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FUNCTIONAL NEURORADIOLOGY

Fractional anisotropy and mean diffusivity in the corpus callosum of patients with multiple sclerosis: the effect of physiotherapy

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Abstract
Introduction Modulation of neurodegeneration by physical activity is an active topic in contemporary research. The purpose of this study was to investigate changes in the brain's microstructure in multiple sclerosis (MS) after facilitation physiotherapy.
Methods Eleven patients with MS were examined using motor and neuropsychological testing and multimodal MRI at the beginning of the study, with second baseline measurement after 1 month without any therapy, and after a 2-month period of facilitation physiotherapy. Eleven healthy controls were examined at the beginning of the study and after 1 month. Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (λ_{ax}), and radial diffusivity (λ_{rad}) were calculated for the whole corpus callosum (CC) in the midsagittal slice of T1W 3D MPRAGE spatially normalized images. Data were analyzed using linear mixed-effect models, paired, and two-sample tests.
Results At the baseline, patients with MS showed significantly lower values in FA ($p<0.001$), and significantly higher values in MD ($p<0.001$), λ_{ax} ($p=0.003$), and λ_{rad} ($p<0.001$) compared to control subjects. The FA, MD, λ_{ax}, and λ_{rad} did not change between the first and second baseline examinations in either group. Differences 2 months after initiating facilitation physiotherapy were in FA, MD, and in λ_{rad} significantly higher than differences in healthy controls ($p<0.001$ for FA, $p=0.02$ for MD, and $p=0.002$ for λ_{rad}). In MS patients, FA in the CC significantly increased ($p<0.001$), MD and λ_{rad} significantly decreased ($p=0.014$ and $p=0.002$), and thus approached the values in healthy controls.
Conclusion The results of the study show that facilitation physiotherapy influences brain microstructure measured by DTI.

Keywords Multiple sclerosis · Rehabilitation · Facilitation physiotherapy · Diffusion tensor imaging · Corpus callosum

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Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by the presence of multifocal inflammatory demyelination lesions and axonal loss. MS-related pathological changes can modify the integrity of white matter tracts, leading to

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diffusion tensor imaging (DTI)-detectable changes. The disruption of myelin sheaths and axons can result in increased water motion that in turn leads to increased mean diffusivity (MD) values and a change in the preferential direction of such motion that is responsible for decreased fractional anisotropy (FA) values [1]. FA and MD are the most frequently used scalar maps in DTI [2]. FA describes the extent to which diffusion is directionally restricted and MD represents a quantitative metric of average molecular diffusion of water in a voxel [3]. Decreased FA and increased MD in MS have been documented in normal-appearing white matter and lesions as well [1, 4–9].

In MS, the corpus callosum (CC) is commonly affected with direct inflammatory processes and secondary Wallerian degeneration located at the callosum or callososeptal interface, as well as callosal atrophy [10]. The CC is the biggest commissural bundle which connects both hemispheres and plays an important role in the interhemispheric communication of the brain.

Patients with MS have a lifelong need for physiotherapy and exercise interventions due to the progressive nature of the disease and their greater risk of complications of inactivity [11], and we suppose that it is very important to offer them the most appropriate and effective treatment, i.e., treatment that leads not only to improving clinical functions but also to inducing adaptive and plastic processes in the CNS—as was confirmed in recent research [12, 13]. It seems that DTI is a good tool for measuring neuroplasticity and repair following rehabilitation and physiotherapy [14–16].

In this work, we compared FA, MD, axial diffusivity (the largest eigenvalue—λ_{ax}), and radial diffusivity (the average of the two smallest eigenvalues—λ_{rad}) in the corpus callosum using DTI in healthy controls and patients with MS. Moreover, we assessed the impact of facilitation physiotherapy. We investigated whether the therapy has an effect on the FA and MD in the corpus callosum, and on clinical status (Paced Auditory Serial Addition Test, PASAT 3 and Expanded Disability Status Scale, EDSS) in patients with MS over time.

Methods

Design of the study

Patients with MS underwent DTI examinations three times. The first examination was at the beginning of the study. The second examination was performed after 1 month without physiotherapy. This measurement of patients without physiotherapy served as a control measurement (second baseline) to assess the effect of physiotherapy over the subsequent period. The third examination was performed at the end of the study after 2 months of physiotherapy. The control subjects (healthy) underwent two DTI examinations 1 month apart without any changes in their habits. Together with all DTI examinations, PASAT was measured to assess cognitive functions. In MS patients, EDSS was also measured during the second and third examinations to assess impairment (Fig. 1, Tables 1 and 2).

Patient selection and characteristics

Eleven right-handed [17] patients with confirmed multiple sclerosis according to the revised McDonald criteria [18] were randomly selected from the MS Center database (Department of Neurology, Third Medical Faculty Charles University and Royal Vinohrady Teaching Hospital in Prague), based on the criteria for inclusion in the study: both genders, suffering from relapsing–remitting (RR) MS, stability of clinical status in the preceding 3 months, minimum of 2 years on immunomodulatory drugs (glatir-

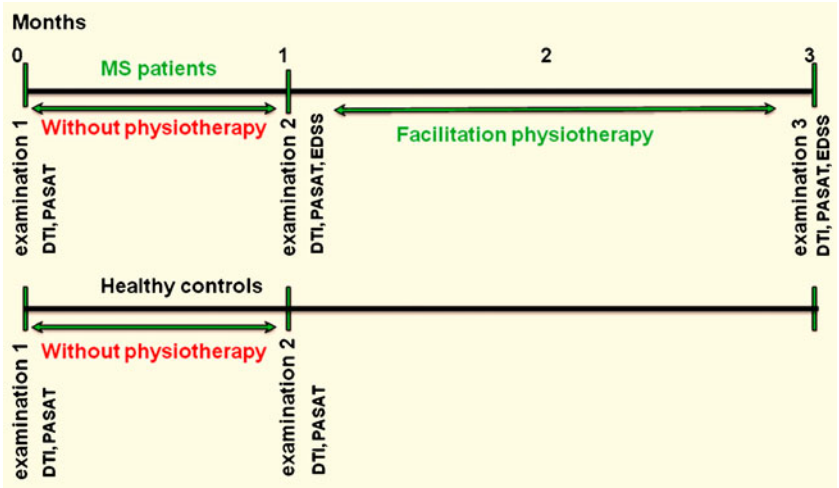

Fig. 1 Design of the study in months (0, 1, 2, and 3). *0* first examination (DTI and PASAT) performed at the beginning of the study in MS and in healthy controls, *1* second examination (DTI and PASAT) performed after 1 month without physiotherapy in patients (this measurement of patients without physiotherapy served as control measurement, second baseline) and in healthy controls, *3* third examination performed at the end of the study 2 months after initiating physiotherapy

