Experimental Neurology 217 (2009) 252–257

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Investigation of the neural correlates underlying action observation in multiple sclerosis patients

Andrea C. Pierno [a], Luca Turella [a], Paola Grossi [b], Federico Tubaldi [a], Massimiliano Calabrese [b], Paola Perini [b], Luigi Barachino [c], Aldo Morra [c], Paolo Gallo [b], Umberto Castiello [a,*]

[a] Department of General Psychology, University of Padova, Via Venezia 8, 35131, Padova, Italy
[b] Department of Neuroscience, University of Padova, Padova, Italy
[c] Euganea Medica, Albignasego, Padova, Italy

ARTICLE INFO

Article history:
Received 17 December 2008
Revised 31 January 2009
Accepted 19 February 2009
Available online 11 March 2009

ABSTRACT

Recent fMRI evidence indicates that both the execution and the observation of hand actions in multiple sclerosis (MS) patients increase recruitment of a portion of the so-called mirror neuron system. However, it remains unclear whether this is the expression of a compensatory mechanism for the coding of observed action or whether such a mechanism represents a rather unspecific functional adaptation process. Here we used fMRI on early relapsing remitting MS (RRMS) patients to clarify this issue. Functional images of 15 right-handed early RRMS patients and of 15 sex- and age-matched right-handed healthy controls were acquired using a 1.5 T scanner. During scanning, participants simply observed images depicting a human hand either grasping an object or resting alongside an object. As shown by a between-group analysis, when compared to controls, RRMS patients revealed a robust increase of activation in an extensive network of brain regions including frontal, parietal, temporal and visual areas usually activated during action observation. However, this pattern of hemodynamic activity was completely independent of the type of observed hand–object interaction as revealed by the lack of any significant between-group interaction. Our findings are in line with previous fMRI evidence demonstrating cortical reorganization in MS patients during action observation. However, based on our findings we go one step further and suggest that such functional cortical changes may be the expression of a generalized and unspecific compensatory mechanism, that is not necessarily involved in action understanding.

Introduction

Research showing that grey matter demyelination starts early and can be extensive in MS (Geurts and Barkhof, 2008; Pirko et al., 2007), stimulated the use of functional magnetic resonance imaging (fMRI) to investigate this disease. For instance, a wealth of studies conducted on MS patients indicated that, compared to healthy controls, the execution of motor tasks markedly increased recruitment not only of motor and sensory regions but also of some frontal and parietal areas usually associated with grasping and manipulating objects (Lee et al., 2000; Filippi et al., 2002, 2004; Reddy et al., 2000; Rocca et al., 2002, 2005; Pantano et al., 2005). This altered pattern of activation has been postulated to be an expression of a functional cortical reorganization following MS-related injuries and to maintain an apparently normal level of functioning.

Building upon the discovery in both non-human and human primates of a particular class of frontal and parietal neurons activated during execution and the observation of a given action (the so-called mirror neurons; e.g. Decety and Grezes, 1999; Rizzolatti et al., 2001; Rizzolatti and Craighero, 2004), a recent study tested the intriguing hypothesis that the over-activation mechanism reported in MS patients during action execution tasks may extend to action observation and action understanding situations (Rocca et al., 2008). Results from this study show that, compared to healthy controls, the observation of a video clip representing flexion–extension movements of the last four fingers of a human hand elicited in MS patients an increased activation of a number of areas known to be involved during hand action observation such as the inferior frontal gyrus and the superior temporal sulcus (Buccino et al., 2001; Gazzola et al., 2007). Although the findings from this study are of great interest for the understanding of the cortical plasticity mechanism operating in MS, an important issue remains unsolved. Specifically, it is still unclear whether the reported increased cortical activation in action observation-related brain areas is the result of a specific deficit in understanding the observed actions or it reflects a rather unspecific and generalized response of the human brain to structural damage of the central nervous system.

The aim of the present study was to address this issue by comparing patterns of hemodynamic activity measured in early RRMS patients and in normal controls during the observation of a human hand either

* Corresponding author. Fax: +39 049 8276600.
E-mail address: umberto.castiello@unipd.it (U. Castiello).

0014-4886/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2009.02.016

interacting with an object or simply resting close to an object. To do so, we conducted a whole-brain fMRI experiment in which subjects were scanned while they observed two types of display depicting a human hand either grasping an object (hand grasping condition), or resting alongside an object (hand resting condition).

