagirone C, Centonze D, Cercignani M, Bozzali M (2013): Functional connectivity changes within specific networks parallel the clinical evolution of multiple sclerosis. *Mult Scler* 20:1050–1057.
- Carpinella I, Cattaneo D, Ferrarin M (2014): Quantitative assessment of upper limb motor function in Multiple Sclerosis using an instrumented Action Research Arm Test. *J Neuroeng Rehabil* 11:67.
- Ceccarelli A, Rocca MA, Valsasina P, Rodegher M, Falini A, Comi G, Filippi M (2010): Structural and functional magnetic resonance imaging correlates of motor network dysfunction in primary progressive multiple sclerosis. *Eur J Neurosci* 31:1273–1280.
- Chen JT, Kuhlmann T, Jansen GH, Collins DL, Atkins HL, Freedman MS, O'Connor PW, Arnold DL (2007): Voxel-based analysis of the evolution of magnetization transfer ratio to quantify remyelination and demyelination with histopathological validation in a multiple sclerosis lesion. *Neuroimage* 36:1152–1158.
- Chen JT, Collins DL, Atkins HL, Freedman MS, Arnold DL, Antel JP, Bar-Or A, Bence-Bruckler I, Duquette P, Huebsch L, Laneuville P, Lapierre Y, Messner H, Pierre Sekaly R, O'Connor P, Halpenny M (2008): Magnetization transfer ratio evolution with demyelination and remyelination in multiple sclerosis lesions. *Ann Neurol* 63:254–262.
- Chen JT-H, Easley K, Schneider C, Nakamura K, Kidd GJ, Chang A, Staugaitis SM, Fox RJ, Fisher E, Arnold DL, Trapp BD (2013): Clinically feasible MTR is sensitive to cortical demyelination in MS. *Neurology* 80:246–252.
- Compston A, Coles A (2008): Multiple sclerosis. *Lancet* 372:1502–1517.
- Crespy L, Zaaraoui W, Lemaire M, Rico A, Faivre A, Reuter F, Malikova I, Confort-Gouny S, Cozzzone PJ, Pelletier J, Ranjeva JP, Audoin B (2011): Prevalence of grey matter pathology in early multiple sclerosis assessed by Magnetization transfer ratio imaging. *PLoS One* 6:2–7.
- Cutter GR, Baier ML, Rudick RA, Cookfair DL, Fischer JS, Petkau J, Syndulko K, Weinshenker BG, Antel JP, Confavreux C, Ellison GW, Lublin F, Miller AE, Rao SM, Reingold S, Thompson A, Willoughby E (1999): Development of a multiple sclerosis functional composite as a clinical trial outcome measure. *Brain* 122:871–882.
- Daams M, Steenwijk MD, Wattjes MP, Geurts JJ, Uitdehaag BM, Tiewarie PK, Balk LJ, Pouwels PJ, Killestein J, Barkhof F (2015): Unraveling the neuroimaging predictors for motor dysfunction in long-standing multiple sclerosis. *Neurology* 85:248–255.
- De Luca M, Smith S, De Stefano N, Federico A, Matthews PM (2005): Blood oxygenation level dependent contrast resting state networks are relevant to functional activity in the neocortical sensorimotor system. *Exp Brain Res* 167:587–594.
- Dellatolas G, Moreau T, Jallon P, Lellouch J (1993): Upper limb injuries and handedness plasticity. *Br J Psychol* 84:201–205.
- Dousset V, Grossman RI, Ramer KN, Schnall MD, Young LH, Gonzalez-Scarano F, Lavi E, Cohen JA (1992): Experimental allergic encephalomyelitis and multiple sclerosis: Lesion characterization with magnetization transfer imaging. *Radiology* 182:483–491.
- Faivre A, Rico A, Zaaraoui W, Crespy L, Reuter F, Wybrecht D, Soulier E, Malikova I, Confort-Gouny S, Cozzzone PJ, Pelletier J, Ranjeva J-P, Audoin B (2012): Assessing brain connectivity at rest is clinically relevant in early multiple sclerosis. *Mult Scler* 18:1251–1258.
- Filippi M, Agosta F (2010): Imaging biomarkers in multiple sclerosis. *J Magn Reson Imaging* 31:770–788.
- Filippi M, Agosta F, Spinelli EG, Rocca MA (2013): Imaging resting state brain function in multiple sclerosis. *J Neurol* 1709–1713.
- Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, Van Der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale AM (2002): Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron* 33:341–355.
- Fisher E, Lee J-C, Nakamura K, Rudick RA (2008): Gray matter atrophy in multiple sclerosis: A longitudinal study. *Ann Neurol* 64:255–265.