Table 1 Group characteristics

Group characteristics	MS patients (mean±SD)	Control subjects (mean±SD)	*p* value
Age (years)	43.27±9.28	39.45±12.2	0.43
Sex (men/women)	(4/7)	(3/8)	
Disease duration (years)	6.10±2.34		
EDSS at the beginning of the study	3.50±0.80		
Number attacks last year	0.20±0.44		
Immunomodulatory drug treatment	2 GA, 1 MX, 1 IFNβ-1a, 7 IFNβ-1b		

GA glatiramer acetate, *MX* mitoxantrone, *IFNβ-1a* interferon beta-1a, *IFNβ-1b* interferon beta-1b, *SD* standard deviations, *EDSS* Expanded Disability Status Scale

amer acetate, interferon beta-1a, interferon beta-1b, mitoxantrone), persisting motor impairment, ability to move independently and walk at least 200 m with two canes (EDSS ≤5), and indication and ability to undergo outpatient physiotherapy (e.g., motivation to actively cooperate, ability to come for regular sessions). For each patient, an age- and sex-matched healthy control was chosen. All subjects agreed with participation in the study and provided written informed consent to the study. The study was approved by the Czech ethics committee of the Third Medical Faculty of Charles University in Prague.

Physiotherapy

Patients with MS underwent the so-called facilitation physiotherapy sessions guided by experienced physiotherapists. Sensorimotor stimuli (adaptive resistance, verbal command, and maximal stretching of muscle groups before movement) were applied repetitively in standard postural positions and motor functions (sitting, standing up, sitting down, standing, and walking). Patients received therapy for the exact period of time (2 h per week with an overall duration of 2 months). Patients were instructed to use the principles learned during physiotherapy sessions at home during daily activities [19, 20].

Examinations

Clinical examinations

Basic characteristics of patients (duration of the disease, number of attacks during the past year, and information about treatment) were determined by an independent neurologist at the beginning of the study (Table 1). All subjects were examined by an independent (blinded to treatment) skilled examiner using the PASAT 3 and the EDSS.

EDSS is a method of quantifying disability in MS in eight functional systems. EDSS steps 1.0–4.5 refer to people with MS who are fully ambulatory. EDSS steps 5.0–9.5 are defined by the impairment of ambulation [21]. PASAT 3 is a measure of cognitive function that assesses

Table 2 DTI, PASAT, and EDSS in MS patiens (MS) and control subjects (C) for measurements 1, 2, and 3: mean values and standard deviations (SD)

		MS1	MS2	MS3	C1	C2
FA	Mean	0.52	0.51	0.55	0.68	0.67
	SD	0.07	0.08	0.08	0.02	0.02
MD	Mean	1.20	1.18	1.13	0.86	0.86
	SD	0.21	0.20	0.21	0.05	0.04
λ_{ax}	Mean	1.89	1.86	1.86	1.64	1.64
	SD	0.21	0.18	0.20	0.07	0.07
λ_{rad}	Mean	0.86	0.84	0.77	0.46	0.47
	SD	0.22	0.21	0.22	0.04	0.04
PASAT	Mean	43.00	45.73	49.36	51.40	51.60
	SD	13.66	12.63	9.65	9.22	8.11
EDSS	Mean		3.59	3.41		
	SD		0.86	0.94		

FA fractional anisotropy, *MD* mean diffusivity ($\times 10^{-3}$ mm^2/s), λ_{ax} axial diffusivity ($\times 10^{-3}$ mm^2/s), λ_{rad} radial diffusivity ($\times 10^{-3}$ mm^2/s), *MS1* diffusion indices measured in MS at the beginning of the study (examination 1), *MS2* diffusion indices measured after 1 month without physiotherapy (this measurement in patients served as their own controls, examination 2), *MS3* diffusion indices measured at the end of the study 2 months after initiating physiotherapy (examination 3), *C1* diffusion indices measured in healthy controls at the beginning of the study (examination 1), *C2* diffusion indices measured in healthy controls at 1 month apart without any changes in their habits (examination 2), *PASAT* Paced Auditory Serial Addition Test, *EDSS* Expanded Disability Status Scale

working memory, auditory information processing speed and flexibility, and calculation ability [22].

MRI acquisition protocol

All subjects in this study underwent MRI examinations on a 3T MR scanner (Siemens Magnetom Trio, Erlangen, Germany) using a 12-channel, phased-array head coil with the following protocol:

1. T_1-weighted (T1W) 3D magnetization-prepared rapid acquisition gradient echo (MPRAGE) with the following parameters: voxel size of $0.85\times0.85\times0.85$ mm^3, 192 sagittal slices, echo time (TE) of 4.73 ms, repetition time (TR) of 2,000 ms, flip angle of 10°, and field of view (FOV) of 326 mm.
2. 3D T_2-weighted fluid-attenuated inversion recovery (FLAIR) with the following parameters: voxel size of $1\times1\times1$ mm^3, 176 sagittal slices, TE of 422 ms, TR of 6,000 ms, and FOV of 256 mm.