Two main predictions were proposed. If the increased cortical activation in action observation areas (if any) is confined to situations involving the viewing of grasping actions, then, such over-activation mechanism can be ascribed to the need to recruit more neural resources with respect to normal controls in order to understand the observed action. Conversely, if this increased pattern of hemodynamic activity is also triggered by the observation of our "hand resting" condition in which hand–object interactions are not present, then the over-activation mechanism is likely to be part of a generic compensatory mechanism occurring in response to brain tissue damage. This is an important issue to consider given that action understanding is fundamental for the quality of patients' social life. For instance, action understanding is a prerequisite for the establishment of cooperative social interactions in which the understanding of other people behavior is an essential building block.

Materials and methods

Patients

Fifteen right-handed early relapsing remitting MS (RRMS) patients (7 males and 8 females, mean age = 30.6 years, range = 19–44; see Table 1) have been recruited for the present study. Their average disease duration was 16.2 months (range = 3–34 months) and the mean Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) score was 1.5 (range = 1–3). All patients were relapse-free for at least 2 months and steroid-free for at least 1 month, when the anatomical and functional MRI scans were performed. The main inclusion criteria were the maximum disease duration of 36 months and the absence of clinical impairment in the visual system and the upper limbs, which could affect the performance of the requested task. Three patients presented a single episode of optic neuritis with complete recovery at the onset of disease. At the time of image acquisition, ten patients were under treatment with immunomodulatory drugs (8 interferon beta, 2 glatiramer acetate) and five patients were therapy free.

Fifteen right-handed volunteers (8 males and 7 females, mean age = 34 years, range = 24–54; see Table 1) with no history of neurological problems and normal or corrected to normal vision served as controls. The study was approved by a local ethics committee. Informed consent was obtained from all of the participants before the testing session in accordance with the declaration of Helsinki.

Table 1
Demographic, clinical and MRI parameters of both early RRMS patients and healthy controls.

	MS patients	Healthy controls
Female/male	8/7	7/8
Mean age in years	30.6 (19–44)	34 (24–54)
Mean disease duration in months	16.2 ± 9.2 (3–34)	n.a.
T2-WM-LV	2.4 ± 1.7 (0.8–5.3)	0
CLs number	3.1 ± 2.1 (0–7)	0
BPf (%)	83.1 ± 3.2 (81.1–86.7)	82.8 ± 3.4 (81.2–85.8)
GMf (%)	39.1 ± 1.6 (36.2–41.1)	40.2 ± 2.1 (38.2–42.3)
WMf (%)	44.0 ± 1.8 (41.0–46.8)	42.6 ± 2.0 (40.9–46.3)
EDSS	1.5 ± 0.6 (1–3)	n.a.
DMT:		n.a.
None	5	
Immunomodulatory	10	

Data are reported as mean, ± standard deviation, and range in brackets. T2-WM-LV = T2 white matter lesion volume; CLs = cortical lesions; BPf = brain parenchimal fraction; GMf = grey matter fraction; WMf = white matter fraction; EDSS = expanded disability status scale; DMT = disease modifying therapy; n.a. = not applicable.

Procedures

Different types of black and white digital photographs (bitmap format, resolution 1024 × 768 pixels), which proved to be effective in eliciting activation within areas concerned with action observation (Johnson-Frey et al., 2003), were utilized as stimuli. During acquisition of functional volumes participants were presented with images depicting: i) a human right hand grasping an object positioned on a dark surface (hand grasping condition) or ii) a human right hand resting alongside an object with the palm adjacent to the dark surface (hand resting condition). Note that for the control condition any sort of hand–object interaction was avoided. For all conditions the same set comprising eleven objects (e.g., a glass, a tin box, a candle; a can; a jar; a tennis ball, etc.) were utilized. All stimuli were presented by means of a laptop PC that ensured synchronization with the MR scanner using the software 'E-prime' (Psychology Software Tools Inc, Pittsburgh USA). An LCD computer-controlled projector was employed to present the stimuli on a screen positioned outside the bore of the magnet and was viewed by the participants through a mirror mounted on the head coil. To minimize head motion, cushions and pads specifically designed to restrain head translations and rotations within the head coil were utilized. In addition, participants were instructed to keep their head still during scanning.

During the experiment participants lay supine in the scanner and were requested to carefully observe all the displayed pictures. The experimental conditions were presented in a block design in which two different types of block (corresponding to the experimental conditions) were implemented. Within each block eleven static images were displayed on the screen for 1100 ms and were separated by 290 ms intervals of blank screen yielding a block duration of 15 s. Consecutive blocks were separated by a 15 s rest period consisting of a blank screen with a white fixation cross. The experiment was split into four functional runs. Within each run eight periods of activation were alternated with nine periods of rest. The two experimental conditions were presented four times per run resulting in a total of sixteen repetitions throughout the entire experiment.

