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A longitudinal fMRI study on motor activity in patients with multiple sclerosis

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Using functional MRI (fMRI), patients with multiple sclerosis showed a greater extent of motor activation than controls. Although functional changes are often interpreted as adaptive and as a contributing factor in limiting the clinical deficit, no longitudinal studies have yet been performed for multiple sclerosis. Sixteen patients with multiple sclerosis, two patients with possible multiple sclerosis and nine age-matched controls underwent two fMRI studies with a time interval of 15–26 months. The motor task consisted of a self-paced sequential finger opposition movement with the right hand. Patients with multiple sclerosis exhibited greater bilateral activation than controls in both fMRI studies. At follow-up, patients showed a reduction in functional activity in the ipsilateral sensorimotor cortex and in the contralateral cerebellum. No significant differences between the two fMRI studies were observed in controls. Activation changes in ipsilateral motor areas correlated inversely with age, extent and progression of T1 lesion load, and occurrence of a new relapse. This study may help the understanding of the evolution of brain plastic changes in multiple sclerosis indicating that, in younger patients with a less structural brain damage and benign clinical course, the brain reorganizes its functional activity towards a more lateralized pattern of brain activation. The tendency towards a normalization of brain functional activity is hampered in older patients and in those developing relapses or new irreversible brain damage.

Keywords: multiple sclerosis; fMRI; motor activity; longitudinal study

Abbreviations: BA = Brodmann area; EDSS = expanded disability status scale; fMRI = functional MRI; L = left; LL = lesion load; MR = magnetic resonance; R = right

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Introduction

A number of functional MRI (fMRI) studies have demonstrated that, in almost all the clinical phenotypes of multiple sclerosis, patients showed greater motor activation than healthy controls. This correlated with the tissue damage revealed by conventional (Lee *et al.*, 2000; Pantano *et al.*, 2002*a*,*b*) or non-conventional MRI (Filippi *et al.*, 2002; Reddy *et al.*, 2000*a*; Rocca *et al.*, 2003).

Brain functional changes may represent a compensatory function designed to maintain a normal performance despite scattered brain lesions. Indeed, patients with multiple sclerosis may show recovery from symptoms while progressively accumulating tissue damage and they often show a discrepancy between lesions on conventional magnetic resonance (MR) and clinical symptoms (Smith *et al.*, 1993). This discrepancy may partially be explained by diffuse abnormality in normal appearing brain tissue as seen on non-conventional MRI (Miller *et al.*, 2003).

Cortical motor reorganization is not specific to multiple sclerosis, since it has been described in various diseases including tumours (Roux *et al.*, 2000), vascular malformations (Schlosser *et al.*, 1997) and stroke. Longitudinal fMRI studies on motor activity in stroke patients have suggested that cortical reorganization is a dynamic phenomenon that evolves with time and may correlate with clinical outcome (Calautti and Baron, 2003).

Dynamic cortical changes paralleling the clinical course have also been described in a multiple sclerosis patient who completely recovered from right hemiplegia (Reddy *et al.*, 2000*b*). No other longitudinal fMRI studies have explored possible changes in the pattern of motor activation over time in multiple sclerosis.

The knowledge of how brain reorganization evolves in response to disease evolution may shed light on the role of brain plasticity in multiple sclerosis and the potentialities of medical or physical therapies in modulating it. The aim of this study was to evaluate longitudinal changes in motor-related brain activation in patients with early multiple sclerosis and their relationship with clinical and conventional MRI features.

Material and methods

Subjects

Patients were recruited from the Multiple Sclerosis Service at the Department of Neurological Sciences, University of Rome 'La Sapienza', Italy. Inclusion criteria were: (i) a single clinical episode; (ii) positive MR findings according to Fazekas's criteria (Fazekas *et al.*, 1988); (iii) no sensory and/or motor deficit at the neurological examination; (iv) no treatment with immunomodulatory or immunosuppressive drugs; (v) time elapsed since the end of steroid treatment, if any, of not less than 2 months; and (vi) no MR lesions in the spinal cord.

Eighteen patients who met these inclusion criteria were included in the study. They had been the object of previous fMRI studies (Pantano *et al.*, 2002*a*,*b*). All patients had suffered from a single clinical attack consisting either of hemiparesis (n = 9: five right hemiparesis and four left hemiparesis) or optic neuritis (n = 9), and they had had a diagnosis of multiple sclerosis (n = 16) or of possible multiple sclerosis (n = 2) according to the recommendations of the international panel on the diagnosis of multiple sclerosis (McDonald *et al.*, 2001). All patients underwent conventional neurological examination and were scored on the expanded disability status scale (EDSS) (Kurtzke, 1983).

Nine healthy gender and age-matched volunteers constituted the control group. They had no history of neurological or psychiatric disease and were free of any medication.

All patients and controls underwent a second fMRI study (fMRI2) with the same modalities within a time interval of 15–26 months (median: patients = 19; controls = 18) since the first fMRI study (fMRI1).