   All patients demonstrated typical multiple ovoid (Fig. 2) or confluent (Fig. 3) lesions in the periventricular and juxtacortical white matter. Two months after initiating facilitation physiotherapy, there were no new lesions visible in FLAIR images (Fig. 4).
3. Diffusion-weighted images using spin-echo echo-planar imaging (SE EPI) sequence with the parameters: voxel size of $2\times2\times2$ mm^3, TR of 6,000 ms, TE of 93 ms, 44 axial slices, three averages, FOV=256 mm, number of diffusion directions 20, and two b values: 0, 1,000 s/mm^2.

DTI analysis

The DTI data were corrected for distortions and eddy current effects using FSL (www.fmrib.ox.ac.uk/fsl/index.html). The b=0 EPI images of the DTI image set were co-registered to T_1-weighted 3D MPRAGE to obtain the co-registration matrix, which was subsequently applied to other EPI diffusion images. The EPI and T1W 3D MPRAGE co-registered images were then normalized to a T1 template. The co-registration and spatial normalization was done using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). The normalized DTI indices (FA, MD, λ_{ax}, and λ_{rad}) were then calculated using MedINRIA (Asclepios Research Project—INRIA Sophia Antipolis, http://www-sop.inria.fr/asclepios/software/MedINRIA). The regions of interest (ROIs) of the corpus callosum for FA and other DTI indices calculation were manually selected in the midsagittal slice of the T1W 3D MPRAGE normalized image (Fig. 5). For accuracy, the same ROI of the CC was used for each subject at the beginning and the end of the study (the ROIs geometries of the CC were copied and pasted).

Visual perception and counting of lesions

For better spatial resolution and counting of the number of brain lesions, the 3D T2-FLAIR images were co-registered to T1-weighted 3D MPRAGE. All detectable hyperintense lesions on T2-FLAIR images matching hypointense lesions on MPRAGE images were identified and counted in the 3D image viewer (MedINRIA, version 1.9.0).

Statistical analysis

The first and the second measurement in MS patients together with both measurements in healthy controls were used to describe the changes without physiotherapy and differences in the two groups at the baseline (Tables 3 and 4): a two-sample t test was used to compare the two groups in differences between measurements. A paired

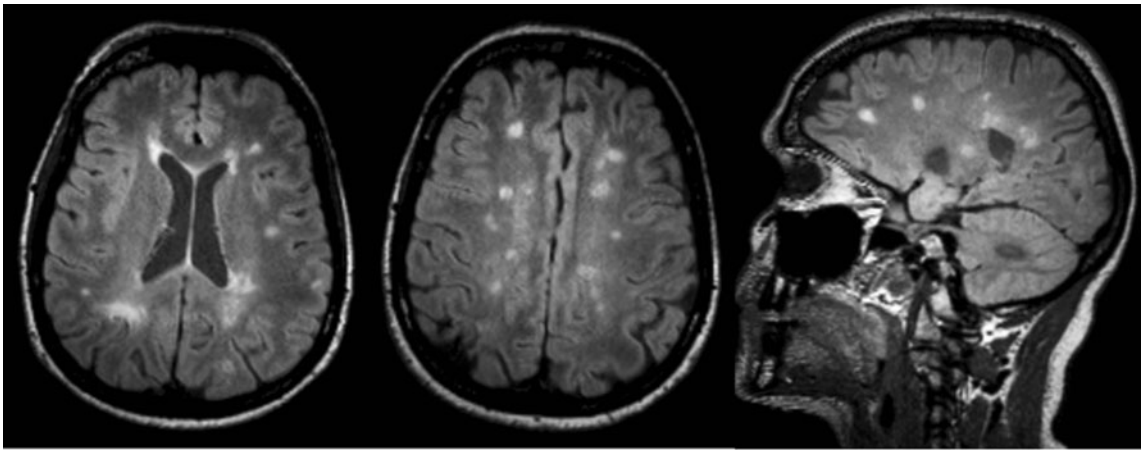

Fig. 2 Axial and sagittal T2-FLAIR images of a 51-year-old woman with MS demonstrate typical multiple ovoid hyperintense lesions in the periventricular and juxtacortical white matter

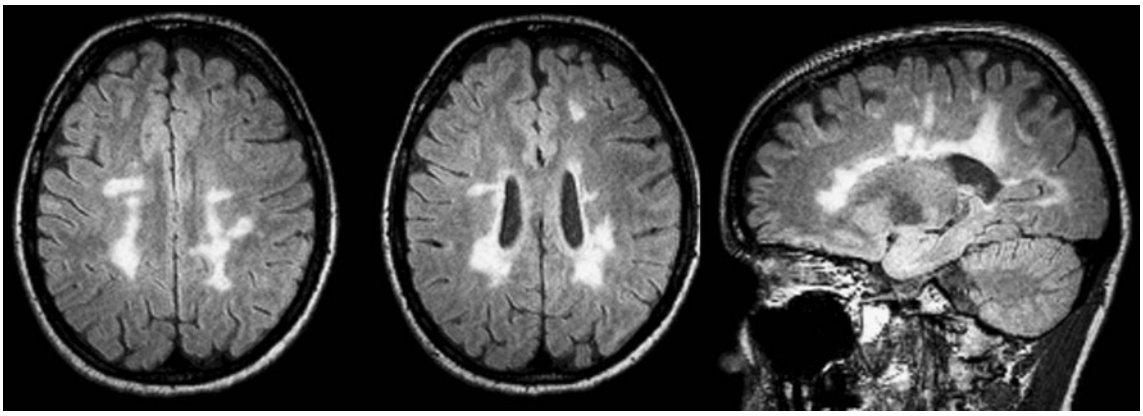

Fig. 3 Axial and sagittal T2-FLAIR images of a 31-year-old man with MS demonstrate typical confluent periventricular lesions in the white matter

t test was used to test the differences between repeated measurements. A two-sample *t* test was used on the average between the two measurements to test the difference between groups at the baseline. Nearly the same *p* values were obtained by *F* tests in the linear mixed-effect model with random patient effect. The three tests mentioned above correspond to tests of the group× measurement interaction, measurement effect, and group effect, respectively.