All functional and structural images collected for the present study were acquired using a whole body 1.5 T Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands) equipped with a standard 8-channel head coil. Functional images were obtained with a standard single shot echo-planar (EPI) T2*-weighted sequence in order to measure blood oxygenation level-dependent (BOLD) contrast throughout the whole brain (TR = 3 s; TE = 50 ms; flip angle = 90°; 32 contiguous axial slices with a slice thickness of 3.5 mm/0.5 mm gap; FOV = 224 × 224 mm; matrix size = 64 × 64; in-plane resolution of 3.5 × 3.5 mm). 356 volumes were acquired in four scanning runs (89 volumes for each run). Immediately after the acquisition of the functional EPI volumes, two different sets of structural images of the brain were acquired for each participant with the following sequences: i) two three-dimensional T1-weighted Fast Field Eco (FFE) sequences (TR = 25; TE = 4.6; flip angle = 30°; 120 contiguous axial slices with a slice thickness 1.2 mm, FOV = 250 × 250 mm^2, matrix size = 256 × 256, in-plane resolution of 0.98 × 0.98 mm); ii) a Fast Fluid Attenuated Inversion Recovery (FLAIR) sequence (TR = 10000 ms; TE = 120 ms; Inversion Time = 2500 ms, Echo Train Length = 23; 50; 50 contiguous axial slices with a slice thickness of 3 mm; FOV = 250 × 200 mm; matrix size = 172 × 288).

Image analysis

Structural images post-processing

WM lesion identification and volume measurements. All images were assessed by consensus of two experienced observers, who were blinded to patients' identity. On FLAIR images, total WM lesion volume (T2-WM-LV) was quantified after lesion identification and using a

semi-automated local thresholding technique based on Fuzzy C-mean algorithm (Pham and Prince, 1999; Pham et al., 2000) part of the Medical Images Processing, Analysis and Visualization (MIPAV) software (http://mipav.cit.nih.gov).

Brain tissue measurements. On 3DFFE images, normalized volumes of the whole of the brain parenchyma (BPf) and neocortical grey matter (GMf) were measured using a method for total and regional brain volume measurements (the cross-sectional version of the SIENA software [SIENAX]) (Smith et al., 2001). SIENAX uses BET (Brain Extraction Tool, part of FSL–FMRIB's Software Library; www.fmrib.ox.ac.uk/fsl) to extract the brain and skull from the MR images, as previously described (Smith et al., 2002). A tissue segmentation program (FAST another part of FSL) (Zhang et al., 2001) is then used to segment the extracted brain image into grey and white matter, CSF and background, yielding an estimate of total brain tissue volume. The brain-extracted MR images are registered on a canonical image in a standardized space (using the skull image to provide the scaling cue), a procedure that also provides a spatial normalization (scaling) factor for each subject. For selective measurements of neocortical volumes, a standard space mask (which includes ventricles, deep grey matter, cerebellum and brain stem) is used to separate segmented grey matter into neocortical and non-neocortical. The estimated volumes for a subject are then multiplied by the normalization factor to yield either the volume of the total brain tissue (NBV) or the normalized cortical volumes (NCV). This fully automated method provides results with an accuracy of 0.5–1% for single-time point (cross-sectional) measurements (Smith et al., 2001, 2002).

fMRI images post-processing and analysis

Before analysis, the initial four functional volumes of each run were discarded to eliminate magnetic saturation effects. Subsequently the functional images were pre-processed using SPM5 (www.fil.ion.ucl.ac.uk/spm). First, EPI images from all sessions were spatially realigned to the first volume of the first session of scanning (Friston et al., 1995). Second, high quality T1 images were co-registered to the mean EPI image. Lastly, the EPI images were normalized (Ashburner and Friston, 1999) to the standard space defined by the Montreal Neurological Institute template and spatially smoothed with an 8 mm full-width at half-maximum isotropic Gaussian kernel. High-pass filtering was also applied to remove low-frequency drifts in signal. Data were subsequently analyzed by applying a General Linear Model (GLM) separately for each individual using SPM5. Additional regressors of no interest were modeled to account for translation and rotation along the three possible dimensions as measured during the realignment stage of the preprocessing. All conditions were modeled using a box-car function convolved with the hemodynamic response function (HRF) and contrasts were defined in order to pick out the main effects of each experimental condition. These contrasts were subsequently entered into a second-level random effects analysis (2×2 ANOVA) in which 'condition' (grasping or control) was manipulated as within-subject factor, and group (healthy controls and RRMS patients) served as a between-subject factor. The main effects and the interactions were then tested by specifying appropriately weighted linear contrasts. Unless specified, the voxel-level threshold for these second-level contrasts was set at $p<0.01$ (FDR corrected for multiple comparisons) (Genovese et al., 2002) and the extent threshold was of at least 15 contiguous voxels. The resulting SPM{*t*} maps reflected areas in which variance related to the experimental manipulation was captured by the HRF adopted in the GLM.

