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Neurobiological mechanisms underlying emotional processing in relapsing-remitting multiple sclerosis

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Affective disorders are frequent and disabling conditions in multiple sclerosis; however, the underlying neurobiological mechanisms are still poorly understood and investigated. Previous structural imaging studies have suggested that damage of frontal and temporal cortices plays an important role in the genesis of emotional disorders in multiple sclerosis, although psychosocial factors have been also implicated. However, this initial research may not have fully characterized the brain's functional dynamics of emotional processes in multiple sclerosis. Functional magnetic resonance imaging (fMRI) appears, therefore, to be a sensible tool to explore neurobiological mechanisms of emotions in multiple sclerosis since it also allows investigation of the functional connectivity or 'communication' between critical regions in affective behaviour [e.g. the prefrontal cortex (PFC) and amygdala]. In the present study, functional imaging was used to investigate the neural substrate of processing emotions in 12 multiple sclerosis patients relative to 12 healthy subjects matched for age and educational level. Only relapsing-remitting multiple sclerosis patients, who were cognitively unimpaired and who did not assume disease-modifying therapies, were included, given the potential confounding effect of these variables in the genesis of emotional symptoms. Brain responses were recorded in all participants while they executed an active task that consisted of processing emotional relative to neutral stimuli. Structural measures (i.e. total lesion load, grey matter, white matter and total brain volume) were also recorded to control for any effect of these variables. Despite similar performances during the task, and no differences in structural measures, multiple sclerosis patients displayed significantly greater responses within the ventrolateral PFC [*t*'s >5, *P*'s <0.02, Family Wise Error (FWE), small volume correction (svc)], compared to controls. Multiple sclerosis patients also showed a lack of functional connectivity between two prefrontal areas and the amygdala, a subcortical region critically involved in the generation of negative feelings (*t*'s >4, *P*'s <0.05, FWE, svc). It is likely that pathological changes related to the disease are reflected in an abnormal 'communication' between key emotional regions and that adaptive processes take place and become evident as enhanced

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responses of task-specific areas (i.e. the ventrolateral PFC). Local reorganizations in the brain can be viewed as compensatory mechanisms aimed to limit the clinical expression of emotional symptoms in multiple sclerosis. Overall our findings offer new insights into the neurobiological mechanisms of emotions in multiple sclerosis and provide evidence that they resemble those described for some psychiatric disorders.

Keywords: multiple sclerosis; fMRI; emotion; prefrontal cortex; amygdala

Abbreviations: BOLD = blood oxygenation level dependant; CMDI = Chicago Multiscale Depression Inventory; DSM = diagnostic and statistical manual of mental disorders; EPI = echo planar image; FWE = family wise error; FMRI = functional magnetic resonance imaging; FWHM = full width half maximum; JLO = judgement of line orientation; MNI = Montreal Neurological Institute; RAVLT = rey auditory-verbal learning test; ROCFT = Rey–Osterrieth complex figure test; SIENA = structural image evaluation using normalization of atrophy; SPM = statistical parametric mapping; WAIS-R = Wechsler Adult Intelligence Scale-Revised

Introduction

Affective disorders are common and disabling conditions in people suffering from multiple sclerosis, a frequent neurological disease in young adults (Hirtz *et al*., 2007). Several studies confirmed that motor and cognitive impairments in multiple sclerosis patients are often accompanied by emotional symptoms, with a prevalence that is three times higher than the general population (Minden and Schiffer, 1990; Sadovnick *et al*., 1996; Patten *et al*., 2005).

Inflammation, demyelination, axonal dysfunction and grey matter (GM) damage are all highly associated with the clinical manifestations of multiple sclerosis, including affective disorders (Foley *et al*., 1992; Bakshi *et al*., 2000; Mohr *et al*., 2001; Feinstein, 2007). Hence, identifying brain markers of emotional dysfunctions in multiple sclerosis represents a fundamental step for increasing our knowledge about the disease's mechanisms and for improving treatments that specifically target these symptoms. One of the first studies on this topic found that, independently of clinical disability, multiple sclerosis patients with lesions affecting the brain parenchyma showed more emotional symptoms compared to subjects with lesions of the spinal cord (Rabins *et al*., 1986). Another study associated lesions in the left arcuate fasciculus to depressive symptoms (Pujol *et al*., 1997) and further experiments showed that depression was correlated with damage in both frontal and temporal cortices (Zorzon *et al*., 2001; Feinstein *et al*., 2004).

However, it is possible that previous research underestimated alterations in the white matter (WM) that are difficult to detect using conventional imaging. More sophisticated techniques (e.g. magnetization transfer, diffusion-weighted imaging or proton magnetic resonance spectroscopy) have demonstrated that the involvement of the WM in multiple sclerosis might be very severe in spite of few and small focal WM lesions (Bonavita *et al*., 1999; Filippi and Rocca, 2007; Zivadinov *et al*., 2008). Another issue is that structural imaging could not fully characterize the brain's dynamics underlying emotional disorders compared to other methods. For example, functional magnetic resonance imaging (fMRI) with blood oxygenation level dependent (BOLD) contrast represents a sensitive measure of disease-related changes. This technique is constantly employed for exploring brain substrates of many psychiatric disorders (Malhi and Lagopoulos, 2008), and for characterizing mechanisms of sensory-motor and cognitive deficits in multiple sclerosis (Mainero *et al*., 2004; Bobholz *et al*., 2006; Rocca *et al*., 2007*a, b*, 2008*b*). Nevertheless, functional studies investigating neural correlates of emotions in multiple sclerosis are surprisingly missing.