All patients and controls were right-handed and gave their written consent in accordance with the declaration of Helsinki. The study was approved by the Local Ethical Committee of the University of Rome 'La Sapienza'.

fMRI data acquisition

Morphological MRI and fMRI data were acquired during the same imaging session using a 1.5 T magnet (Philips Gyroscan NT 15 Philips, the Netherlands) with echo planar capabilities and a head volume radio frequency coil. Each subject lay supine in the scanner with eyes closed. Head movements were minimized through use of foam padding and a restraining strap. The acquisition of a multiplanar T1-weighted localizer at the beginning of each study assured the same slice orientation (parallel to the bi-commissural plane) and the same brain volume acquisition (last slice tangent to the cortical mantle surface) in different fMRI sessions.

T2*-weighted echo planar images were acquired (64 × 64 matrix over a 24-cm field of view). These consisted of 25 consecutive, 4-mm thick axial sections, with TR/TE (repetition time/echo time) = 3000/50 ms, a 90° flip angle and one excitation.

Each functional study lasted 225 s, during which a total of 75 consecutive dynamics were acquired.

Motor task paradigm

During fMRI acquisition, both patients and healthy subjects performed a self-paced sequential finger opposition task in which the thumb repeatedly touched the other four fingers in a sequential order with the right hand. Seven periods of hand movement and seven periods of rest were alternated (each period lasting for 15 s). 'Start' and 'stop' acoustic signals were given during the acquisition. Subjects were required to perform the task as quickly as possible, opening their hand wide. Correct execution of the task was confirmed by an operator who was present in the magnet room throughout the session and who recorded the rate of hand movements both for patients and controls.

fMRI data analysis

fMRI data were analysed using SPM99 software (Wellcome Department of Cognitive Neurology, Institute of Neurology, University College London, London, UK) according to the following procedure. Images were realigned, normalized and spatially smoothed using a Gaussian kernel of 8 mm.

At the first stage, the time series of functional MR images obtained from each participant was analysed separately. The effects of the experimental paradigm were estimated on a voxel-by-voxel basis using the principles of the general linear model extended to allow the analysis of fMRI data as a time series (Friston *et al.*, 1994, 1995). The data regarding each subject were modelled using a boxcar design, convolved with the haemodynamic response function chosen to represent the relationship between neuronal activation and blood flow changes.

Four contrast images were created for each subject: (i) task-related activation at fMRI1; (ii) task-related activation at fMRI2; (iii) task-related activity increase between the two fMRI studies (fMR1 < fMRI2); and (iv) task-related activity decrease between the two fMRI studies (fMR1 > fMRI2).

These contrast images were then used for a second level random effect analysis, according to a 2 × 2 design with time (fMR1 and fMRI2) and group (patients and controls) as factors. We assessed main effects, interactions and simple main effects using subject-specific contrasts as the response variable and one or two sample t-tests. Clusters of voxels (corrected $P < 0.05$) that had a peak Z score >3.7 were considered to show significant changes.

We also performed a within-group multiple regression analysis to look at the effects of age, disease progression and related measures on the extent of activations by including 11 clinical and radiological variables (see Results). Clusters of voxels (corrected $P < 0.05$) which had a peak Z score >2.4 were considered to be significantly correlated.

Within each region of statistical significance, local maxima of signal increase were determined (the voxels of maximum significance) and their location was expressed in terms of x, y, and z coordinates. Montreal Neurological Institute (MNI) coordinates were converted to the Talairach space (Talairach and Tournoux, 1988) using a linear transformation (www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html).

Morphological MRI acquisition and data analysis

After the fMRI study, we performed a morphological MRI protocol that included proton density and T2-weighted spin-echo images (T2-WI) (TR = 2000 ms; TE = 20/90 ms), and T1-weighted spin-echo images (T1-WI) (TR = 550 ms; TE = 12 ms) before and after injection of an intravenous bolus of 0.3 mmoles/kg gadolinium

diethyltriamine pentaacetic acid (Gd-DTPA). For proton density, T2-WI and T1-WI, 40 contiguous axial slices were acquired with 4-mm thickness, 256 × 256 matrix and 24-cm field of view.

On conventional MR images, hyperintense T2 and hypointense T1 lesion load (LL) were calculated in each patient using the display program Dispunc (D.L. Plummer, University College London, London, UK) with a semi-automated contouring technique (Grimaud *et al.*, 1996).

Results

Clinical data

Patients comprised 14 females and four males aged from 21 to 51 years (mean ± SD = 31 ± 8 years). Controls comprised seven females and two males aged from 24 to 50 years (mean ± SD = 31 ± 8). EDSS score at the time of fMRI1 ranged from 0 to 2.5 (median = 1.0; mean ± SD = 0.81 ± 0.75).

Patients underwent a second fMRI study 15–26 months later (mean ± SD = 19.8 ± 3.4 months).

No mirror movements were noted during right-hand movement in any of the patients at either fMRI study. The rate of hand movement did not differ significantly between patients and controls and between fMRI1 and fMRI2 (patients: fMRI1 = 1.98 ± 0.18 Hz.; fMRI2 = 2.00 ± 0.19 Hz.; controls: fMRI1 = 2.02 ± 0.20 Hz.; fMRI2 = 2.01 ± 0.18 Hz.). There were no significant differences in the rate of right hand movements between patients who had had a right hemiparesis and those who had had a left hemiparesis.