Anatomical details of significant signal changes were obtained by superimposing the SPM{*t*} maps on the T1 canonical MNI (Montreal Neurological Institute) template image. Results were also checked against normalized structural T1 images of each participant. We used two atlases as a general neuroanatomical reference (Duvernoy and Bourgouin, 1999; Mai et al., 2004). Further, the SPM Anatomy Toolbox (Eickhoff et al., 2005) based on three-dimensional probabilistic cytoarchitectonic maps was used to determine the cytoarchitectonic probability (when available) of the peak activity voxels.

Results

Structural MRI findings

As expected, neither cortical lesions nor white matter lesions were found in healthy controls. After age correction, GMf resulted significantly lower in early RRMS patients (39.1%, SD = 1.6%) than in healthy controls (40.2%, SD = 2.1%, $p=0.002$; see Table 1). In contrast, no statistical differences were found in BPf between patients and NC ($p=0.326$; see Table 1).

Functional MRI findings

First we tested for possible differences between RRMS patients and controls independently from the type of observed stimuli by exploring the main effect of the factor 'group'. Results from this contrast [(RRMS patients/hand grasping + RRMS patients/hand resting) − (Controls/hand grasping + Controls/hand resting)] indicated robust differential activation in a widespread network of areas including occipital, parietal, temporal and frontal regions. In the occipital lobe, RRMS patients were more activated than controls in two extensive clusters that also extended to the parietal cortex (see Fig. 1). Specifically, RRMS patients showed increased activations in a number of areas including the fusiform gyrus, the inferior, the middle and the superior occipital gyri, and the calcarine gyrus (see Table 1 and Fig. 1). All these activations were bilateral. In the parietal lobe RRMS patients were more activated than controls in both the inferior and the superior parietal lobules bilaterally including the angular and the supramarginal gyri (see Fig. 1). In the temporal lobe RRMS patients showed increased activation in the inferior and the middle temporal gyri

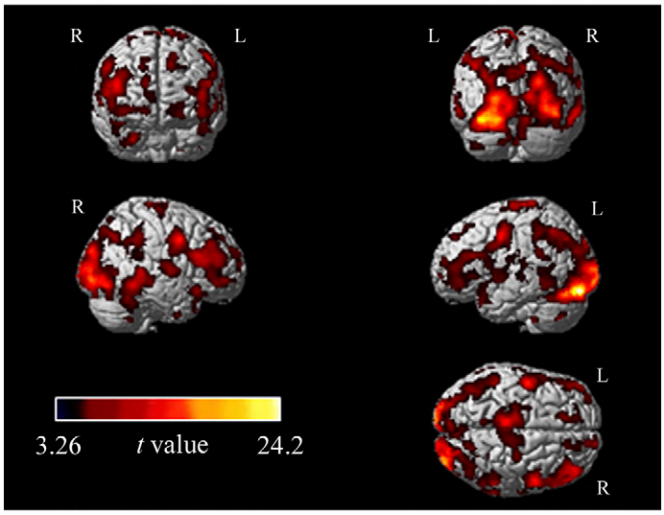

Fig. 1. Regions of increased activation for the main effect of 'group' (RRMS patients>Controls). Regardless of the type of the performed task (i.e., observing a human hand grasping an object or resting in proximity of an object) patients with RRMS and no disability showed a robust increased of activation with respect to healthy controls in a widespread network of areas including occipital, parietal, temporal and frontal regions. The activation map for this contrast is overlaid on the three-dimensional surface of the MNI (Montreal Neurological Institute) standard brain. Note that this projection renders onto the surface activity which may in fact be located in the sulci. (L) left, (R) right.

bilaterally and in the left superior temporal gyrus (see Table 1 and Fig. 1). Increased activation for RRMS patients was also reported in the insular cortex and in the hippocampus bilaterally (see Table 2 and Fig. 1). In the frontal and prefrontal cortices differential activations were found in the precentral gyrus (premotor cortex), in the paracentral lobule, and in both the inferior and the superior frontal gyri (see Table 2 and Fig. 1). Finally, RRMS patients also showed increased activation in the left amygdala and in the cerebellum (see Table 2 and Fig. 1). The reverse contrast [(Controls/hand grasping + Controls/hand resting) − (RRMS patients/hand grasping + RRMS patients/hand resting)] did not show any significant activations. Thus there were no regions activated in neurologically healthy controls compared with RRMS patients.