- Fisniku LK, Brex PA, Altmann DR, Miszkil KA, Benton CE, Lanyon R, Thompson AJ, Miller DH (2008): Disability and T2 MRI lesions: A 20-year follow-up of patients with relapse onset of multiple sclerosis. *Brain* 131:808–817.
- Fisniku LK, Altmann DR, Cercignani M, Tozer DJ, Chard DT, Jackson JS, Miszkil K. a, Schmierer K, Thompson a. J, Miller DH (2009): Magnetization transfer ratio abnormalities reflect clinically relevant grey matter damage in multiple sclerosis. *Mult Scler* 15:668–677.
- Foerster B, Tomasi D, Caparelli EC (2005): Magnetic field shift due to mechanical vibration in functional magnetic resonance imaging. *Magn Reson Med* 54:1261–1267.
- Francis SJ (2004): Automatic Lesion Identification in MRI of Multiple Sclerosis Patients. New York: McGill University.
- Glickstein M (2000): How are visual areas of the brain connected to motor areas for the sensory guidance of movement? *Trends Neurosci* 23:613–617.
- Glover S (2004): Separate visual representations in the planning and control of action. *Behav Brain Sci* 27:3–24.
- Goodkin DE, Hertsgaard D, Seminary J (1988): Upper extremity function in multiple sclerosis: Improving assessment sensitivity with box-and-block and nine-hole peg tests. *Arch Phys Med Rehabil* 69:850–854.
- Greve DN, Fischl B (2009): Accurate and robust brain image alignment using boundary-based registration. *Neuroimage* 48:63–72.
- Habas C, Kamdar N, Nguyen D, Prater K, Beckmann CF, Menon V, Greicius MD (2009): Distinct cerebellar contributions to intrinsic connectivity networks. *J Neurosci* 29:8586–8594.
- Harirchian MH, Rezvanizadeh A, Fakhri M, Oghabian MA, Ghoreishi A, Zarei M, Firouznia K, Ghanaati H (2010): Non-invasive brain mapping of motor-related areas of four limbs in

- patients with clinically isolated syndrome compared to healthy normal controls. *J Clin Neurosci* 17:736–741.
- Hayton T, Furby J, Smith KJ, Altmann DR, Brenner R, Chataway J, Hughes R. a C, Hunter K, Tozer DJ, Miller DH, Kapoor R (2009): Grey matter magnetization transfer ratio independently correlates with neurological deficit in secondary progressive multiple sclerosis. *J Neurol* 256:427–435.
- Holper L, Coenen M, Weise A, Stucki G, Cieza A, Kesselring J (2010): Characterization of functioning in multiple sclerosis using the ICF. *J Neurol* 257:103–113.
- Janssen AL, Boster A, Patterson BA, Abduljalil A, Prakash RS (2013): Resting-state functional connectivity in multiple sclerosis: An examination of group differences and individual differences. *Neuropsychologia* 51:2918–2929.
- Jenkins IH, Brooks DJ, Nixon PD, Frackowiak RS, Passingham RE (1994): Motor sequence learning: A study with positron emission tomography. *J Neurosci* 14:3775–3790.
- Jenkinson M (1999): Measuring transformation error by RMS deviation. Internal Technical Report TR99MJ1, FMRIB Centre, University of Oxford. Available at www.fmriv.ox.ac.uk/analysis/techrep for downloading.
- Jenkinson M, Bannister P, Brady M, Smith S (2002): Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 17:825–841.
- Johansson S, Ytterberg C, Claesson IM, Lindberg J, Hillert J, Andersson M, Widén Holmqvist L, Von Koch L (2007): High concurrent presence of disability in multiple sclerosis: Associations with perceived health. *J Neurol* 254:767–773.
- Jolles DD, Van Buchem MA, Crone EA, Rombouts SARB (2011): A comprehensive study of whole-brain functional connectivity in children and young adults. *Cereb Cortex* 21:385–391.
- Jure L, Zaaraoui W, Rousseau C, Reuter F, Rico A, Malikova I, Confort-Gouny S, Cozzzone PJ, Pelletier J, Ranjeva JP, Audoin B (2010): Individual voxel-based analysis of brain magnetization transfer maps shows great variability of gray matter injury in the first stage of multiple sclerosis. *J Magn Reson Imaging* 32:424–428.
- Khaleeli Z, Cercignani M, Audoin B, Ciccarelli O, Miller DH, Thompson AJ (2007): Localized grey matter damage in early primary progressive multiple sclerosis contributes to disability. *Neuroimage* 37:253–261.
- Kierkegaard M, Einarsson U, Gottberg K, von Koch L, Holmqvist LW (2012): The relationship between walking, manual dexterity, cognition and activity/participation in persons with multiple sclerosis. *Mult Scler J* 18:639–646.