Five patients had presented a new clinical event in the interval time between the two fMRI studies and begun interferon-beta (IFNβ) therapy. Relapses consisted of optic neuritis ($n = 2$), hypoesthesia in both legs ($n = 1$), diplopia with mild ataxia ($n = 1$) and left upper limb hyposthenia ($n = 1$). Six patients had a subclinical progression of the disease (appearance of new T2 hyperintense lesions without a new clinical event). The remaining seven patients were in a stationary phase. The EDSS score at the time of fMRI2 ranged from 0 to 2.5 (median = 1.0; mean ± SD = 1.22 ± 0.75).

Clinical and conventional MR data of the 18 patients at the time of the two fMRI studies are shown in Table 1. No significant differences were observed in EDSS, T1 and T2 lesion load between time 1 and time 2.

Table 1 Clinical and conventional MR data of the 18 patients with multiple sclerosis at the time of the two fMRI studies

	fMRI1	fMRI2
EDSS	0.81 ± 0.75(0–2.5)	1.22 ± 0.75(0–2.5)
Number of patients with a second clinical event	0	5
T2 LL (ml)	7.29 ± 7.1(0.73–33.3)	7.55 ± 6.7(0.91–28.9)
T1 LL (ml)	1.03 ± 0.9(0.15–3.24)	1.09 ± 1.2(0.07–4.81)
Number of T2 hyperintense lesions	41 ± 35(7–120)	43 ± 36(7–120)
Number of patients with new T2 lesions	—	11

EDSS, T2 and T1 lesion load, and the number of T2 hyperintense lesions are expressed as mean ± SD (range).

Changes in motor-related brain activation as a function of time

Foci of significant activation during right-hand movement at both the first and the second fMRI studies in nine control subjects and 18 patients with multiple sclerosis are shown in Tables 2 and 3, respectively. Motor activation was significantly greater in patients than in controls at both fMRI studies in primary and secondary sensori-motor areas ($P < 0.05$ corrected for multiple comparisons, two-sample t-test). Task-related differences between patients and controls at the time of the first fMRI are not reported explicitly in the current study since we have described them previously (Pantano *et al.*, 2002*a*,*b*). At the time of the follow-up study, patients showed greater activation than controls in the lentiform nucleus, thalamus and insula bilaterally, in the ipsilateral premotor and superior parietal cortices.

Patients with multiple sclerosis showed significant task-related activity changes between the two fMRI studies (fMR1 and fMRI2). Functional changes consisted of a decreased motor activity (fMRI1 > fMRI2) in the left cerebellar hemisphere (x, y, z coordinates = −22, −56, −10; $Z = 4.44$, corrected $P < 0.05$) and in the right sensorimotor cortex (x, y, z coordinates = 18, −27, 48; $Z = 3.74$, corrected $P < 0.05$) (Fig. 1).

No focus of increased motor activity was observed at follow-up (fMR1 < fMRI2) in the group of 18 multiple sclerosis patients. No significant differences between the two fMRI studies performed with a time interval of 20.0 ± 2.1 months were observed in the group of nine healthy subjects.

Analysis of group contrast between (change in patients) – (changes in controls) revealed results which were very similar in the anatomical profile to those provided by the simple main effect of changes in patients, i.e. the difference in activation between fMRI1 and fMRI2 in patients was significantly greater than changes in controls in the ipsilateral sensorimotor cortex (x, y, z coordinates = 40, −2, 52; $Z = 4.27$) and in the contralateral cerebellum (x, y, z coordinates = −21, −50, −17; $Z = 4.09$).

Relationship between time-dependent changes in motor activation and clinical and conventional MRI findings

We tested which of the various clinical and conventional MR features in 18 patients with multiple sclerosis correlated significantly with changes in the brain in motor activity between the two fMRI studies. The following clinical and radiological variables were included in the multiple regression analysis: (i) age; (ii) EDSS score at fMRI1; (iii) disease duration; (iv) type of clinical syndrome at presentation; (v) occurrence of a relapse; (vi) time interval between fMRI1 and fMRI2;

Table 2 Within group analysis (one-sample *t*-test, SPM99). Location of significant activations ($P < 0.05$ corrected at the cluster level) during right-hand movement in nine control subjects at baseline and at follow-up

Brain area	Baseline		Follow-up	
	Talairach coordinates (*x*, *y*, *z*)	*Z*	Talairach coordinates (*x*, *y*, *z*)	*Z*
L sensorimotor cortex (BA 1–4)	−33, −17, 52	5.78	−45, −10, 45	5.11
			−42, −18, 50	4.80
L inferior parietal lobule (BA 40)	−27, −40, 50	4.30	−50, −35, 45	4.76
	−40, −40, 55	4.22		
L lateral premotor cortex (BA 6)	−59, −9, 27	4.01	−47, 0, 45	4.36
	−47, 2, 45	3.46		
L supplementary motor area (BA 6)	−2, 0, 60	4.36	−3, 1, 55	4.46
R sensorimotor cortex (BA 1–4)	29, −19, 52	3.94	35, −20, 52	4.37
R inferior parietal lobule (BA 40)	40, −36, 49	4.59	41, −39, 45	3.90
R supplementary motor area (BA 6)	3, 0, 55	4.46	8, −5, 56	4.22
R cerebellum	15, −63, −12	5.58	28, −70, −16	5.77
Vermis	6, −67, −18	5.53	5, −50, −8	5.26
	8, −65, −25	5.11	8, −56, −10	4.44

Z = voxel level.