We next investigated the effects of viewing pictures depicting grasping transitive actions by exploring the main effect of the factor 'condition'. Specifically, for both groups we compared activation elicited by the 'hand grasping' condition with that elicited by the 'hand resting' condition [(Controls/hand grasping − Controls/hand resting) + (RRMS patients/hand grasping − RRMS patients/hand resting)]. As shown in Table 3 and Fig. 2 this contrast revealed significant differential activation in a number of occipital, parietal and frontal areas classically known to be activated during action observation (Gazzola et al., 2007). With respect to our 'hand resting' condition the sight of a human hand grasping an object determined increased activation within the inferior and the middle occipital gyri, the superior and the inferior parietal lobules including the intraparietal sulcus and the postcentral gyrus, and finally within the precentral (ventral premotor cortex) and the inferior frontal gyri (see Table 3 and Fig. 2). All these activations were bilateral except for the precentral gyrus, which was confined to the left hemisphere.

Table 2
Local maxima of the activation foci for the main effect of 'group' in a random effects analysis.

Brain region	Probabilistic cytoarchitecture	T^*	MNI coordinates† (x,y,z) mm		
RRMS patients>controls					
Frontal cortex					
Precentral gyrus	Area 6 (80%)	12.72	−50	−4	46
Paracentral lobule	Area 4a (50%) Area 6 (40%) Area 3b (20%)	11.28	−10	−28	70
Precentral gyrus	Area 6 (10%) Area 44 (10%)	10.57	50	2	36
Superior medial gyrus	–	6.78	10	66	8
Superior frontal gyrus	–	6.17	−14	32	48
Superior frontal gyrus	–	5.49	−20	64	0
Superior frontal gyrus	–	4.36	18	46	38
Temporal cortex					
Middle temporal gyrus	–	8.46	58	−38	2
Hippocampus	Hipp. EC (80%)	8.04	28	2	−36
Hippocampus	Hipp. Hata (40%)	6.96	18	−10	−12
Inferior temporal gyrus	–	6.66	−62	−56	−6
Insula	–	5.32	28	18	−12
Middle temporal gyrus	–	5.24	−66	−46	4
Superior temporal gyrus	–	4.87	−66	−26	12
Insula	–	3.98	−32	−6	12
Inferior temporal gyrus	–	10.53	58	−46	−12
Visual cortex					
Fusiform gyrus	Area 18 (20%)	24.22	−22	−86	−18
Middle occipital gyrus	Area 18 (30%)	16.65	−22	−94	4
Cerebellar cortex					
Cerebellar cortex (crus II)	–	5.03	−38	−66	−46
Cerebellar vermis (9)	–	4.98	−2	−62	−40
Subcortica regions					
Amygdala	Amyg. LB (80%) Amyg. SF (30%) Amyg. CM (30%)	4.10	−24	−6	−14

* Results from a two-way ANOVA, $p<0.01$ FDR corrected.
† Positive coordinate values on the *x* axis indicate right lateralization, negative values indicate left lateralization.

Table 3
Local maxima of the activation foci for the main effect of 'condition' in a random effects analysis.

Brain region	Probabilistic cytoarchitecture	T^*	MNI coordinates† (x,y,z) mm		
Hand grasping>hand resting					
Frontal cortex					
Middle frontal gyrus	–	4.61	−34	60	14
Inferior frontal gyrus (pars opercularis)	Area 45 (80%) Area 44 (20%)	4.42	54	20	28
Inferior frontal gyrus (pars orbitalis)	Area 45 (20%)	4.23	52	36	−8
Inferior frontal gyrus (pars triangularis)	Area 45 (40%)	4.11	−48	40	14
Precentral gyrus	Area 44 (30%) Area 3b (10%)	3.99	−54	10	30
Parietal cortex					
Postcentral gyrus	Area 2 (40%) Area 3b (20%) Area 1 (10%)	5.82	50	−22	38
Visual cortex					
Inferior occipital gyrus	Area 18 (60%)	11.89	28	−90	−6
Middle occipital gyrus	Area 18 (10%)	11.06	−36	−90	0
Superior occipital gyrus		4.6	26	−80	32
Hand resting>hand grasping					
Visual cortex					
Calcarine gyrus	Area 17 (100%) Area 18 (10%)	8.40	4	−86	−2

* Results from a two-way ANOVA, $p<0.01$ FDR corrected.
† Positive coordinate values on the *x* axis indicate right lateralization, negative values indicate left lateralization.

The reverse contrast [(Controls/hand resting − Controls/hand grasping) + (RRMS patients/hand resting − RRMS patients/hand grasping)] revealed significant differential activation only in the left calcarine gyrus (primary visual cortex; Table 3). Finally the interaction aimed at localizing the modulating effect of the factor 'group' on the factor 'condition' was not significant even when explored at a more liberal threshold (i.e., $p<0.001$ uncorrected).