- Kister I, Bacon TE, Chamot E, Salter AR, Cutter GR, Kalina JT, Herbert J (2013): Natural history of multiple sclerosis symptoms. *Int J MS Care* 15:146–158.
- Kristo G, Rutten GJ, Raemaekers M, de Gelder B, Rombouts SARB, Ramsey NF (2014): Task and task-free fMRI reproducibility comparison for motor network identification. *Hum Brain Mapp* 35:340–352.
- Kutzelnigg A, Lucchinetti CF, Stadelmann C, Brück W, Rauschka H, Bergmann M, Schmidbauer M, Parisi JE, Lassmann H (2005): Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 128:2705–2712.
- Lamers I, Feys P (2014): Assessing upper limb function in multiple sclerosis. *Mult Scler J* 20:775–784.
- Larson-Prior LJ, Zempel JM, Nolan TS, Prior FW, Snyder AZ, Raichle ME (2009): Cortical network functional connectivity in the descent to sleep. *Proc Natl Acad Sci U.S.A* 106:4489–4494.
- Lowe MJ, Phillips MD, Lurito JT, Mattson D, Dzemidzic M, Mathews VP (2002): Multiple sclerosis: Low-frequency temporal blood oxygen level-dependent fluctuations indicate reduced functional connectivity initial results. *Radiology* 224:184–192.
- Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M, Reynolds R, Aloisi F (2007): Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain* 130:1089–1104.
- Mallik S, Muhlert N, Samson RS, Sethi V, Wheeler-Kingshott CA, Miller DH, Chard DT (2015): Regional patterns of grey matter atrophy and magnetisation transfer ratio abnormalities in multiple sclerosis clinical subgroups: A voxel-based analysis study. *Mult Scler J* 21:423–432.
- Matthews, W (1991): Multiple sclerosis and other demyelinating diseases: Clinical features. In: Swash M, Oxbury J, editors. *Clinical Neurology*. New York: Churchill Livingstone. pp 1106–1116.
- Oldfield RC (1971): The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia* 9:97–113.
- Oxford Grice K, Vogel KA, Le V, Mitchell A, Muniz S, Vollmer MA (2003): Adult norms for a commercially available nine hole peg test for finger dexterity. *Am J Occup Ther* 57:570–573.
- Pantano P, Iannetti GD, Caramia F, Mainero C, Di Legge S, Bozzao L, Pozzilli C, Lenzi GL (2002): Cortical motor reorganization after a single clinical attack of multiple sclerosis. *Brain* 125:1607–1615.
- Pike GB, De Stefano N, Narayanan S, Worsley KJ, Pelletier D, Francis GS, Antel JP, Arnold DL (2000): Multiple sclerosis: Magnetization transfer MR imaging of white matter before lesion appearance on T2-weighted images. *Radiology* 215:824–830.
- Price DD (2000): Psychological and neural mechanisms of the affective dimension of pain. *Science* 288:1769–1772.
- Ranjeva J-P, Audoin B, Au Duong M, Van Duong MVA, Ibarrola D, Confort-Gouny S, Malikova I, Soulier E, Viout P, Ali-Chérif A, Pelletier J, Cozzzone P (2005): Local tissue damage assessed with statistical mapping analysis of brain magnetization transfer ratio: Relationship with functional status of patients in the earliest stage of multiple sclerosis. *AJNR Am J Neuroradiol* 26:119–127.
- Reddy H, Narayanan S, Arnoutelis R, Jenkinson M, Antel J, Matthews PM, Arnold DL (2000): Evidence for adaptive functional changes in the cerebral cortex with axonal injury from multiple sclerosis. *Brain* 123:2314–2320.
- Reddy H, Narayanan S, Woolrich M, Mitsumori T, Lapierre Y, Arnold DL, Matthews PM (2002): Functional brain reorganization for hand movement in patients with multiple sclerosis: Defining distinct effects of injury and disability. *Brain* 125:2646–2657.
- Rocca MA, Matthews PM, Caputo D, Ghezzi A, Falini A, Scotti G, Comi G, Filippi M (2002): Evidence for widespread movement-associated functional MRI changes in patients with PPMS. *Neurology* 58:866–872.
- Rocca MA, Absinta M, Moiola L, Ghezzi A, Colombo B, Martinelli V, Comi G, Filippi M (2010): Functional and structural connectivity of the motor network in pediatric and adult-onset relapsing-remitting multiple sclerosis. *Radiology* 254:541–550.
- Rocca MA, Valsasina P, Martinelli V, Misci P, Falini A, Comi G, Filippi M (2012): Large-scale neuronal network dysfunction in relapsing-remitting multiple sclerosis. *Neurology* 79:1449–1457.