Table 3 Within group analysis (one-sample *t*-test, SPM99). Location of significant activations ($P < 0.05$ corrected at the cluster level) during right-hand movement in 18 patients with multiple sclerosis at baseline and at follow-up

Brain area	Baseline		Follow–up	
	Talairach coordinates (*x*, *y*, *z*)	*Z*	Talairach coordinates (*x*, *y*, *z*)	*Z*
L sensorimotor cortex (BA 1–4)	−44, −19, 43	5.80	−38, −19, 47	7.57
L inferior parietal lobule (BA 40)	−46, −32, 52	5.24	−53, −30, 24	4.15
			−49, −38, 48	3.87
L lateral premotor cortex (BA 6)	−34, −5, −55	5.14	−59, 6, 32	6.69
L supplementary motor area (BA 6)	−2, −1, 55	4.27	−2, 1, 53	5.44
L lentiform nucleus	−12, −12, −1	5.22	−26, −3, 9	5.21
L thalamus	−12, −11, 13	4.72	−16, −17, 3	5.09
L insula	−55, 12, 3	4.69	−49, −20, 16	5.65
L cerebellum	−18, −55, −17	5.17	—	—
R sensorimotor cortex (BA 1–4)	42, 0, 52	4.06	45, −27, 40	4.55
R inferior parietal lobule (BA 40)	32, −48, 54	5.23	40, −33, 40	4.63
			61, −2, 19	4.39
R lateral premotor cortex (BA 6)	30, −7, 57	5.08	61, 7, 29	5.77
	57, 8, 36	4.81	36, −11, 58	5.26
R superior parietal cortex (BA 7)	32, −48, 54	5.23	36, −52, 56	4.67
R lentiform nucleus	—	—	22, −2, 2	4.37
R thalamus	12, −7, 13	4.17	12, −6, 13	4.17
R insula	57, 19, −4	4.78	57, 16, 1	4.22
	47, 4, −1	3.91		
R cerebellum	18, −55, −17	6.59	18, −53, −18	6.22
Vermis	−2, −67, −10	5.89	2, −50, −3	4.68

Z = voxel level.

(vii) T2 LL and (viii)T1 LL at fMRI1; (ix) T2LL and (x) T1LL changes between fMRI1 and fMRI2; and (xi) number of new T2 hyperintense lesions at fMRI2.

Age, occurrence of a new relapse, T1 LL at fMRI1 and T1LL changes between fMRI1 and fMRI2 were inversely correlated with decreased activity in different motor areas, mainly located in the ipsilateral hemisphere (Table 4).

Age correlated significantly with activation foci located in the ipsilateral premotor [Brodmann area (BA) 6] and sensorimotor (BA 1–4) cortex, indicating that the younger the patients, the greater the decrease in motor activation in these areas at follow-up.

The occurrence of a new relapse correlated significantly with activation foci in the ipsilateral sensorimotor cortex (BA 1–4) and in the supplementary motor area (BA 6), indicating that the decrease in motor activation in these areas was smaller in patients with a worse clinical course.

T1 lesion load at baseline correlated significantly with activation foci located in the ipsilateral parietal cortex (BA 40) and in the contralateral sensorimotor cortex