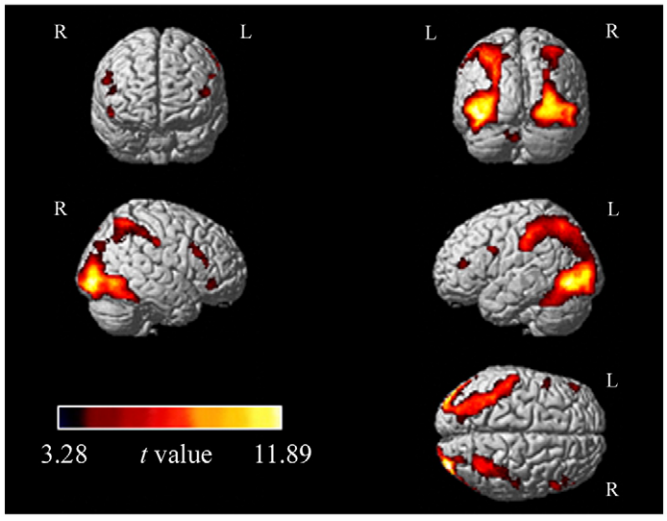

Fig. 2. Regions of increased activation for the main effect of 'condition' (Grasping hand>Resting hand). When comparing brain activation elicited by the sight of a human hand grasping an object with that elicited by the sight of the same hand simply resting in proximity of an object, both RRMS patients and healthy controls showed robust activation in occipital, parietal, and inferior frontal areas. The activation map for this contrast is overlaid on the three-dimensional surface of the MNI (Montreal Neurological Institute) standard brain. Note that this projection renders onto the surface activity which may in fact be located in the sulci. (L) left, (R) right.

Discussion

Potential differences in the pattern of hemodynamic activity evoked by the observation of grasping actions were investigated in early RRMS patients and in healthy controls by means of fMRI. The aim of the present work was to ascertain whether possible differences in cortical activation between MS patients and controls during the viewing of other people actions could be genuinely interpreted as the expression of a specific deficit in the ability to understand observed actions or represent a rather unspecific and generalized adaptive response of the human brain put in place to compensate for structural damage of the central nervous system. To this end, early RRMS patients and controls were invited to observe stimuli representing the hand of a human model either grasping an object or resting in proximity of an object.

A previous action observation study conducted on MS patients (Rocca et al., 2008) revealed that observing hand actions elicited in MS patients significantly higher activation than controls within the inferior frontal gyrus and the superior temporal sulcus, two neural markers of action observation. For action observation these results are important in that they extend the notion that during the execution of actions MS patients tend to show an increased recruitment of neural resources in terms of both the level of activity and the number of involved areas (Filippi et al., 2004; Reddy et al., 2000; Rocca et al., 2002; Pantano et al., 2005). These findings are noteworthy when considering that understanding quickly and effortlessly what another person is doing can be a fundamental block for the social life of early MS patients. If the above-mentioned activation pattern in action observation areas is the expression of a difficulty in performing such function, this should be carefully considered at both clinical and rehabilitative levels.

It is unclear whether the reported effects are due to a compensatory mechanism specific for the understanding of observed actions or whether such mechanism represents a less specific functional adaptation process. The study by Rocca et al. (2008) does not provide a definite answer to this question because a control condition aside from simple rest was missing in their experimental setting. Indeed, simply comparing neural activation of MS patients following the observation of an action with the neural activation found in healthy controls performing the same task (Rocca et al., 2008) does not allow to fully ascertain whether the reported differences can be ascribed to functional changes which reflect action observation mechanisms. In order to draw such conclusion what is needed is a control situation in which patients observe the hand and an object, but presented in a non interactive fashion. In such circumstances no differences amongst groups (MS patients and controls) should emerge.

