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Functional response to active and passive ankle movements with clinical correlations in patients with primary progressive multiple sclerosis

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Abstract Patients with multiple sclerosis (MS) activate a more diffuse cortical network than do healthy subjects when they perform motor tasks. This brain functional reorganisation might contribute to the limiting of disability, but it is unclear whether there is a loss of regional activation in more advanced disease. The aim of this study was to assess whether functional reorganisation diminishes in more disabled patients with primary progressive (PP) MS. The differences in the fMRI response to active and passive movements of the dominant ankle of 13 patients and 16 controls were assessed. The relationships between functional activation and disability and brain lesion load and atrophy were investigated.

Patients showed greater fMRI activation than controls with passive movements in the superior temporal gyrus, rolandic operculum, and putamen. The fMRI response to active and passive movements in the ipsilateral inferior frontal gyrus was lower in patients with greater disability and greater brain T2 lesion load, respectively. Furthermore, the fMRI activation with active movements in the contralateral cerebellum was lower in patients with worse mobility.

The increased activity with passive movements in regions that participate in sensori-motor integration, such as the putamen, reflects true functional reorganisation, since passive movements induce brain activation through sensory afferents only. The inverse correlation between the fMRI response in regions that are associated with motor control, and clinical or MRI measures of disease progression, suggests that there is a loss of distributed activation in more disabled patients. This may inform future treatment strategies.

Key words fMRI · multiple sclerosis · brain · disability · passive movements

Introduction

Recent functional magnetic resonance imaging (fMRI) studies have shown that in patients with multiple sclerosis (MS) the pattern of brain activation during motor tasks differs from that of normal controls, in both location and extent [1, 2]. Previous fMRI studies, aiming to assess whether these functional changes contribute to the maintenance of clinical function, reported increased

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functional activation with increasing brain pathology measured by MRI [3–8]. Since patients performed the motor task with clinically unimpaired limbs, the authors suggested that cortical reorganisation following MS tissue damage has an adaptive role. However, it is unclear whether this functional reorganisation diminishes in patients with greater disability. Primary progressive (PP) MS [9] presents an opportunity to investigate brain functional changes in the progressive phase of the disease and in patients with disability, without the confounding effect of clinical attacks and high MRI activity [10, 11].

A common confounding factor in the interpretation of the fMRI studies in patients with disability is that the motor task may not be matched for performance between patients and controls, which could contribute to differences in fMRI activation between the groups. A possible solution is to employ passive movements, which activate the motor cortex primarily via sensory afferents and do not depend on voluntary planning or on ability to execute the task. A previous fMRI study in patients with relapsing-remitting MS used passive digit movements and reported activation changes that were felt to reflect true functional reorganisation of the motor pathways [12].

Previous studies mainly employed active movement of the hand, which has greater spatial representation in the motor cortex than the foot [13]. The fMRI response to ankle movements in patients with MS has not been widely investigated, although movement at the ankle joint is critical for walking, and can be used to assess motor control of walking during rehabilitation [14].

Thus, we employed both active and passive movements of the dominant (right) ankle in patients with PP MS to assess functional reorganisation and to investigate whether patients with greater disability and greater brain structural damage have a smaller functional brain activity in the motor network, suggesting that adaptive functional mechanisms diminish with more advanced disease.

Methods

Subjects

We recruited 14 patients with primary progressive MS (PP MS) [9] (mean age 46.6, SD 11.3, 9 men and 5 women), who attended the National Hospital for Neurology and Neurosurgery in London. All patients underwent conventional neurological assessment, and were scored on the EDSS [15], and 25foot Timed Walk Test (TWT) [16]. Patients with PP MS affected by spinal cord syndrome, with lower limb spasticity ≤3 on the Ashworth scale [17], and normal or slight proprioceptive deficit, were recruited. One patient could not complete the fMRI experiment and was then excluded from the analysis, leaving a total of 13 patients (mean age 46, SD 11.5, 9 men and 4 women).

Eighteen healthy controls (mean age 36.22, SD 11.7, 12 men and 6 women) were also recruited, but two of them were excluded from the analysis because their scans showed movement artefacts (to be discussed later). Thus, 16 controls (mean age 37.3, SD 11.9, 10 men and 6 women) were studied.

All patients and controls were right-foot dominant (the dominance was established on the basis of the answer to the question: "Which foot do you prefer to kick with?" – from the Edinburgh Handedness Inventory scale [18]. All subjects gave informed, written consent before the study, which was approved by the local research ethics committee.

Motor paradigm

The motor paradigm was a pseudo-random interleaved block design of 20 second periods of active and passive movement of the right (dominant) ankle. Each active and each passive period was followed by 20 seconds of rest. The motor task was dorsi-plantar flexion of the foot. Subjects were studied supine with their knees flexed and their thighs supported. Both feet were strapped into a custom-made wooden manipulandum with the rotation axes aligned to the ankle joints, and the right foot was made free to move, while the left foot was maintained in the resting position. The active task was auditory-cued by a metronome at 1.25Hz. This rate cued the movement from dorsi to plantar-flexion (or vice versa), and was also followed by the operator (O. C.), who elicited the passive movements. The operator visually monitored the execution of the active task to ensure accurate performance throughout both sessions. Subjects were instructed to relax as much as possible during the rest and passive periods, without mentally rehearsing the movement. To avoid activation in the visual cortex, subjects were told to keep their eyes closed throughout.