The second and the third measurement in MS patients together with the two measurements in healthy controls were used to describe the changes in patients after physiotherapy (Table 5): the two-sample *t* test was used on differences to show that the changes are significantly different (higher) in MS patients than in healthy controls. The *p* value was almost the same as in the *F* test of group× measurement interaction in the mixed-effect model. The paired *t* test with one-sided alternative was used in MS patients to test the improvement after therapy. The same test with two-sided alternative was used in healthy controls to test the change between the two measurements.

Since we do not assume normality of EDSS, paired Wilcoxon test was used to test the difference after physiotherapy. PASAT measurement in one healthy control was detected to be significantly outlying observation (Grubbs test [23], $p<0.001$) and was therefore deleted from analyses.

Statistical analyses were performed in environment R (http://www.R-project.org) [24], using package nlme [25] and outliers (http://CRAN.R-project.org/package=outliers) [26]. Differences were judged statistically significant for *p* value less than 0.05.

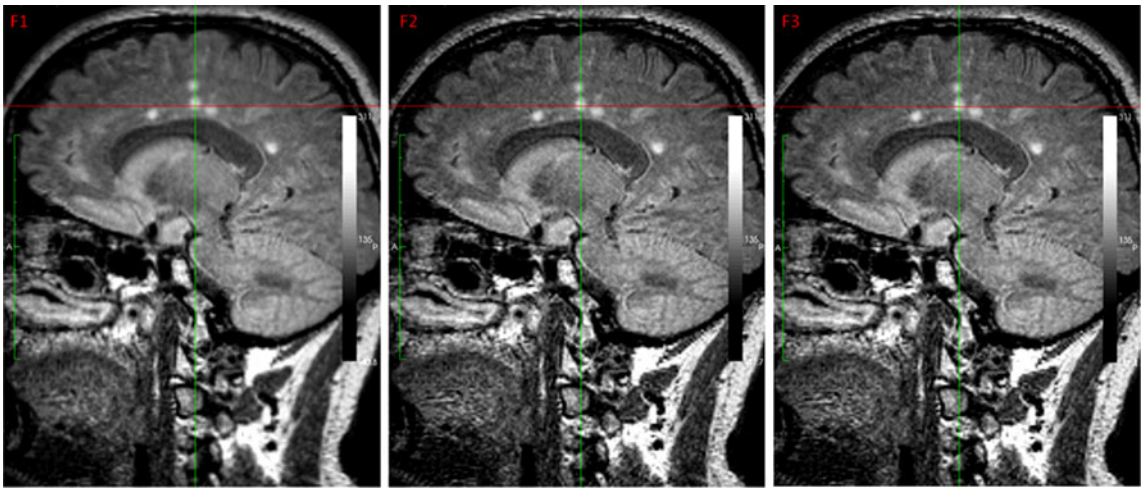

Fig. 4 Sagittal FLAIR images of a 55-year-old woman with MS show multiple ovoid hyperintense lesions in the white matter with immediate contact to the lateral ventricles. Sagittal FLAIR images acquired at the beginning of the study (*F1*), after 1 month without physiotherapy (*F2*), and at the end of the study 2 months after initiating physiotherapy (*F3*) demonstrate the stable number of the lesions during this study

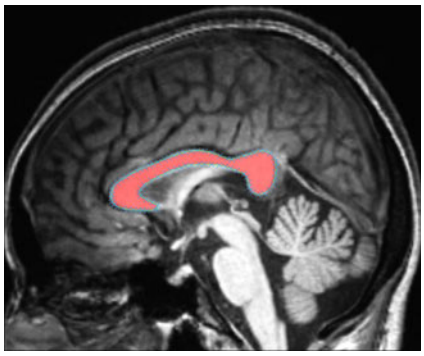

Fig. 5 The regions of interest of the corpus callosum were manually selected in the midsagittal slice of the T1W 3D MPRAGE normalized image

Results

All patients finished the study, and no patients manifested relapses during the follow-up. The basic characteristics of the controls and patients are shown in Table 1. There were no significant differences in basic characteristics between patients and control subjects in sex and age at the beginning of the study.

Comparison of patients and controls without changing their habits

FA and MD in the ROI of the corpus callosum

There were no significant changes observed in FA, MD, λ_{ax}, and λ_{rad} between the first and the second examination in either group. At the baseline, patients with MS showed significantly lower values in FA in the selected region of the CC ($p<0.001$) and significantly higher values in MD ($p<0.001$), λ_{ax} ($p=0.001$), and in λ_{rad} ($p<0.001$) compared to control subjects (Table 4).

PASAT 3

At the baseline, the PASAT 3 mean score was lower in patients compared to control subjects; however, due to high variability, the differences were not significant ($p=0.231$). There was a trend for different change in the two groups between the two measurements ($p=0.085$, Table 4). While the healthy controls did not change between the two measurements ($p=0.842$, Table 5), the MS patients improved significantly ($p=0.02$), reflecting a possible learning effect of this test.

The number of lesions per patient

The total number of lesions per patient in all regions: mean± SD (range) was 39.3±21.8 (11–80). The lesions were found predominantly in periventricular regions 15.6±7.4 (4–27), deep WM 12.4±11.7 (2–37), juxtacortical regions 2.3±2.1 (0–4), subcortical WM regions 6.0±6.5 (1–20), corpus callosum 2.4±1.2 (0–4), and in infratentorial regions 1.1± 1.0 (0–2).

Changes in patients after physiotherapy and comparison to changes in controls

FA and MD in the ROI of the corpus callosum

Differences 2 months after initiating facilitation physiotherapy were in FA, MD, and in λ_{rad} significantly higher ($p<0.001$ for FA, $p=0.024$ for MD, $p=0.002$ for λ_{rad}) than differences in healthy controls. In MS patients, FA in the CC significantly increased ($p<0.001$) and MD and λ_{rad} significantly decreased ($p=0.014$ and $p=0.002$), and thus approached the values in healthy controls (Table 5). There were no significant changes in axial diffusivity in MS after facilitation physiotherapy.