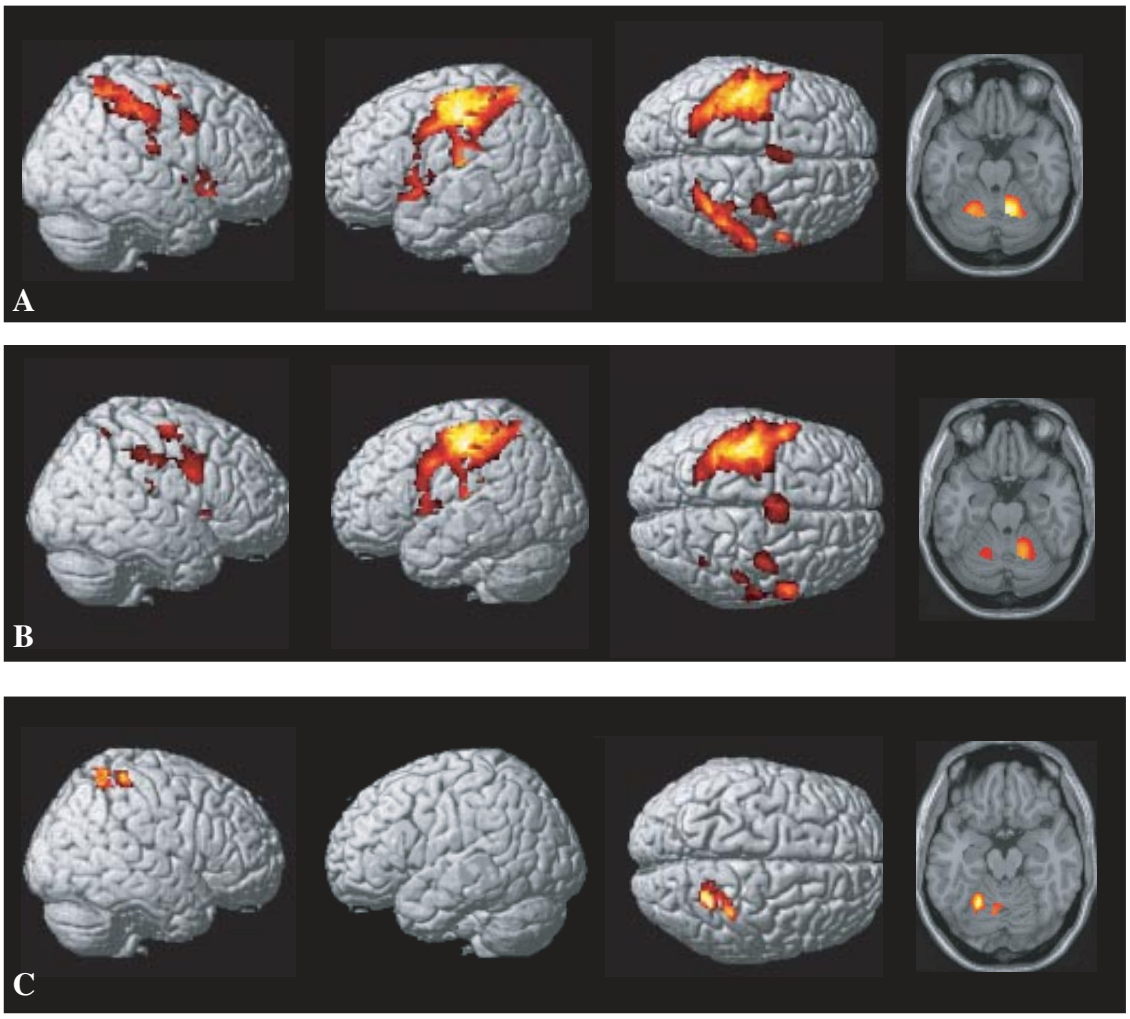

Fig. 1 Group maps generated from random effect analysis showing (**A**) task-related activation at fMRI1, (**B**) task-related activation at fMRI2 and (**C**) task-related activity decrease between the two fMRI studies during right hand movement in 18 patients with multiple sclerosis. Significant areas of activation (in colour) are superimposed on 3D brain rendering and slices ($z = -18$). Areas of decreased activity (fMRI1 > fMRI2) (**C**) included the right (ipsilateral) sensorimotor cortex and the left (contralateral) cerebellum. One-sample *t*-test ($P < 0.05$) corrected at the cluster level. Images are displayed according to the neurological convention.

(BA 1–4), indicating that the decrease in motor activation in these areas was smaller in patients with a higher T1 lesion load at baseline.

T1 lesion load changes correlated significantly with activation foci in the ipsilateral sensorimotor (BA 1–4) and parietal cortex (BA 40), showing that the decrease in motor activation was smaller in patients with a greater increase in T1 lesion load between the two fMRI studies.

Discussion

Motor activity in patients with multiple sclerosis was significantly reduced in the follow-up fMRI study compared with the first fMRI study, which had been performed 15–26 months earlier. No significant longitudinal differences were observed in controls in the same time interval.

Some caution must be used in longitudinal fMRI studies because the blood oxygenation level-dependent (BOLD) contrast, which reflects a complex interaction between blood flow, blood volume and haemoglobin oxygenation (Ogawa *et al.*, 1990; Kwong *et al.*, 1992), may be modified by various physiological and environmental factors (Levin *et al.*, 2001; Laurienti *et al.*, 2003). This could affect the reliability of fMRI data obtained across different sessions. However, a good reproducibility has been demonstrated in multiple cerebral areas specific to the motor system in healthy subjects (Mattay *et al.*, 1996; Yetkin *et al.*, 1996), particularly after a time interval of some months (Loubinoux *et al.*, 2001).

None of our control subjects demonstrated an increase or a decrease in task-related activation between the two fMRI sessions. Conversely, patients with multiple sclerosis exhibited a significantly reduced activity in the ipsilateral sensorimotor cortex and in the contralateral cerebellum.

At follow-up, however, our patients still showed a higher degree of activation in motor areas of both the cerebral hemispheres than controls, confirming our previous data

Table 4 Significant inverse correlations between changes in brain motor activation patterns (fMRI1 > fMRI2) during right-hand movement in 18 patients with multiple sclerosis, together with clinical and conventional MRI data (multiple regression analysis, SPM99, $P < 0.05$ corrected at the cluster level)

Variable	Cluster size	*Z*	Talairach coordinates (*x*, *y*, *z*)	Brodmann area (BA)
Age	118	4.08	51, 4, 37	R BA 6
	298	3.75	63, −23, 32	R BA 1–4
	113	3.73	48, 3, 15	R BA 4/6
New relapse	106	4.38	10, −22, 51	R BA 6
	306	4.20	55, −21, 31	R BA 1–4
	159	3.44	38, −22, 47	R BA 1–4
T1 LL baseline	143	4.40	55, −24, 29	R BA 40
	111	3.61	38, −42, 54	R BA 40
	79	3.03	−36, −16, 60	L BA 1–4
T1 LL changes	567	4.57	55, −23, 31	R BA 40
	400	4.01	55, −18, 36	R BA 1–4

Z = voxel level.