Assessment of muscular activity

All subjects underwent a training session prior to the fMRI that involved simultaneous recording of surface EMG from tibialis anterior (for dorsi-flexion) and soleus (for plantar-flexion) of the right (dominant) limbs. Subjects were positioned in the same way as during the subsequent fMRI experiment, and their feet were strapped into an identical custom-made wooden manipulandum. First, they performed a maximal voluntary contraction of tibialis anterior and soleus for five seconds. Subjects then performed unilateral active and passive ankle movements for two periods of 20 seconds each. Movements were paced by a metronome at 1.25Hz.

The EMG was sampled at 2 KHz, filtered (30–1000 Hz) and amplified x 2000 (D360 Digitimer Ltd Welwyn Garden City, UK). Data were AD converted (1410-plus CED Cambridge, UK) and recorded using the SPIKE programme (Version 4 CED Cambridge, UK).

All subjects were able to relax completely during passive movements. The EMG analysis was performed for active and passive movements. Firstly, the maximum EMG signal was obtained by averaging the signal from a 1 second period of maximum contraction. Following this, the average EMG signal, recorded over 10 dorsi- and plantar-flexion cycles was calculated and then expressed as a percentage of the maximum EMG signal (EMG-percentage).

Ankle joint angle measurements

The amplitude of the right foot dorsi- and plantar-flexion during active movements was marked during the training session, after subjects were asked to move the foot in the most comfortable way. The same angle was maintained by the operator during passive movements. Although each subject was told to maintain the same angle during the fMRI experiment, the ankle joint angle was re-measured during the first active movement, and this new angle was maintained by the operator throughout the subsequent passive periods.

To assess differences in movement amplitude between patients and controls, a Mann-Whitney U test was used. Furthermore, the ankle joint angle was taken into account (as a covariate) in the following

analysis, as a possible factor contributing to the differences in the fMRI response between the two groups.

fMRI protocol and analysis

All imaging was performed on a 1.5 T Signa Echospeed MRI system (GE Medical System, Milwaukee, Wisconsin, USA). The functional protocol consisted of 27 interleaved ascending slices covering the whole brain using a single shot gradient echo EPI [slice thickness 4.6 mm, slice gap 0.4 mm, in-plane resolution 3x3 mm, TR 4 seconds, matrix 128x64, 220 scans acquired over 14 min and 40 s].

The analysis was performed using Statistical Parametric Mapping (SPM99; Wellcome Department of Imaging Neuroscience, London, UK) [19, 20]. The images were realigned to the first image of each experiment in order to correct for inter-scan movement. Realignment parameters were checked in all subjects, and it was noted that in two controls movement was greater than 2 mm in at least one direction; these two subjects were therefore excluded from the analysis. For each realigned subject's time series, a mean image was obtained and reoriented. The reorientation parameters were then applied to all the images. The images were normalized into a standardized space for the remaining 16 controls and 13 patients (as defined by the Montreal Neurological Institute (MNI)) and then smoothed using a full width half maximum Gaussian filter of 8 mm. By the central limit theorem, smoothing the data renders the errors more normal in their distribution, thereby validating the use of standard parametric statistics. Smoothing also helps to compensate for inter-subjects neuroanatomical variability. A fixed effects approach was adopted, where the individual smoothed images were entered into a design matrix, in which the realignment parameters were included as covariates of no interest. The contrasts of parameter estimates for active and passive were then generated.

The difference in age between patients and controls was not significant ($p = 0.07$, two sample t test). A preliminary analysis assessed the age effects on the fMRI response. Separate linear regression models were specified for patients and controls with age entered as the covariate of interest. There was no significant effect for age on the fMRI response ($p < 0.05$ corrected across the image volume). We consequently omitted age from subsequent fMRI statistical modelling.

Two types of analyses were performed for active and passive movements. Firstly, to investigate the behaviour of the fMRI response in patients and controls, one-sample t tests were performed for each contrast in each group using a random-effects approach [21, 22]. Statistical SPM(T) images were thresholded at $p < 0.05$ corrected at voxel level for multiple comparisons across the whole brain to determine the significant main effects. Secondly, to investigate differences in the fMRI response between patients and controls, a conditions and covariates model was employed, entering the subject specific contrasts; patient and control groups were considered as conditions and ankle joint angle as covariate. Active and passive movements were assessed with separate models. For each model, two contrasts, e. g. patients greater than controls and controls greater than patients, were estimated. Statistical SPM(T) images were thresholded at $p < 0.05$ corrected at voxel level for multiple comparisons within volumes of interest (VOIs) which were chosen a priori [22]. These VOIs were created as spheres or boxes and centered on brain structures which are known to be part of the motor network and were reported in previous publications investigating ankle movements in PPMS patients or healthy controls [23, 24], using MRIcro as reference [25] (Table 1).