- Roosendaal SD, Schoonheim MM, Hulst HE, Sanz-Arigita EJ, Smith SM, Geurts JJG, Barkhof F (2010): Resting state networks change in clinically isolated syndrome. *Brain* 133:1612–1621.
- Sacco R, Bonavita S, Esposito F, Tedeschi G, Gallo A (2013): The contribution of resting state networks to the study of cortical reorganization in MS. *Mult Scler Int* 2013:1–7.
- Schmierer K, Scaravilli F, Altmann DR, Barker GJ, Miller DH (2004): Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain. *Ann Neurol* 56:407–415.
- Schmierer K, Tozer DJ, Scaravilli F, Altmann DR, Barker GJ, Tofts PS, Miller DH (2007): Quantitative magnetization transfer imaging in postmortem multiple sclerosis brain. *J Magn Reson Imaging* 26:41–51.
- Schmierer K, Parkes HG, So P-W, An SF, Brandner S, Ordidge RJ, Yousry TA, Miller DH (2010): High field (9.4 Tesla) magnetic resonance imaging of cortical grey matter lesions in multiple sclerosis. *Brain* 133:858–867.
- Siffrin V, Vogt J, Radbruch H, Nitsch R, Zipp F (2010): Multiple sclerosis – candidate mechanisms underlying CNS atrophy. *Trends Neurosci* 33:202–210.
- Smith SM, Zhang Y, Jenkinson M, Chen J, Matthews PM, Federico A, De Stefano N (2002): Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 17:479–489.
- Tomassini V, Matthews PM, Thompson AJ, Fuglø D, Geurts JJ, Johansen-Berg H, Jones DK, Rocca MA, Wise RG, Barkhof F, Palace J (2012): Neuroplasticity and functional recovery in multiple sclerosis. *Nat Rev Neurol* 8:635–646.
- Traboulsee A, Dehmshki J, Peters KR, Griffin CM, Brex PA, Silver N, Ciccarelli O, Chard DT, Barker GJ, Thompson AJ, Miller DH (2003): Disability in multiple sclerosis is related to normal appearing brain tissue MTR histogram abnormalities. *Mult Scler* 9:566–573.
- Valsasina P, Rocca MA, Absinta M, Sormani MP, Mancini L, De Stefano N, Rovira A, Gass A, Enzinger C, Barkhof F, Wegner C, Matthews PM, Filippi M (2011): A multicentre study of motor functional connectivity changes in patients with multiple sclerosis. *Eur J Neurosci* 33:1256–1263.
- Van den Heuvel MP, Pol HEH (2010): Exploring the brain network: A review on resting-state fMRI functional connectivity. *Eur Neuropsychopharmacol* 20:519–534.
- Wegner C, Filippi M, Korteweg T, Beckmann C, Ciccarelli O, De Stefano N, Enzinger C, Fazekas F, Agosta F, Gass A, Hirsch J, Johansen-Berg H, Kappos L, Barkhof F, Polman C, Mancini L, Manfredonia F, Marino S, Miller DH, Montalban X, Palace J, Rocca M, Ropele S, Rovira A, Smith S, Thompson A, Thornton J, Yousry T, Matthews PM (2008): Relating functional changes during hand movement to clinical parameters in patients with multiple sclerosis in a multi-centre fMRI study. *Eur J Neurol* 15:113–122.
- Windischberger C, Langenberger H, Sycha T, Tschernko EM, Fuchsjaeger-Mayerl G, Schmetterer L, Moser E (2002): On the origin of respiratory artifacts in BOLD-EPI of the human brain. *Magn Reson Imaging* 20:575–582.
- Woo C-W, Krishnan A, Wager TD (2014): Cluster-extent based thresholding in fMRI analyses: Pitfalls and recommendations. *Neuroimage* 91:412–419.
- Xiong J, Parsons LM, Gao JH, Fox PT (1999): Interregional connectivity to primary motor cortex revealed using MRI resting state images. *Hum Brain Mapp* 8:151–156.
- Yousry TA, Schmid UD, Alkadhi H, Schmidt D, Peraud A, Buettner A, Winkler P (1997): Localization of the motor hand area to a knob on the precentral gyrus. A new landmark. *Brain* 120:141–157.
- Yozbatiran N, Baskurt F, Baskurt Z, Ozakbas S, Idiman E (2006): Motor assessment of upper extremity function and its relation with fatigue, cognitive function and quality of life in multiple sclerosis patients. *J Neurol Sci* 246: 117–122.
- Zar JH (2010): *Biostatistical Analysis*. New Jersey: Prentice Hall.