(Pantano *et al.*, 2002*a*,*b*) and findings from other cross-sectional fMRI studies during motor stimulation in patients with multiple sclerosis (Lee *et al.*, 2000; Pantano *et al.*, 2002*a*; Reddy *et al.*, 2000*a*). The role of this increased motor activation and, in particular, that of the ipsilateral motor pathway in patients with multiple sclerosis is not completely clear. Increased ipsilateral activation may have a vicarious function owing to a variety of mechanisms such as bilateral representation of motor function, creation of new connections, release of inhibition and synaptic sprouting.

What is still unknown is the evolution of these functional changes over time in patients with multiple sclerosis. To our knowledge, only one previous report has described longitudinal fMRI changes in a single multiple sclerosis patient studied with serial magnetic resonance spectroscopy (MRS) and fMRI while recovering from an acute relapse of hemiplegia (Reddy *et al.*, 2000*b*). The clinical improvement paralleled the recovery of N-acetylaspartate and progressive reduction in abnormally increased fMRI motor activation.

None of our patients was in an acute phase at the time of each fMRI study. All the patients had recovered from a single clinical event at the time of the first fMRI and few had suffered a new relapse in the time between the two fMRI studies. Only five patients had presented a new clinical event at least 4 months before the second fMRI study. The rate of clinical conversion in our sample of patients with a single clinical event and MR findings suggestive of multiple sclerosis is in agreement with the rate of ~30% in 18 months reported by the Controlled High-Risk Subjects Avonex Multiple Sclerosis Prevention Study (CHAMPS) group (CHAMPS, 2002).

In six patients, we observed the appearance of new T2 lesions during the follow-up period despite the absence of new symptoms. In the remaining seven patients, the disease was in an apparent phase of stability. However, the knowledge that microscopic damage exists even in the so-called 'normal appearing white matter' of patients with multiple sclerosis (Fernando *et al.*, 2004) does not exclude disease progression at an ultrastructural level, as demonstrated with more sophisticated MR techniques (Caramia *et al.*, 2002).

Given the heterogeneity of the sample studied in terms of both clinical manifestations and MRI features, we were interested in assessing which factors could determine the observed pattern of reduced brain motor activity over time.

Multiple regression analysis indicated that age, occurrence of a new relapse, T1 LL at baseline and T1 LL changes between the two fMRI studies had an independent effect upon changes in motor activation patterns. All these factors were inversely correlated with motor areas mostly located in the ipsilateral hemisphere.

Age is known to influence motor recovery after brain damage: experimental and clinical data suggest that younger subjects have a greater ability to recover motor function and are likely to benefit substantially from treatments that facilitate plasticity-mediated recovery (Goldstein and Bullman, 1999; Gwak *et al.*, 2004; Katz *et al.*, 2004; Stein, 2004). Ipsilateral sensorimotor cortex has been found to be more activated in older than in younger healthy subjects during a motor task, suggesting that a recruitment of motor cortical units accompanies aging (Hutchinson *et al.*, 2002).

The greater activation of the ipsilateral motor cortex is one of the most important effects of neuroplasticity in patients with multiple sclerosis (Lee *et al.*, 2000; Pantano *et al.*, 2002*a*; Reddy *et al.*, 2000*a*), although its beneficial effect has never been demonstrated. Younger patients showed a decrease in the extent of ipsilateral activation, regardless of disease burden, indicating a natural tendency towards a more physiological pattern of cortical activation.

The occurrence of a relapse may also contribute to the modulation of cortical plastic changes over time. Interestingly, cortical activation in patients who developed a new clinical episode was substantially the same in both fMRI studies. Conversely, the absence of new clinical events appears to be one of the conditions required for brain motor organization to return to more normal activity levels.

The severity of tissue damage, as evaluated by T1 LL, was also inversely correlated with motor activity changes between the two fMRI studies, i.e. the greater the T1 LL at baseline, the smaller the decrease in motor activated areas in the ipsilateral hemisphere. Moreover, the decrease in ipsilateral motor activation was also inversely correlated with the increase in T1 LL at the second fMRI study.

Hypointense lesions on T1-weighted images represent a marker of severe tissue destruction since they are associated with both extensive demyelination and loss of axons (van Walderveen *et al.*, 1998; van Waesberghe *et al.*, 1999). Axonal transection is a consistent feature of the lesions in multiple sclerosis, which can already be found in the early phase of the disease (Trapp *et al.*, 1998). In our previous work (Pantano *et al.*, 2002*b*), we did not find any significant correlation between total T1 LL and the level of cortical motor activation

in patients with multiple sclerosis. However, T1 LL specifically located along the corticospinal tract correlated positively with activated foci in the ipsilateral premotor cortex. Therefore, the total amount of axonal damage does not seem to influence the initial level of motor activity, but it can influence the subsequent reorganization of the motor network. The tendency of brain motor activity to return towards a more lateralized activation pattern seems to be affected by the amount of axonal damage and by how much this damage increases over time.