Table 3 Mean and standard deviation (SD) of differences between measurements in MS patients and healthy controls

		MS2-MS1	MS3-MS2	C2-C1
FA	Mean	−0.01	0.04	0.00
	SD	0.02	0.02	0.01
MD	Mean	−0.02	−0.05	0.00
	SD	0.07	0.06	0.03
λ_{ax}	Mean	−0.04	0.00	0.00
	SD	0.07	0.07	0.05
λ_{rad}	Mean	−0.02	−0.08	0.01
	SD	0.07	0.07	0.02
PASAT	Mean	3.30	3.64	0.20
	SD	4.37	6.02	3.08
EDSS	Mean		−0.18	
	SD		0.34	

MS2-MS1 differences between measurements 2 and 1 in MS patients, *MS3-MS2* differences between measurements 3 and 2 in MS patients, *C2-C1* differences between measurements 2 and 1 in healthy controls

Table 4 The p values of statistical tests performed on data of the first two measurements in MS patients (MS1, MS2) and the two measurements of healthy controls (C1, C2)

Tested effect	Used test	FA	MD	λ_{ax}	λ_{rad}	PASAT
Group×measurement	Two-sample t test used on differences	0.763	0.262	0.170	0.394	0.085
Measurement	Paired t test for measurement 1 vs. 2 for all together	0.098	0.388	0.163	0.668	0.066
Group	Two-sample t test used on mean of measurements	<0.001	<0.001	0.003	<0.001	0.231

Group× measurement interaction between group effect and measurement effect

PASAT 3 and EDSS

Although the MS patients did not differ significantly in the differences after physiotherapy from differences between the two measurements in healthy controls (p=0.116) in PASAT 3, the MS patients improved significantly after the physiotherapy (p=0.037), while healthy controls did not change (p=0.842, Table 5). There was a trend towards improvement in EDSS (p=0.087).

Discussion

The CC is a very well-organized and densely packed fiber structure having high FA in the normal condition, so it is likely that changes may also be more markedly manifested once pathology occurs [27]. For these reasons, this study focused on the DTI measurement and evaluation of the CC.

The baseline results in our study are consistent with previous FA and MD findings in the CC of patients with MS in most studies [8, 28–30] that show ultrastructural damage characterized as increased MD and reduced FA values. Lenzi et al. [1] found increased MD and reduced FA values in particular in the CC part through which connections between sensorimotor and premotor areas pass.

Several longitudinal DTI studies in patients with MS have been published [31–34]; however, their results have not provided a clear pattern—some found DTI changes over time in grey matter [33], some in white matter [34]. Some did not detect any changes when comparing patients with early MS and controls at baseline, but diffusion abnormalities were markedly apparent over 12 months [35]. In recent years, longitudinal studies gained higher power to detect subtle abnormalities due to the development of improved DTI acquisition sequences.

From the literature results, it follows that DTI in healthy controls [6] and MS patients [7], mainly early relapsing–remitting multiple sclerosis (RR MS) [35], gives reproducible quantitative results of FA and also that FA in RR MS does not change over time, whereas in progressive MS [31, 32], DTI parameters do change over time. Cassol et al. (2004) even described fluctuating changes in FA during six measurements in 1 year. At 0 and 3 months, the FA was abnormal, while at 1, 6, 9, and 12 months, there was a return towards control histogram values [29].

In this study, we confirmed that FA and MD in the ROIs of the CC did not change over a month in healthy controls. To our best knowledge, there is no published study monitoring FA and MD before and after facilitation physiotherapy in patients with MS. Based on Le Bihan's [36] finding that short-term changes in diffusion can occur upon brain activation, we expected changes of the FA and MD in MS patients. In agreement with this expectation, an increase in the FA and a decrease in MD values after 2 months of facilitation physiotherapy were observed in the whole selected ROI of the CC.

Clinical and neuropsychological measures

Over the treatment period, MS patients showed a trend for improvement in the major clinical parameter, EDSS. There is also evidence that CC dysfunction in MS may have a negative impact on cognitive performance, e.g., in callosal-mediated tasks where the impairment correlates with CC atrophy [37]. Because of the reported associations between dysfunction of interhemispheric cooperation and neuropsy-

Table 5 p values of statistical tests performed on data of the last two measurements in MS patients (MS2, MS3) and the two measurements of healthy controls (C1, C2)

Tested effect	Used test	FA	MD	λ_{ax}	λ_{rad}	PASAT
Group×measurement	Two-sample t test used on differences	<0.001	0.024	0.910	0.002	0.116
Measurement in C	Paired for measurement 1 vs. 2 in controls, two sided	0.937	0.693	0.966	0.336	0.842
Measurement in MS	Paired for measurement 2 vs. 3 in MS, one sided	<0.001	0.014	0.543	0.002	0.037

Group× measurement interaction between group effect and measurement effect

chological [38] or cognitive [39] functions, we decided to test cognitive functions, using PASAT 3, a test frequently used in MS, which shows lower values in patients with MS than in controls [40, 41]. In this study, at baseline, we found lower values of PASAT 3 in MS patients than in healthy controls (Table 2). However, these differences were not significant, partially because of the high variability of values and low number of patients. While the healthy controls did not change significantly between measurements, the MS patients showed significant improvement. Since the MS patients also improved without therapy, we ascribe this partially to a learning effect [42]. We assume that the learning effect is smaller in healthy controls because they can use their potential for learning improvement before the first measurement during the instruction procedure given to both groups. Observed improvement in clinical functions after therapy corresponds to our previous studies [13, 43]. In future, patients should be tested for PASAT several times before entering the study to eliminate learning effect.