The results of the present study have the potential to provide some answers to this question. If, as it is tempting to assume, we are in the presence of a compensatory mechanism specifically tailored as to contribute to the maintenance of a suitable level of action understanding, then such over-activation mechanism should have been found only for our 'hand grasping' but not for our 'hand resting' condition. This is because the adopted 'hand resting' condition does not entail any hand–object interaction. Our results clearly indicate that this is not the case. Although our experimental design was able to reveal robust main effects of both the factors 'group' and 'condition', we did not find any significant 'group' by 'condition' interaction. Specifically, results from the main effect of the factor 'group' (RRMS patients Vs controls) revealed a widespread network of areas in which activation was indeed greater for early RRMS patients than for healthy controls. Furthermore, results from the main effect of the factor 'condition' (grasping hand Vs resting hand) indicated that in early RRMS patients as well as in healthy controls observing a human hand grasping an object as compared to the same hand resting nearby the object triggered differential significant activation in areas known to play a pivotal role in action understanding such as the inferior frontal gyrus, the precentral gyrus, and both the inferior and the superior sectors of the parietal cortex including the intraparietal sulcus. The most important result, however, is the lack of any interaction between the two manipulated factors ('group' and 'condition'). This indicates that the difference in terms of hemodynamic activation between observing a hand grasping an object and a hand resting in proximity of an object is similar for early RRMS patients and healthy controls. In other words, action understanding mechanisms seem to operate in a comparable fashion across the two tested groups. This suggests that the robust increased activation showed by early RRMS patients regardless of the type of the observed hand–object interaction does not represent a compensatory mechanism to overcome a specific deficit in action understanding. Conversely, it is more likely to be the expression of rather unspecific adaptive functional cortical changes that may help maintain a normal level of function despite the presence of brain tissue damage.

Altogether these findings demonstrate the presence in RRMS patients of a robust increased recruitment of neural resources within an extensive and widespread network of brain regions including visual, parietal, temporal and frontal regions. Although some of the over-activated areas do play a role during the observation of other people actions, our results clearly indicate that such increase in neural activity is not specifically related to action observation. Rather it seems to be the expression of an adaptive but unspecific functional cortical change needs to be put in place by MS patients as to maintain a suitable level of function whatever the task at hand. The possible neurophysiological causes for such over-activation might be mainly related to two interrelated factors. The first factor relies on the evidence that MS causes demyelination of axons which consequently determines a deficit in neuronal transmission (e.g., Franklin and ffrench-Constant, 2008). The second factor is concerned with the continuous repairing mechanism process triggered by the pathology (e.g., Franklin and ffrench-Constant, 2008). Both a deficit in neural transmission and repairing require higher metabolism with respect to a normal brain and therefore may account for the abnormal level of functioning reported for these patients.

The fact that our data did not reveal specific deficits to action observation brain areas in RRMS patients is good news in rehabilitation terms. Recently the observation of actions has been tested as a tool for neurorehabilitation. Specifically, the ability of the neural system underlying action observation to re-enact stored motor representations has been utilized as a mean for rehabilitating motor control (action observation therapy) (Ertelt et al., 2007; Buccino et al., 2006). For instance, stroke patients with moderate, chronic motor deficit of the upper limb underwent an action observation therapy program consisting of the observation of daily actions with concomitant physical training of the observed actions. A significant improvement of motor functions in the course of the treatment has been found. Additionally, the effects of action observation therapy on the reorganization of the motor system have also been investigated by functional magnetic resonance imaging, using an independent sensorimotor task consisting of object manipulation (Ertelt et al., 2007). The direct comparison of neural activations between experimental and control groups after training with those elicited by the same task before training yielded a significant rise in activity within key motor and premotor areas. Therefore it might well be that the action observation therapy may help in bringing back to normal values the brain activity related to action understanding in RRMS patients.

References

Ashburner, J., Friston, K., 1999. Nonlinear spatial normalization using basis functions. Hum. Brain Mapp. 7, 254–266.

Buccino, G., Binkofski, F., Fink, G.R., Fadiga, L., Fogassi, L., Gallese, V., Seitz, R.J., Rizzolatti, G., Freund, H.J., 2001. Action observation activates premotor and parietal areas in a somatotopic manner: an fMRI study. Europ. J. Neurosci. 1, 400–404.