The decrease in motor activation in the ipsilateral sensorimotor cortex we observed over time in patients with multiple sclerosis is consistent with results reported in stroke patients. Indeed, longitudinal studies have shown a general trend towards a reduction in motor activation and a focusing on the damaged hemisphere, i.e. in contralateral motor areas after a cerebrovascular lesion (Marshall *et al.*, 2000; Feydy *et al.*, 2002). Moreover, a task-related brain activation decrease over time has been found to correlate with motor recovery (Ward *et al.*, 2003).

Our results suggest that brain functional changes in multiple sclerosis are a dynamic phenomenon dependent on age, clinical course and brain tissue damage. The altered pattern of brain activation in patients with multiple sclerosis may partially be reversed during non-active periods of disease, especially in the ipsilateral hemisphere, focusing on a contralateral activation pattern. Conversely, the persistence of increased motor activation in patients with multiple sclerosis is associated with either clinical or subclinical disease progression.

In this study, we present longitudinal fMRI changes in a cohort of 18 patients with multiple sclerosis observed over a long period of time. Previous studies have demonstrated acute fMRI changes in multiple sclerosis in response to drugs (Parry *et al.*, 2003; Mainero *et al.*, 2004) and motor training (Morgen *et al.*, 2004). Our data may represent a useful reference for further studies aimed at investigating the effects of treatments on cerebral plasticity in multiple sclerosis in the long term.

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References

Calautti C, Baron JC. Functional neuroimaging studies of motor recovery after stroke in adults: a review. Stroke 2003; 34: 1553–66.

Caramia F, Pantano P, Di Legge S, Piattella MC, Lenzi D, Paolillo A, et al. A longitudinal study of MR diffusion changes in normal appearing white matter of patients with early multiple sclerosis. Magn Reson Imaging 2002; 20: 383–8.

Controlled High-Risk Subjects Avonex Multiple Sclerosis Prevention Study (CHAMPS) Group. MRI predictors of early conversion to clinically definite multiple sclerosis in the CHAMPS placebo group. Neurology 2002; 59: 998–1005.

Fazekas F, Offenbacher H, Fuchs S, Schmidt R, Niederkorn K, Horner S, et al. Criteria for an increased specificity of MRI interpretation in elderly subjects with suspected multiple sclerosis. Neurology 1988; 38: 1822–1825.

Fernando KT, McLean MA, Chard DT, MacManus DG, Dalton CM, Miszkiel KA, et al. Elevated white matter myo-inositol in clinically isolated syndromes suggestive of multiple sclerosis. Brain 2004; 127: 1361–9.

Feydy A, Carlier R, Roby-Brami A, Bussel B, Cazalis F, Pierot L, et al. Longitudinal study of motor recovery after stroke: recruitment and focusing of brain activation. Stroke 2002; 33: 1610–7.

Filippi M, Rocca MA, Falini A, Caputo D, Ghezzi A, Colombo B, et al. Correlations between structural CNS damage and functional MRI changes in primary progressive multiple sclerosis. Neuroimage 2002; 15: 537–46.

Friston KJ, Holmes AP, Worsley K, Poline J-B, Frith C, Frackowiak RSJ. Statistical parametric maps in functional imaging: a general linear approach. Hum Brain Mapp 1995; 2: 189–210.

Friston KJ, Jezzard P, Turner R. Analysis of functional MRI time series. Hum Brain Mapp 1994; 1: 153–71.

Goldstein LB, Bullman S. Age but not sex affects motor recovery after unilateral sensorimotor cortex suction-ablation in the rat. Restor Neurol Neurosci 1999; 15: 39–43.

Grimaud J, Lai M, Thorpe J, Adeleine P, Wang L, Barker GJ, et al. Quantification of MRI lesion load in multiple sclerosis: a comparison of three computer-assisted techniques. Magn Reson Imaging 1996; 14: 495–505.

Gwak YS, Hains BC, Johnson KM, Hulsebosch CE. Locomotor recovery and mechanical hyperalgesia following spinal cord injury depend on age at time of injury in rat. Neurosci Lett 2004; 362: 232–5.

Hutchinson S, Kobayashi M, Horkan C, Pascual-Leone A, Alexander MP, Schlaug G. Age-related differences in movement representation. Neuroimage 2002; 17: 1720–8.

Katz DI, White DK, Alexander MP, Klein RB. Recovery of ambulation after traumatic brain injury. Arch Phys Med Rehabil 2004; 85: 865–9.

Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983; 33: 1444–52.

Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, Poncelet BP, et al. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. Proc Natl Acad Sci USA 1992; 89: 5675–9.

Laurienti PJ, Field AS, Burdette JH, Maldjian JA, Yen YF, Moody DM. Relationship between caffeine-induced changes in resting cerebral perfusion and blood oxygenation level-dependent signal. AJNR Am J Neuroradiol 2003; 24: 1607–11.

Lee M, Reddy H, Johansen-Berg H, Pendlebury S, Jenkinson M, Smith S, et al. The motor cortex shows adaptive functional changes to brain injury from multiple sclerosis. Ann Neurol 2000; 47: 606–13.

Levin JM, Frederick Bde B, Ross MH, Fox JF, von Rosenberg HL, Kaufman MJ, et al. Influence of baseline hematocrit and hemodilution on BOLD fMRI activation. Magn Reson Imaging 2001; 19: 1055–62.