The mechanisms of facilitation physiotherapy can be understood as follows: stimuli applied during the treatment aim at the activation of deeply encoded programs of the CNS. These programs integrate genetically determined factors of motor behavior, muscle activity encoded in motor patterns, and automatic reactions of the motor system to afferent stimulation that mature during the course of postural ontogenesis [20, 44]. For example, there is evidence for the existence of central pattern generators of locomotion on spinal, brainstem, and higher CNS levels [45]. Repetitive activation of the motor program modifies and strengthens the cortical engram—a widely distributed group of neurons comprising multiple smaller functional groupings that tend to fire synchronously during a movement [46–49]. This mechanism probably initiates plastic and adaptive processes in the CNS [49–52]. In this study, significant changes of FA and MD have been confirmed. We are convinced that it is probably due to the introduction of the role of facilitation physiotherapy on plastic and adaptive processes of CNS.

Neuroplasticity is an important mechanism that enables the CNS to limit clinical manifestations of MS by reorganizing itself [53, 54]. Plasticity can occur at the axon, neuron, and synapse or system level. DTI is a technique that can provide a marker of axonal integrity [55], and its repetition during rehabilitation and correlation with long clinical outcome may add to understanding of the mechanisms of neuroplasticity and repair operating during recovery [14, 56].

Matthews et al. [57] considered brain changes with spontaneous recovery after brain injury or induced recovery of neurorehabilitation in the context of healthy brain changes with learning—brain structure and function can change with shifting goals or strategies or injury to a functional system. Facilitation physiotherapy in this and our previous studies is based on sensorimotor learning and uses its stimuli to activate programs in the CNS [58, 59]. Sensorimotor learning can be perceived as a form of synaptic plasticity where the changes in synaptic connection become a physiological substrate for retaining a piece of information in one's memory. Sensorimotor learning, i.e., rehabilitation, may influence the interconnection of neural networks [60] in the context of general molecular, cellular, and behavioral mechanisms for recovery: (1) repair (new oligodendroglial cells from progenitors and remyelination, restoring normal conduction and glial trophic support for axons), (2) compensation (behavioral changes leading to an altered strategy for completing a task), and (3) adaptation (recruitment of new systems) [57]. Adaptive changes involve three main mechanisms: (1) the unmasking of existing but latent horizontal connections, (2) the modulation of synaptic efficacy, such as long-term potentiation or long-term depression, and (3) experience-dependent increases in dendritic spines and synaptogenesis [61].

In MS, neuroplasticity on a system level as a result of neurorehabilitation has been studied. Morgen et al. (2004) showed that when training the motor functions with MS patients, there happens cortical reorganization of motor neuron networks, but on a lesser scale than in healthy subjects. In our pilot study [19], we were able to prove interesting changes when evaluating the amplitude of the signal in relation to neurological treatment which we interpreted as an improvement in the cooperation of brain hemispheres. Similarly, in today's research [62], we confirmed the dysfunction of interhemispheric cooperation in multiple sclerosis and showed the possibilities of influencing it through neurorehabilitation as well as aerobic training.

The significant increase in the radial diffusivity in MS at baseline compared to controls and its following decrease after 2 months of physiotherapy observed in our study may be associated with demyelination and remyelination processes in axons as described by Song et al. [63, 64].

The possibility to actively and purposely induce neuroplasticity is very promising for the future to promote functional recovery but needs to be verified in further research. For the future, it would be useful to add another follow-up examination several months after cessation of physiotherapy to evaluate the long-term maintenance of the observed effects and to evaluate potential effect. Moreover, correlations with more clinical outcomes should be analyzed. The time period between measurements should be identical. Finally, a higher number of probands should be included in the study.

Conclusions

At baseline, we observed significant differences in FA (decreases) and MD (increases) values in the selected ROI of the CC in MS patients compared to control subjects. Diffusion indices (FA, MD, λ_{ax}, and λ_{rad}) did not change in healthy controls, whereas they significantly improved (FA increased, and MD and λ_{rad} decreased) in patients who underwent 2-month facilitation physiotherapy. The results of this study show that facilitation physiotherapy influences brain microstructure measured by DTI—MRI measures that have been proposed as biomarkers of tissue damage in MS.

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Conflict of interest We declare that we have no conflict of interest.