Table 1 Volumes of interest (VOIs) defined a priori for the SPM99 analysis

Regions	Shape and radius (r) or dimensions (d) in mm	x, y, z of the centre
* Paracentral lobule (SI-MI or B. areas 3, 1, 2 and 4)	Sphere, r = 15	10, −32, 68 (R) −10, 32, 68 (L)
* Postcentral gyrus (SII or B. areas 5 and 7)	Sphere, r = 12	20, −36, 72 (R) −20, −36, 72 (L)
* Supplementary motor area (SMA)	Sphere, r = 15	8, −6, 64 (R) −8, −6, 64 (L)
* Premotor cortex	Sphere, r = 15	20, −8, 64 (R) −20, −8, 64 (L)
Sup. frontal gyrus (ant. part) (B. area 10)	Sphere, r = 12	28, 60, 13 (R) −28, 60, 13 (L)
Middle frontal gyrus	Sphere, r = 12	37, 34, 40 (R) −37, 34, 40 (L)
* Inf. frontal gyrus	Sphere, r = 12	46, 31, 12 (R) −46, 31, 12 (L)
Rolandic operculum	Sphere, r = 12	55, 9, 4 (R) −55, 9, 4 (L)
Middle cingulate cortex (B. area 24)	Sphere, r = 12	7, −12, 44 (R) −7, −12, 44 (L)
Inf. parietal gyrus (B. area 40)	Sphere, r = 12	48, −24, 20 (R) −48, −24, 20 (L)
Sup. temporal gyrus	Sphere, r = 10	54, 15, −12 (R) −54, 15, −12 (L)
Precuneus	Sphere, r = 15	8, −54, 64 (R) −8, 54, 64 (L)
Insula	Sphere, r = 10	44, 12, −2 (R) −44, 12, −2 (L)
Thalamus	Sphere, r = 12	13, −17, 7 (R) −13, −17, 7 (L)
Post. putamen	Sphere, r = 12	30, 0, 9 (R) −30, 0, 9 (L)
Caudate	Sphere, r = 12	14, 13, 13 (R) −14, 13, 13 (L)
* Cerebellum	Sphere, r = 15	25, −48, −37 (R) −25, −48, −37 (L)
Sup. vermis	Box, d = 15x25x20	0, −55, −16

* Volumes of interest (VOIs) which were also used to test for correlations between the BOLD response and the clinical or MRI measures
R Right; *L* Left; *B* Brodmann; *Inf.* Inferior; *Sup.* Superior

Structural MRI protocol and analysis

Brain lesion load

All subjects had axial brain dual echo fast spin echo (FSE) imaging acquired prior to the fMRI experiment, providing proton density (PD) and T2-weighted images [TR 2000ms, TE 30/120ms, field of view (FOV) 24x24 cm, matrix 256x256, 28 contiguous axial slices, 5 mm slice thickness]. These images were displayed on a Sun workstation (Sun Mycrosystems, Mountain View, CA using the DispImage software package). Lesion loads were calculated by a single observer (O. C.) (who was blinded to the clinical details) on the PD images (with reference to the T2 images) using a semiautomated local contour thresholding technique [26].

Brain atrophy

Patients underwent also a 3D inversion-prepared fast spoiled gradient recall (FSPGR) sequence of the brain [TR 13.3ms, TE 4.2ms, FOV 300x225, matrix 256x160 reconstructed as 256x256 for a final in plane

Table 2 Additional patients' clinical, radiological and physiological data

Patients	CSF findings	EDSS	Pyramidal FSs	Bowel/ Bladder FSs	Cerebral FSs	Current medications	Number of spinal cord lesions	EMG-% of active m. (TA/Sol.)	EMG-% of passive m. (TA/Sol.)	Ankle joint angle during training session	Ankle joint angle during fMRI
1	OCB + ve	6.0	3	2	0	Tolterodine	2	2.7/8.8	0.3/1.9	15	10
2	OCB – ve	4.0	3	1	0	Tolterodine, Desmopressin	Diffuse signal change	9.2/7.1	2.2/1.7	33	33
3	OCB + ve	6.5	4	2	0	Oxybutynin	Diffuse signal change	8.0/12.1	0.7/9.0	25	50
4	OCB + ve	3.5	1	0	0	None	1	25.6/22.0	0.4/1.3	32	23
5	OCB + ve	6.0	3	2	0	Baclofen, Oxybutynin	6	11.8/19.3	0.4/4.2	18	22
6	OCB + ve	5.0	3	2	1	None	2	7.4/2.9	0.4/0.7	21	12
7	OCB + ve	3.5	2	0	0	None	6	10.2/5.1	0.8/0.9	22	20
8	OCB + ve	5.5	3	1	0	None	6	29.5/32.9	0.9/9.4	24	24
9	OCB + ve	4.0	3	2	0	Tolterodine	5	23.1/61.7	3.0/6.7	30	35
10	OCB + ve	3.0	2	1	0	None	3	34.9/58.7	0.5/2.6	25	25
11	OCB + ve	4.0	3	3	0	Baclofen, Desmopressin	6	22.7/61.5	1.3/6.1	20	33
12	OCB + ve	3.0	2	0	0	None	3	16.6/15.3	0.6/1.9	35	11
13	OCB + ve	6.0	3	0	0	None	1	27.2/25.4	2.6/5.8	35	35