Loubinoux I, Carel C, Alary F, Boulanouar K, Viallard G, Manelfe C, et al. Within-session and between-session reproducibility of cerebral sensorimotor activation: a test–retest effect evidenced with functional magnetic resonance imaging. J Cereb Blood Flow Metab 2001; 21: 592–607.

Marshall RS, Perera GM, Lazar RM, Krakauer JW, Constantine RC, DeLaPaz RL. Evolution of cortical activation during recovery from corticospinal tract infarction. Stroke 2000; 31: 656–61.

Mattay VS, Frank JA, Santha AK, Pekar JJ, Duyn JH, McLaughlin AC, et al. Whole-brain functional mapping with isotropic MR imaging. Radiology 1996; 201: 399–404.

McDonald WI, Compston A, Edan G, Goodkin D, Hartung H-P, Lublin FD, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. Ann Neurol 2001; 50: 121–7.

Mainero C, Inghilleri M, Pantano P, Conte A, Lenzi D, Frasca V, et al. Enhanced brain motor activity in patients with multiple sclerosis after a single dose of 3,4-diaminopyridine. Neurology 2004; 62: 2044–2050.

Miller DH, Thompson AJ, Filippi M. Magnetic resonance studies of abnormalities in the normal appearing white matter and grey matter in multiple sclerosis [Review]. J Neurol 2003; 250: 1407–19.

Morgen K, Kadom N, Sawaki L, Tessitore A, Ohayon J, McFarland H, et al. Training-dependent plasticity in patients with multiple sclerosis. Brain 2004; 127: 2506–17.

Ogawa S, Lee TM, Kay AR, Tank DW. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci USA 1990; 87: 9868–72.

Pantano P, Iannetti GD, Caramia F, Mainero C, Di Legge S, Bozzao L, et al. Cortical motor reorganization after a single clinical attack of multiple sclerosis. Brain 2002a; 125: 1607–15.

Pantano P, Mainero C, Iannetti GD, Caramia F, Di Legge S, Piattella MC, et al. Contribution of corticospinal tract damage to cortical motor reorganization after a single clinical attack of multiple sclerosis. Neuroimage 2002b; 17: 1837–43.

Parry AM, Scott RB, Palace J, Smith S, Matthews PM. Potentially adaptive functional changes in cognitive processing for patients with multiple sclerosis and their acute modulation by rivastigmine. Brain 2003; 126: 2750–60.

Reddy H, Narayanan S, Arnoutelis R, Jenkinson M, Antel J, Matthews PM, et al. Evidence for adaptive functional changes in the cerebral cortex with axonal injury from multiple sclerosis. Brain 2000a; 123: 2314–20.

Reddy H, Narayanan S, Matthews PM, Hoge RD, Pike GB, Duquette P, et al. Relating axonal injury to functional recovery in multiple sclerosis. Neurology 2000b; 54: 236–9.

Rocca MA, Mezzapesa DM, Falini A, Ghezzi A, Martinelli V, Scotti G, et al. Evidence for axonal pathology and adaptive cortical reorganization in patients at presentation with clinically isolated syndromes suggestive of multiple sclerosis. Neuroimage 2003; 18: 847–55.

Roux FE, Boulanouar K, Ibarrola D, Tremoulet M, Chollet F, Berry I. Functional MRI and intraoperative brain mapping to evaluate brain plasticity in patients with brain tumours and hemiparesis. J Neurol Neurosurg Psychiatry 2000; 69: 453–63.

Schlosser MJ, McCarthy G, Fulbright RK, Gore JC, Awad IA. Cerebral vascular malformations adjacent to sensorimotor and visual cortex. Functional magnetic resonance imaging studies before and after therapeutic intervention. Stroke 1997; 28: 1130–7.

Smith M, Stone L, Albert Plbert PS, Frank JA, Martin R, Armstrong M, et al. Clinical worsening in multiple sclerosis is associated with increased frequency and area of gadopentetate dimeglumine-enhancing magnetic resonance imaging lesions. Ann Neurol 1993; 33: 480–9.

Stein J. Motor recovery strategies after stroke. Top Stroke Rehabil 2004; 11: 12–22.

Talairach J, Tournoux P. Coplanar stereotaxic atlas of the human brain: Stuttgart: Thieme; 1988.

Trapp BD, Peterson J, Ransohoff R, Rudick RA, Mork S, Bo L. Axonal transection in the lesions of multiple sclerosis. N Engl J Med 1998; 338: 278–85.

van Waesberghe JH, Kamphorst W, De Groot CJ, van Walderveen MA, Castelijns JA, Ravid R, et al. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. Ann Neurol 1999; 46: 747–54.

van Walderveen MA, Kamphorst W, Scheltens P, van Waesberghe JH, Ravid R, Valk J, et al. Histopathologic correlate of hypointense lesions on T1 weighted spin echo MRI in multiple sclerosis. Neurology 1998; 50: 1282–8.

Ward NS, Brown MM, Thompson AJ, Frackowiak RSJ. Neural correlates of motor recovery after stroke: a longitudinal fMRI study. Brain 2003; 126: 2476–96.

Yetkin FZ, McAuliffe TL, Cox R, Haughton VM. Test-retest precision of functional MR in sensory and motor task activation. AJNR Am J Neuroradiol 1996; 17: 95–98.