References

1. Lenzi D, Conte A, Mainero C, Frasca V, Fubelli F, Totaro P, Caramia F, Inghilleri M, Pozzilli C, Pantano P (2007) Effect of corpus callosum damage on ipsilateral motor activation in patients with multiple sclerosis: a functional and anatomical study. Hum Brain Mapp 28:636–644
2. Basser PJ, Pierpaoli C (1996) Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. J Magn Reson B 111:209–219
3. Warlop NP, Fieremans E, Achten E, Debruyne J, Vingerhoets G (2008) Callosal function in MS patients with mild and severe callosal damage as reflected by diffusion tensor imaging. Brain Res 1226:218–225
4. Nusbaum AO, Tang CY, Wei T, Buchsbaum MS, Atlas SW (2000) Whole brain diffusion MR histograms differ between MS subtypes. Neurology 54:1421–1427
5. Filippi M, Iannucci G, Cercignani M, Assunta RM, Pratesi A, Comi G (2000) A quantitative study of water diffusion in multiple sclerosis lesions and normal appearing white matter using echo-planar imaging. Arch Neurol 57:1017–1021
6. Ciccarelli O, Werring DJ, Barker GJ, Griffin CM, Wheeler-Kingshott CA, Miller DH, Thompson AJ (2003) A study of the mechanisms of normal-appearing white matter damage in multiple sclerosis using diffusion tensor imaging—evidence of Wallerian degeneration. J Neurol 250:287–292
7. Hasan KM, Gusta RK, Santos RM, Dolinsky JS, Narayana PA (2005) Diffusion tensor fractional anisotropy of the normal appearing seven segments of the corpus callosum in healthy adults and relapsing–remitting multiple sclerosis patients. J Magn Reson Imaging 21:735–743
8. Roosendaal SD, Geurts JJ, Vrenken H, Hulst HE, Cover KS, Castelijns JA, Pouwels PJ, Barkhof F (2009) Regional DTI differences in multiple sclerosis patients. Neuroimage 44:1397–1403
9. Nucifora PG, Verma R, Lee SK, Melhem ER (2007) Diffusion-tensor MR imaging and tractography: exploring brain microstructure and connectivity. Radiology 245:367–384
10. Evangelou N, Konz D, Esiri MM, Smith S, Palace J, Matthews PM (2000) Regional axonal loss in the corpus callosum correlates with cerebral white matter lesion volume and distribution in multiple sclerosis. Brain 123:1845–1849
11. Coote S, Garrett M, Hogan N, Larkin A, Saunders J (2009) Getting the balance right: a randomised controlled trial of physiotherapy and Exercise Interventions for ambulatory people with multiple sclerosis. BMC Neurol 9:34
12. Morgen K, Kadom N, Sawaki L, Tessitore A, Ohayon J, McFarland H, Frank J, Martin R, Cohen LG (2004) Training-dependent plasticity in patients with multiple sclerosis. Brain 127 (11):2506–2517
13. Rasova K, Krasensky J, Havrdova E, Obenberger J, Seidel Z, Dolezal O, Rexova P, Zalisova M (2005) Is it possible to actively and purposely make use of plasticity and adaptability in the neurorehabilitation treatment of multiple sclerosis patients? A pilot project. Clin Rehabil 19:170–181
14. Sidaros A, Engberg AW, Sidaros K, Liptrot MG, Herning M, Petersen P, Paulson OB, Jernigan TL, Rostrup E (2008) Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: a longitudinal study. Brain 131(Pt 2):559–572
15. Eliassen JC, Boespflug EL, Lamy M, Allendorfer J, Chu WJ, Szaflarski JP (2008) Brain-mapping techniques for evaluating poststroke recovery and rehabilitation: a review. Top Stroke Rehabil 15(5): 427–450
16. Luccichenti G, Sabatini U (2009) Colouring rehabilitation with functional neuroimaging. Funct Neurology 24(4):189–193
17. Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:97–113
18. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 58:840–846
19. Rasova K (2007) Physiotherapy in neurological diseases (aimed at multiple sclerosis). Ceros o.p.s, Praha: 6–75 (in Czech)
20. Kolar P (2007) Facilitation of agonist–antagonist co-activation by reflex stimulation methods. In: Liebenson C (ed) Rehabilitation of the spine, 2nd edn. Lippincott, Philadelphia, pp 532–565
21. Kurtzke JF (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33:1444–1452
22. Morris LC (2000) Clinical and rehabilitation outcome measures. In: Burks JS, Johnson KP (eds) Multiple sclerosis: diagnosis, medical management and rehabilitation. Demos, New York, pp 236–290
23. Grubbs FE (1950) Sample criteria for testing outlying observations. Ann Math Stat 21(1):27–58
24. R Development Core Team (2010) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. ISBN 3-900051-07-0
25. Pinheiro J, Bates D, DebRoy S, Sarkar D and the R Development Core Team (2010) NLME: Linear and Nonlinear Mixed Effects Models. R package version 3.1-97
26. Komsta L (2010). outliers: tests for outliers. R package version 0.13-3
27. Ge Y, Law M, Grossman RI (2005) Applications of diffusion tensor MR imaging in multiple sclerosis. Ann N Y Acad Sci 1064:202–219
28. Cassol E, Ranjeva JP, Ibarrola D, Mékies C, Manelfe C, Clanet M, Berry I (2004) Diffusion tensor imaging in multiple sclerosis: a tool for monitoring changes in normal-appearing white matter. Mult Scler 10:188–196
29. Coombs BD, Best A, Brown MS, Miller DE, Corboy J, Baier M, Simon JH (2004) Multiple sclerosis pathology in the normal and abnormal appearing white matter of the corpus callosum by diffusion tensor imaging. Mult Scler 10:392–397