OCB Oligoclonal bands; *FSs* functional system score; *M* movement; *TA* tibialis anterior; *Sol.* soleus
Note: The bowel/bladder functional system and the cerebral functional scores are derived by the EDSS. The spinal cord lesions were identified on the printed FSE images of the whole spinal cord by an expert radiologist at the time of the fMRI scanning [TR 2500ms, TE 45/90ms, echo train length 16, FOV 48x48, matrix 512x512, 9 contiguous sagittal slices, 3 mm slice thickness].

resolution of 1.17 mm, 124 contiguous axial slices, 1.5 mm slice thickness]. Images were segmented into white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF) using SPM99 accordingly to a protocol previously described [27]. Images were displayed on a Sun workstation and T1 hypointense lesions were outlined using a semi-automated local thresholding contour technique [26] (with reference to T2 images) and blindly to clinical data. Using in-house software, the lesion contours derived from SPGR were then subtracted from WM, GM and CSF masks, producing four mutually exclusive masks (i. e. WM, GM, CSF, and lesions) with their associated volumes in ml. Volumes were estimated with a caudal cut-off point which was defined as the most cranial slice of the cord not containing cerebellum. Brain parenchymal fraction (BPF) was calculated as WM + GM + lesion mask volumes divided by the total intracranial volume, which was defined as WM + GM + CSF + lesion mask volumes. Although the impact of lesions masks on brain tissue fraction is known to be small (lesion mask volume calculated on the SPGR images in our cohort was of 0.6 % of total intracranial volume), considering all lesions within WM further reduces this miscalculation (all lesions outlined on the FSPGR were indeed in the WM).

Correlations between all the above mentioned structural MRI measures and EDSS were investigated using Spearman's correlation coefficient. Only significant correlations ($p < 0.05$) are reported.

Correlation between fMRI and clinical and structural MRI measures

In order to investigate whether the fMRI signal was associated with any structural MRI or clinical measure (EDSS, TWT, T2 lesion load or BPF), a linear regression analysis using SPM99 was performed separately for each measure. Both negative and positive correlations between the fMRI response to active and passive movements and the clinical or MRI measure were separately investigated. Statistical inferences were performed by correcting at voxel level for multiple comparisons within the volumes of interest (VOIs) chosen a priori [22], based on previous fMRI studies that reported significant correlations between the BOLD response to a motor task and clinical or MRI measures in patients with MS [6, 8, 12, 24, 28]. Seven VOIs for each hemisphere were selected (see Table 1). The p value used for multiple comparisons' correction at voxel level within each VOI was < 0.01. For the most significant suprathresholded voxel within the VOI, the value of R-squared and the plot of parameter estimates against the variable of interest were obtained using SPSS 11.5 for Windows.

Results

Clinical, radiological and physiological findings

Patients' characteristics are reported in Tables 2 and 3. There was a correlation between EDSS and brain T2 lesion load ($r = 0.57$, $p = 0.04$).

There was no significant difference in the EMG activity and ankle joint angle between patients and controls,

Table 3 Patients' characteristics

Mean disease duration (y.)	8.69 (SD 7.49)
Median EDSS	4.0 (range 3–6.5)
Mean TWT	7.42 sec. (SD 3.44)
Mean brain T2 lesion load	10.7 ml (SD 11.18)
Mean parenchymal fraction	0.80 (SD 0.03)

although patients had a smaller mean movement amplitude than controls (Table 4).

FMRI differences between PPMS patients and controls

Patients and controls activated several brain regions during active and passive movements, confirming that the motor task employed in this study was valid (Table 5 and Fig. 1). Statistical comparisons of patients and controls revealed that patients activated more than controls during both active and passive movements in several areas, including motor control regions and polymodal sensory regions (regions are listed in Table 6 and a few examples are shown in Fig. 2). No regions were identified in which healthy controls showed greater activation than patients.

Correlation between fMRI response and clinical or MRI measures of disease progression

Negative correlations were observed between brain activation and clinical or MRI measures (Fig. 3). In particular, the fMRI activation associated with active movements in the ipsilateral inferior frontal gyrus correlated inversely with EDSS (Rsq = 0.80, p = 0.001), and that in the contralateral cerebellum correlated inversely with TWT (Rsq = 0.84, p = 0.004). The fMRI response to passive movements in the ipsilateral inferior frontal gyrus correlated inversely with T2 lesion load (Rsq = 0.82, p = 0.006), in line with the significant correlation between EDSS and fMRI activity with active movements in the same region. No positive relationship was found between the fMRI response and clinical and MRI measures.