Buccino, G., Solodkin, A., Small, S., 2006. Functions of the mirror neuron system: implications for neurorehabilitation. Cogn. Behav. Neurol. 19, 55–63.
Decety, J., Grezes, J., 1999. Neural mechanisms subserving the perception of human actions. Trends Cogn. Sci. 3, 172–178.
Duvernoy, H.M., Bourgouin, P., 1999. The Human Brain: Surface, Three-Dimensional Sectional Anatomy and MRI. Springer-Verlag, Wein.
Eickhoff, S.B., Stephan, K.E., Mohlberg, H., Grefkes, C., Fink, G.R., Amunts, K., Zilles, K., 2005. A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. NeuroImage 25, 1325–1335.
Ertelt, D., Small, S., Solodkin, A., Dettmers, C., McNamara, A., Buccino, G., 2007. Action observation has a positive impact on rehabilitation of motor deficits after stroke. NeuroImage 36, T164–173.
Filippi, M., Rocca, M.A., Colombo, B., Falini, A., Codella, M., Scotti, G., Comi, G., 2002. Functional magnetic resonance imaging correlates of fatigue in multiple sclerosis. NeuroImage 15, 559–567.
Filippi, M., Rocca, M.A., Mezzapesa, D.M., 2004. A functional MRI study of cortical activations associated with object manipulation in patients with MS. NeuroImage 21, 1147–1154.
Franklin, R.J.M., ffrench-Constant, C., 2008. Remyelination in the CNS: from biology to therapy. Nat. Rev. Neurosci. 9, 839–855.
Friston, K.J., Ashburner, J., Frith, C.D., Poline, J.B., Heather, J.D., Frackowiak, R.S.J., 1995. Spatial registration and normalization of images. Hum. Brain Mapp. 3, 165–189.
Gazzola, V., Rizzolatti, G., Wicker, B., Keysers, C., 2007. The anthropomorphic brain: the mirror neuron system responds to human and robotic actions. NeuroImage 35, 1674–1684.
Genovese, C.R., Lazar, N.A., Nichols, T., 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. NeuroImage 15, 870–878.
Geurts, J.J.G., Barkhof, F., 2008. Grey matter pathology in multiple sclerosis. Lancet Neurol. 7, 841–851.
Johnson-Frey, S.H., Moloof, F.R., Newman-Norlund, R., Farrerm, C., Inati, S., Grafton, S.T., 2003. Action or hand–object interactions? Human inferior frontal cortex and action observation. Neuron 39, 1053–1058.
Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale EDSS. Neurol 33, 1444–1452.
Lee, M., Reddy, H., Johansen-Berg, H., Pendlebury, S., Jenkison, M., Smith, S., Palace, J., Mattews, P.M., 2000. The motor cortex shows adaptive functional changes to brain injury from multiple sclerosis. Ann. Neurol. 47, 606–613.
Mai, J.K., Assheuer, J., Paxinos, G., 2004. Atlas of the Human Brain 2nd ed. Elsevier Academic Press, San Diego.
Pantano, P., Maniero, C., Lenzi, D., Caramia, F., Iannetti, G.D., Piattella, M.C., Pestalozza, I., Di Legge, S., Bozzao, L., Pozzilli, C., 2005. A longitudinal fMRI study on motor activity in patients with multiple sclerosis. Brain 128, 2146–2153.
Pham, D.L., Prince, J.L., 1999. Adaptive fuzzy segmentation of magnetic resonance images. IEEE Trans. Med. Imaging 18, 737–752.
Pham, D.L., Xu, C., Prince, J.L., 2000. Current methods in medical image segmentation. Annu. Rev. Biomed. Eng. 2, 315–337.
Pirko, I., Lucchinetti, C.F., Sriram, S., Bakshi, R., 2007. Gray matter involvement in multiple sclerosis. Neurol 68, 634–642.
Reddy, H., Narayanan, S., Arnoutelis, R., Jenkinson, M., Antel, J., Mattews, P.M., Arnold, D.L., 2000. Evidence for adaptive functional changes in the cerebral cortex with axonal injury from multiple sclerosis. Brain 123, 2314–2320.
Rizzolatti, G., Craighero, L., 2004. The mirror-neuron system. Ann. Rev. Neurosci. 27, 169–192.
Rizzolatti, G., Fogassi, L., Gallese, V., 2001. Neurophysiological mechanisms underlying the understanding and imitation of action. Nat. Rev. Neurosci. 2, 661–670.
Rocca, M.A., Colombo, B., Falini, A., Grezzi, A., Martinelli, V., Scotti, G., Comi, G., Filippi, M., 2005. Cortical adaptation in patients with MS: a cross-sectional functional MRI study of disease phenotypes. Lancet Neurol. 4, 618–626.
Rocca, M.A., Falini, A., Colombo, B., Scotti, G., Filippi, M., 2002. Adaptive functional changes in the cerebral cortex of patients with non-disabling MS correlate with the extent of brain structural damage. Ann. Neurol. 51, 330–339.
Rocca, M.A., Tortorella, P., Ceccarelli, A., 2008. The “mirror-neuron system” in MS: a 3 Tesla fMRI study. Neurol 70, 255–262.
Smith, S.M., De Stefano, N., Jenkinson, M., Matthews, P.M., 2001. Normalized accurate measurement of longitudinal brain change. J. Comput. Assist. Tomogr. 25, 466–475.
Smith, S.M., Zhang, Y., Jenkinson, M., Chen, J., Mattews, P.M., Federico, A., De Stefano, N., 2002. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. NeuroImage 17, 479–489.
Zhang, Y., Brady, M., Smith, S., 2001. Segmentation of brain MR images through a hidden Markov random field model and the expectation–maximization algorithm. IEEE Trans. Med. Imag. 20, 45–57.