30. Guo AC, MacFall JR, Provenzale JM (2002) Multiple sclerosis: diffusion tensor MR imaging for evaluation of normal appearing white matter. Radiology 222:729–736
31. Rovaris M, Gallo A, Valsasina P, Benedetti B, Caputo D, Ghezzi A, Montanari E, Sormani MP, Bertolotto A, Mancardi G, Bergamaschi R, Martinelli V, Comi G, Filippi M (2005) Short-term accrual of gray matter pathology in patients with progressive multiple sclerosis: an in vivo study using diffusion tensor MRI. Neuroimage 24:1139–1146
32. Schmierer K, Altmann DR, Kassim N, Kitzler H, Kerskens CM, Doege CA, Aktas O, Lünemann JD, Miller DH, Zipp F, Villringer A (2004) Progressive changes in primary progressive multiple sclerosis normal appearing white matter: a serial diffusion magnetic resonance imaging study. Mult Scler 10:182–187
33. Oreja-Guevara C, Rovaris M, Iannucci G, Valsasina P, Caputo D, Cavarretta R, Sormani MP, Ferrante P, Comi G, Filippi M (2005) Progressive gray matter damage in patients with relapsing–remitting multiple sclerosis: a longitudinal diffusion tensor magnetic resonance imaging study. Arch Neurol 62:578–584
34. Gallo A, Rovaris M, Riva R, Ghezzi A, Benedetti B, Martinelli V, Falini A, Comi G, Filippi M (2005) Diffusion-tensor magnetic resonance imaging detects normal appearing white matter damage unrelated to short-term disease activity in patients at the earliest clinical stage of multiple sclerosis. Arch Neurol 62:803–808
35. Caramia F, Pantano P, Di Legge S, Piattella MC, Lenzi D, Paolillo A, Nucciarelli W, Lenzi GL, Bozzao L, Pozzilli C (2002) A longitudinal study of MR diffusion changes in normal appearing white matter of patients with early multiple sclerosis. Magn Reson Imaging 20:383–388
36. Le Bihan D (2006) Looking into the functional architecture of the brain with diffusion MRI. Int Congr Ser 1290:1–24
37. Manson SC, Palace J, Frank JA, Matthews PM (2006) Loss of interhemispheric inhibition in patients with multiple sclerosis is related to corpus callosum atrophy. Exp Brain Res 174:728–733
38. Dietemann JL, Beigelman C, Rumbach L, Vouge M, Tajahmady T, Faubert C, Jeung MY, Wackenheim A (1988) Multiple sclerosis and corpus callosum atrophy: relationship of MRI findings to clinical data. Neuroradiology 30:478–480
39. Mesaros S, Rocca MA, Riccitelli G, Pagani E, Rovaris M, Caputo D, Ghezzi A, Capra R, Bertolotto A, Comi G, Filippi M (2009) Corpus callosum damage and cognitive dysfunction in benign MS. Hum Brain Mapp 30:2656–2666
40. Rao SM, Leo GJ, Bernardin L, Unverzagt F (1991) Cognitive dysfunction in multiple sclerosis: I. Frequency, patterns, and prediction. Neurology 41:685–691
41. Patti F, Amato MP, Trojano M, Bastianello S, Tola MR, Goretti B, Caniatti L, Di Monte E, Ferrazza P, Brescia Morra V, Lo Fermo S, Picconi O, Luccichenti G, COGIMUS Study Group (2009) Cognitive impairment and its relation with disease measures in mildly disabled patients with relapsing-remitting multiple sclerosis: baseline results from the Cognitive Impairment in Multiple Sclerosis (COGIMUS) study. Mult Scler 15:779–788
42. 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(Pt 5):871–882
43. Rasova K, Brandejsky P, Havrdova E, Zalisova M, Foubikova B (2006) Comparison of the influence of different rehabilitation programs on clinical spirometric and spiroergometric parameters in patients with multiple sclerosis. Mult Scler 12:227–234
44. Vojta V (1973) Early diagnosis and therapy of cerebral movement disorders in childhood. C. Reflexogenous locomotion—reflex creeping and reflex turning. C1. The kinesiologic content and connection with the tonic neck reflexes. Z Orthop Ihre Grenzgeb 111:268–291
45. Grillner S (1985) Neurobiological bases of rhythmic motor acts in vertebrates. Science 228:143–149
46. Merzenich MM, Sameshima K (1993) Cortical plasticity and memory. Curr Opin Neurobiol 3:187–196
47. Daoudal G, Debanne D (2003) Long-term plasticity of intrinsic excitability: learning rules and mechanisms. Learn Mem 10:456–465
48. Niemann J, Winker T, Gerling J, Landwehrmeyer B, Jung R (1991) Changes of slow cortical negative DC-potentials during the acquisition of a complex finger motor task. Exp Brain Res 85:417–422
49. Sochurkova D, Rektor I, Jurak P, Stancak A (2006) Intracerebral recording of cortical activity related to self-paced voluntary movements: a Bereitschaftspotential and event-related desynchronization/synchronization. SEEG study. Exp Brain Res 173:637–649
50. Dobkin HB (2004) Neurobiology of rehabilitation. Ann N Y Acad Sci 1038:148–170
51. Nelles G, Jentzen W, Jueptner M, Müller S, Diener HC (2001) Arm training induced brain plasticity in stroke studied with serial positron emission tomography. Neuroimage 13:1146–1154
52. Johansen-Berg H, Dawes H, Guy C, Smith SM, Wade DT, Matthews PM (2002) Correlation between motor improvements and altered fMRI activity after rehabilitative therapy. Brain 125:2731–2742
53. Lee M, Reddy H, Johansen-Berg H, Pendlebury S, Jenkinson M, Smith S, Palace J, Matthews PM (2000) The motor cortex shows adaptive functional changes to brain injury from multiple sclerosis. Ann Neurol 47:606–613
54. Reddy H, Narayanan S, Arnoutelis R, Jenkinson M, Antel J, Matthews PM, Arnold DL (2000) Evidence for adaptive functional changes to brain injury from multiple sclerosis. Brain 123:2314–2320
55. Giacomini PS, Arnold DL (2008) Non-conventional MRI techniques for measuring neuroprotection, repair and plasticity in multiple sclerosis. Curr Opin Neurol 21:272–277
56. Sterr A, Shen S, Szameitat AJ, Herron KA (2010) The role of corticospinal tract damage in chronic motor recovery and neurorehabilitation: a pilot study. Neurorehabil Neural Repair 24 (5):413–419
57. Matthews PM, Johansen-Berg H, Reddy H (2004) Non-invasive mapping of brain functions and brain recovery: applying lessons from cognitive neuroscience to neurorehabilitation. Restor Neurol Neurosci 22(245–260):245
58. Leonard ChT (1998) The neuroscience of motor learning. In: The neuroscience of human movement. Mosby, St. Louis 203–229
59. Mier H (2000) Human learning. In: Toga AW, Mazziotta JC (eds) Brain mapping. The systems. Academic Press, London, pp 605–620
60. Squire LR (1986) Mechanisms of memory. Science 232:1612–1669
61. Bütefisch CM (2006) Neurobiological bases of rehabilitation. Neurol Sci 27:18–23
62. Rasova K, Brandejsky P, Tintera J, Krasensky J, Zimova D, Medova E, Herbenova A, Kalistova H, Sobotkova L, Jech R, Rasova M, Zemanova P, Zeman J, Ibrahim I, Martinkova P, Dolezil D, Jandova D (2009) Bimanual tandem motor task with multiple sclerosis in functional magnetic resonance imaging: effect of physiotherapeutic techniques—a pilot study. Česká a slovenská neurologie a neurochirurgie 4(72/105):350–359 (in Czech)
63. Song SK, Yoshino J, Le TQ, Lin SJ, Sun SW, Cross AH, Armstrong RC (2005) Demyelination increases radial diffusivity in corpus callosum of mouse brain. Neuroimage 26(1):132–140
64. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH (2002) Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 17 (3):1429–1436