Table 4 Physiological data in patients and controls

	Controls		Patients	
	Tibialis ant. mean (SD)	Soleus mean (SD)	Tibialis ant. mean (SD)	Soleus mean (SD)
EMG-% of active movements during the training session mean (SD)	12.3 (7.5)	17 (11.7)	17.6 (10.1)	25.6 (21.7)
EMG-% of passive movements during the training session mean (SD)	0.8 (0.9)	2.6 (3.3)	1.1 (0.9)	4.02 (3.1)
Ankle joint angle during the training session mean (SD)	29.3° (12.2)		25.8° (6.65)	
Ankle joint angle during the fMRI session mean (SD)	28.9° (12.9)		25.6° (11.5)	

Table 5 Brain regions activated by active and passive movements of the right (dominant) foot in patients and controls

	Controls			Patients		
	x, y, z (mm)	Z	P	x, y, z (mm)	Z	P
Active						
Left paracentral lobule (SI-MI)	–6, –10, 68	5.48	0.004	–6, –28, 70	5.52	0.003
Left postcentral gyrus (SII)	–10, –40, 64	5.08	0.038			
Right SMA	8, 2, 56	5.44	0.005			
Left thalamus	–16, –16, 10	5.26	0.014			
Right cerebellum	18, –34, –30	5.52	0.003	14, –36, –30	4.98	0.05
Vermis	2, –48, –8	5.46	0.005			
Passive						
Left paracentral lobule (SI-MI)	–4, –20, 64	5.25	0.015	–2, –34, 60	5.35	0.009
Left postcentral gyrus (SII)	–8, –36, 62	5.80	0.001			

Coordinates are given in mm in MNI space. *P* values are corrected at $p < 0.05$ at voxel level for multiple comparisons across the whole brain

MI primary motor cortex; *SI and SII* primary and secondary somatosensory cortices; *SMA* supplementary

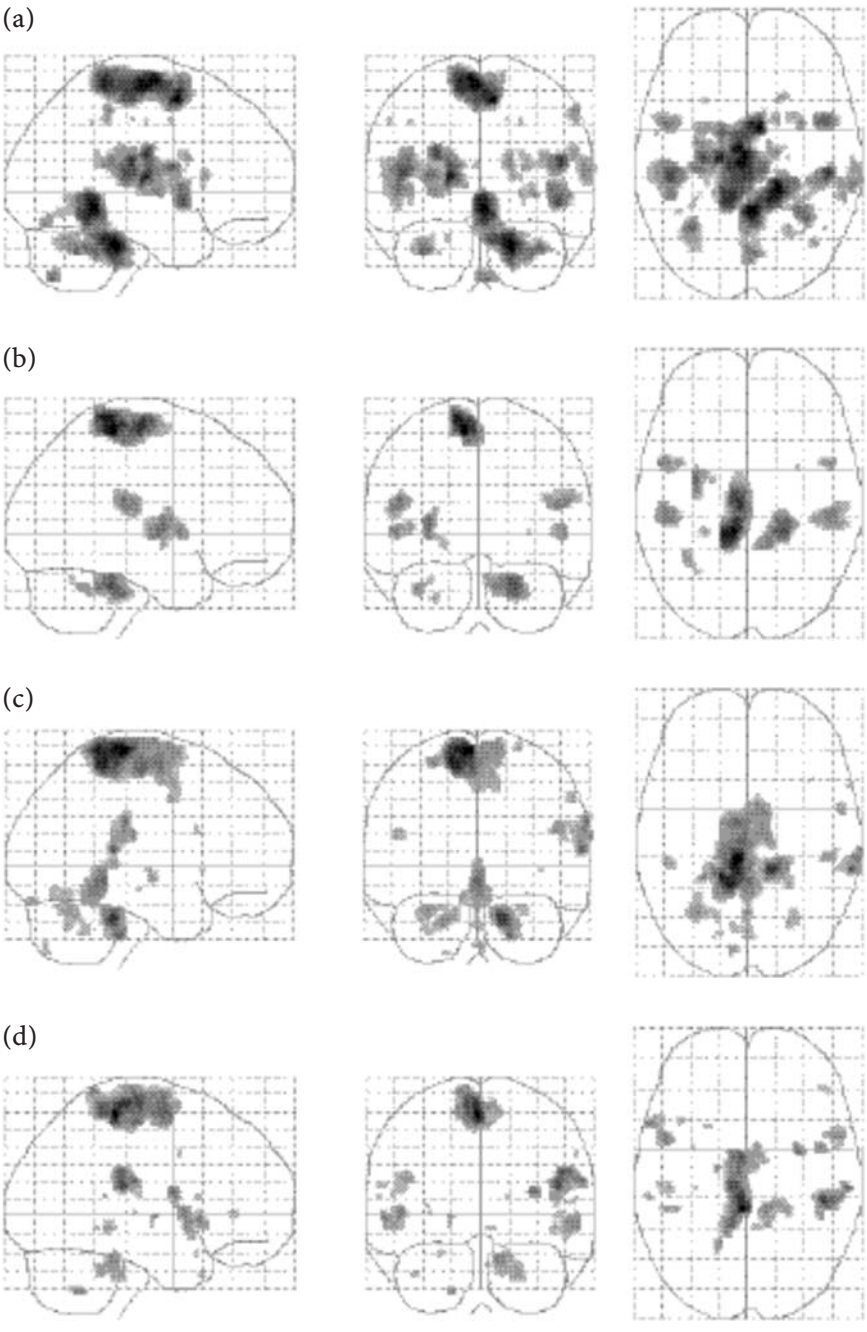


Fig. 1 FMRI response to active movements in controls (**a**) and patients (**c**) and to passive movements in controls (**b**) and patients (**d**). Results are displayed onto a glass brain. The activated voxels have been thresholded at $p < 0.0001$ (uncorrected) for display purposes. Brain regions that showed significant activation after correction for multiple comparisons across the whole brain ($p < 0.05$) are reported in Table 5

Discussion

FMRI differences between PPMS patients and controls associated with active and passive movements

The present study identified brain functional reorganisation associated with ankle dorsi-plantar flexion in patients with PPMS, using active and passive ankle movements. The advantage of using passive movements is that they obviate the need to control for possible task performance differences between groups.

As in previous fMRI studies in MS investigating hand movements [4, 5, 8, 12, 24, 29], we found significant differences in the fMRI response to active movements between patients and controls. Patients significantly activated more than controls in a highly distributed pattern, which included regions which activate when healthy controls perform complex or novel tasks, such as the ip-

Table 6 Regions that showed greater activation in patients than in controls during active and passive movements

	Active			Passive	
	x, y, z (mm)	P (Z score)		x, y, z (mm)	P (Z score)
Right postcentral g. (SII)	26, –46, 72	0.003 (4.14)			
Right sup.frontal g.	28, 64, 12	0.016 (3.61)			
			Left rolandic op.	–56, 16, 0	0.047 (3.32)
Right cingulate c.	10, –6, 50	0.022 (3.49)			
Left sup.temporal g.	–56, 6, –14	0.03 (3.20)	Left sup.temporal g.	–46, 10, –12	0.008 (3.76)
Left precuneus	–12, –50, 72	0.032 (3.58)			
Left cerebellum	–28, –54, –40	0.02 (3.73)			
	–20, –38, –32	0.027 (3.63)			
			Right putamen	32, 0, 14	0.012 (3.80)

Coordinates are given in mm in MNI space. P values are corrected at voxel level for multiple comparisons within the VOIs. Only regions with $p < 0.05$ are listed

SII secondary somatosensory cortex; *g.* gyrus; *op.* operculum; *c* cortex; *sup.* superior

silateral postcentral gyrus, ipsilateral superior frontal gyrus, contralateral cerebellum, and ipsilateral cingulate cortex [23, 30]. Patients also showed greater activation in polymodal sensory areas, such as the contralateral precuneus and contralateral superior temporal gyrus.

Significant differences in the fMRI response between patients and controls were also detected during passive movements, demonstrating that the functional reorganisation occurring in patients was independent of volitional planning and the ability to execute the task. This true functional reorganisation involved the contralateral superior temporal gyrus, contralateral rolandic operculum and ipsilateral putamen. The contralateral superior temporal gyrus has been shown in animal studies to be part of the sensory associative network, and to have widespread corticostriatal projections [31]; in patients with PP MS, it has consistently shown greater activation during active movements of the right impaired foot [24] or unimpaired hand [8]. The rolandic operculum is known as a premotor region, and has been found to participate in the volitional hand motor network [32]. Activation of the putamen has been previously demonstrated mainly during active movements in the side contralateral to the limb [33, 34]. Therefore, the increased fMRI response to passive movements found in patients with PP MS might reflect the contribution of these premotor and subcortical structures in processing sensory information for foot movements [35, 36].

A methodological comment for these results pertains to the observation that some brain regions activated more in patients than controls, when compared using two sample t-tests (Table 6), but did not activate significantly either in patients or in controls with the one sample t-test (Table 5). This has probably occurred because we corrected at the voxel level for multiple comparisons across the whole brain for the one- sample t-tests, whereas two sample comparisons between patients and controls were performed with correction for multiple comparisons within volumes of interest (VOIs) applying, therefore, a smaller number of independent tests.

Functional mechanisms diminish in more disabled patients with PPMS

We found few negative correlations between the BOLD signal and measures of MRI and clinical disease progression. In particular, the fMRI response in the inferior frontal gyrus and cerebellum was lower in patients with higher EDSS and worse mobility and/or greater brain T2 lesion load. Both these brain regions contribute to motor control, and these findings suggest that their activation is smaller as MS progresses and disability increases. In particular, the inferior frontal gyrus is associated with the planning and control of motor tasks, and has reciprocal connections with other premotor and cortical areas [37]. We interpret the inferior frontal gyrus association with caution because this region did not show greater activation in the patient group compared with controls. Nevertheless, we feel it may still be of relevance because it has been extensively reported in studies of cortical reorganisation for MS patients performing motor tasks [6, 30, 38, 39].

A recent study, investigating the functional connectivity of the motor cortex, found increased regional cerebral blood flow in the contralateral cerebellum with low frequency transcranial magnetic stimulation of the primary motor cortex, consistent with potential facilitatory connections between the motor cortex and the contralateral cerebellum [40].

Our data cannot discern whether this loss of brain function occurs within the inferior frontal gyrus and cerebellum, or in brain areas projecting to these regions. Moreover, a reduced activation in these regions may also be induced by pathological changes affecting the connections between brain regions. For example, the re-

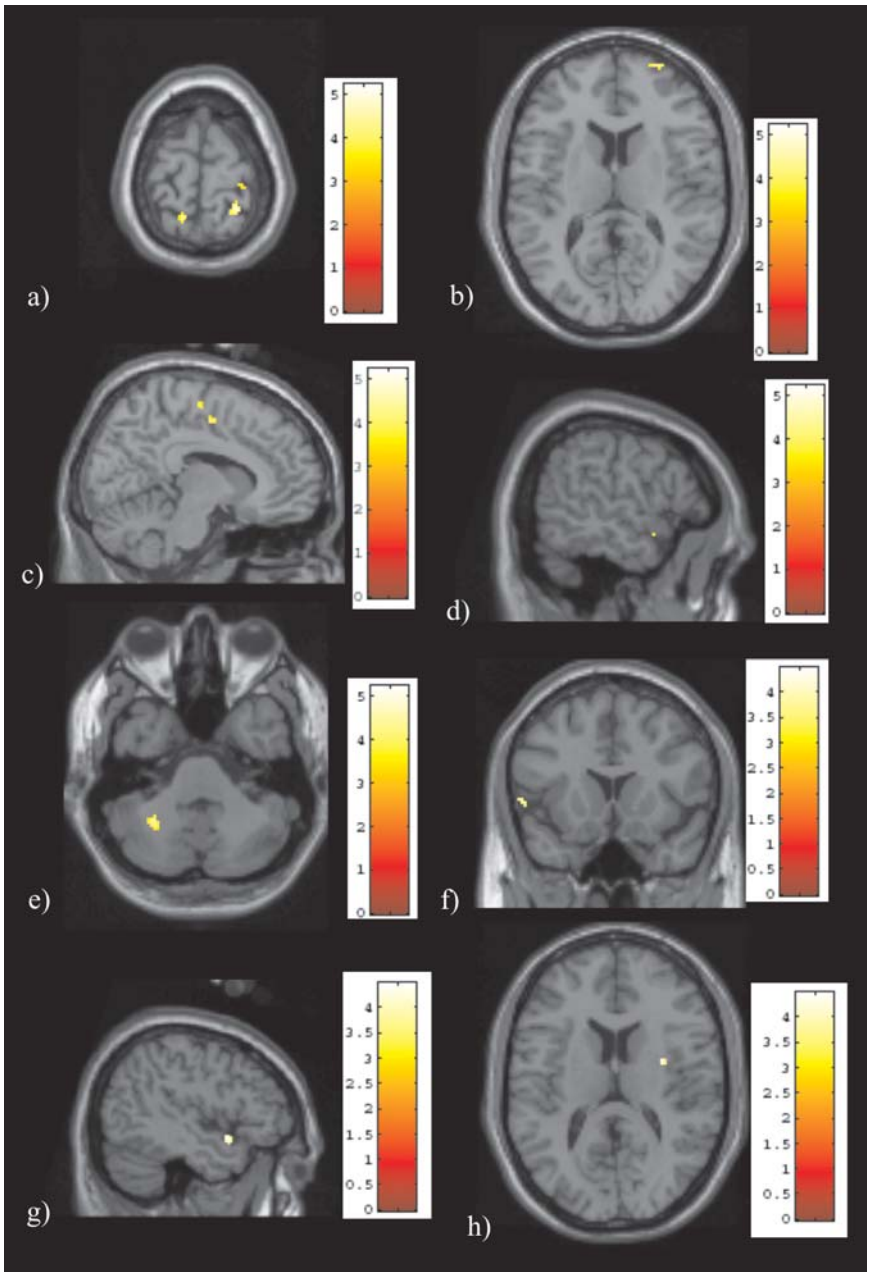
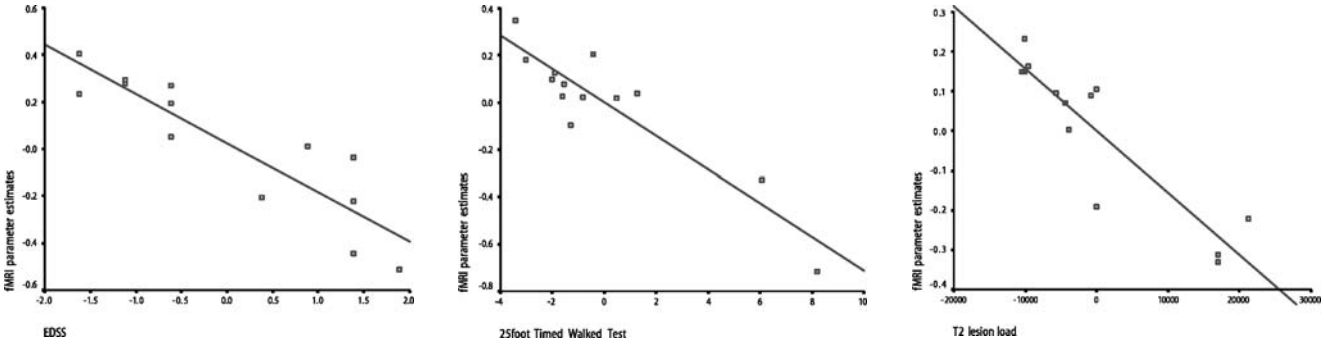


Fig. 2 *Regions that activated more in patients than controls during active movements:* (**a**) the right postcentral gyrus and left precuneus (MNI coordinates x = 26, y = –46, z = 72); (**b**) the right superior frontal gyrus (x = 28, y = 64, z = 12); (**c**) the right cingulate cortex (x = 10, y = –6, z = 50); on this slice it is also possible to see the right SMA, which showed a greater activation in patients with a trend only (p = 0.068, Z score 3.31); (**d**) the left superior temporal gyrus (x = –56, y = 6, z = –14); (**e**) the left cerebellum (x = –28, y = –54, z = –40). *Regions that activate more in patients than controls during passive movements:* (**f**) the left rolandic operculum (x = –56, y = 16, z = 6); (**g**) the left superior temporal gyrus (x = –46, y = 10, z = –12); (**h**) the right putamen (x = 32, y = 0, z = 14). Results are overlaid onto T1-weighted template and corrected for multiple comparisons. The colour scale indicates the T score

duced activation in the inferior frontal gyrus in patients with greater disability may reflect the impaired cortico-cortical and cortico-subcortical connections. This hypothesis is supported by the inverse relationship between the fMRI activity in the inferior frontal gyrus and the T2 lesion load, mostly affecting the cerebral white matter.

A recent longitudinal study in patients with relapsing-remitting MS found that measures of disease progression during 1-year follow-up, such as the occurrence of a new relapse and increased T1 lesion load, were greater in patients who showed a smaller decrease of brain activation in the ipsilateral motor areas, suggesting that the persistence of increased motor activation is associated with disease progression [41]. On the other hand, recent evidence suggests that in MS patients, the lack or the progressive exhaustion of the "classical" adaptive mechanisms, which involve activation of the primary sensori-motor cortex, and the need of "second order" compensatory areas, such as the fronto-parietal regions, might be among the factors that contribute to the accumulation of irreversible clinical deficits [42]. Although these findings can appear to disagree with one another, and with our results of reduced activation in re-



(a) (b) (c)

Fig. 3 Plots of the significant correlations between fMRI responses and clinical or structural MRI measures. (**a**) Plot of the parameter estimates for the most significant voxel in the right inferior frontal gyrus (MNI coordinates x = 42, y = 30, z = 12) during active movements against EDSS (Rsq = 0.80, p = 0.01). (**b**) Plot of the parameter estimates for the most significant voxel in the left cerebellum (x = −34, y = −42, z = −46) during active movements against TWT (Rsq = 0.84, p = 0.04). (**c**) Plot of the parameter estimates for the most significant voxel in the right inferior frontal gyrus (x = 42, y = 30, z = 22) during passive movements against T2 lesion load (Rsq = 0.82, p = 0.06). In all plots, x and y are mean corrected

gions outside the primary sensori-motor cortex in patients with greater disability, it is difficult to compare studies when different types of patients are recruited, and different fMRI paradigms and study designs are employed.

Taking together all these results, it is clear that functional changes constitute a dynamic phenomenon, and might vary according to accumulation of disability and different stages of MS [43]. Future longitudinal studies will address whether there is a tendency towards a "normalization" of brain activation in PP MS patients with accumulation of disability.

In conclusion, longitudinal structural and fMRI studies, which employ passive movements, are needed to understand the complex dynamics of functional brain reorganisation and to elucidate whether a loss of brain function might occur in brain regions as a primary abnormality within the grey matter, or as a consequence of damage to the white matter connections. Future work, which will include several clinical and radiological variables, such as measures of cognitive impairment and spinal cord damage, might use a multiple regression analysis in order to determine which clinical or radiological variable is associated with the fMRI response. A fuller understanding of the role of cortical reorganisation, and its inter-action with the underlying pathology, should help to inform therapeutic strategies that aim to sustain the activity of these brain regions during the execution of movements which are critical for walking.

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