A consistent finding amongst fMRI studies in multiple sclerosis is that, during the execution of sensory-motor or cognitive tasks, patients engage additional brain regions or exhibit a greater response within similar neural networks recruited by controls (Filippi *et al*., 2002; Mainero *et al*., 2004; Rocca *et al*., 2008*b*). This has been interpreted as an adaptive change, i.e. the brain reacts to injuries via compensatory reorganizations that limit behavioural deficits despite neural damage, in particular at early stages of the disease (Pantano *et al*., 2002). Adaptive processes are likely to be triggered by pathological changes related to multiple sclerosis and become evident as enhanced responses of task-specific brain regions or by the formation/reshaping of connections between them. A combination of these two mechanisms of plasticity is also possible; however, it is important to bear in mind that this plasticity could, in contrast, induce maladaptive mechanisms due to diffuse neural disorganization (Morgen *et al*., 2004; Citri and Malenka, 2008). Ultimately, increased brain responses are not expected when the task complexity and the burden of the disease increase, with reduced or maladaptive plasticity associated with impaired function and exhaustion of the functional reserve (i.e. the ability of brain responses to match behavioural demands) (Pantano *et al*., 2005; Cader *et al*., 2006).

In the present study, we used fMRI to investigate brain correlates of processing emotional stimuli in multiple sclerosis patients without affective disorders. We hypothesized that if adaptive reorganizations appear early, to limit the clinical expression of the disease, then differences in emotional processing between multiple sclerosis patients and controls should be evident even when emotional symptoms are not manifested. We, therefore, restricted our study to individuals with multiple sclerosis who did not present affective disorders; furthermore, we did not include patients with cognitive impairment, or who recently assumed disease-modifying therapies, given the potential role of these two latter variables in the genesis of emotional symptoms (Feinstein *et al*., 2002; Feinstein, 2006). Following previous fMRI findings, we expected greater brain responses or increased recruitment of regions implicated in emotional behaviour in multiple sclerosis patients compared to controls. In particular, differences were predicted in the orbital, medial and ventrolateral prefrontal

cortex (PFC) given its critical role in emotional regulation (Davidson *et al*., 2000; Quirk and Beer, 2006), and in the amygdala, a subcortical region consistently implicated in processing emotional stimuli and in the generation of negative feelings (Davis and Whalen, 2001; LeDoux, 2003). At the same time, we expected significant differences between multiple sclerosis patients and controls in the functional connectivity (i.e. 'communication') between these areas, as a function of processing emotional relative to neutral stimuli. This 'functional disconnection' might reflect differences in the organization of cortico–subcortical interactions responsible for emotional behaviour.

Participants and methods

Participants

From a sample of 50 individuals (Liguori *et al*., 2007) with relapsing-remittent multiple sclerosis (Poser *et al*., 1983), 12 subjects (seven female), who met the following criteria, were included: (i) no evidence of major depressive episodes or other psychiatric disorders according to the Structured Clinical Interview of the DSM-IV (Steinberg, 1994); (ii) no history of traumatic brain injury, past or current history of substance abuse, or other coexisting medical conditions; (iii) no clinical relapses for at least 6 months prior to study entry; (iv) no assumption of antidepressant, anxiolytic, antipsychotic or antiepileptic drugs; (v) no assumption of steroids, or disease-modifying therapy in the 6 months before recruitment; (vi) no evidence of cognitive impairment as evaluated by a detailed neuropsychological assessment (see next section); (vii) expanded disability status scale (EDSS) ranging from 1 to 3 (Kurtzke, 1983); (viii) right-handedness according to the Edinburgh handedness inventory (Oldfield, 1971); and (ix) completely normal functioning of the right upper limb and optimal visual acuity.

Twelve right-handed healthy volunteers (four female) with no previous history of neurological or psychiatric diseases and with a normal MRI of the brain (as assessed by structural MRI scanning) were matched for age and education with multiple sclerosis patients. The demographic and clinical characteristics of all participants are summarized in Table 1.

All participants gave written informed consent, which was approved by the Ethical Committee of the University 'Magna Graecia' of Catanzaro, according to the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm).

Neuropsychological assessment

A trained neuropsychologist (M.C.G.) administered a detailed battery of tests to all participants. It was divided in two sessions of 45 min each to minimize fatigue and loss of concentration (note also that the order between sessions was counterbalanced across subjects). The following cognitive functions were evaluated: (i) verbal and spatial memory [Rey Auditory-Verbal Learning Test (RAVLT), and Rey–Osterrieth Complex Figure Test (ROCFT)](Rey, 1958, 1968); (ii) executive functions (Modified Card Sorting Test and Word List Generation) (Benton and Hamsher, 1964; Nelson, 1976); (iii) attention [Digit Symbol, Span and Arithmetic: subtests of the revised Wechsler Adult Intelligence Scale-Revised (WAIS-R)] (Wechsler, 1981); and (iv) visuo-spatial processing [Judgement of Line Orientation (JLO), and Block Design of WAIS-R] (Benton *et al*., 1978; Wechsler, 1981).

Two expert neurologists (P.V. and R.N. with 25 and 12 years of experience, respectively), unaware of any other result, evaluated clinical signs and symptoms in multiple sclerosis patients, including fatigue according to the Fatigue Severity Scale (FSS) (Krupp *et al*., 1989). As previously reported by our group and by others (Amato *et al*., 2001; Cerasa *et al*., 2006; Gioia *et al*., 2007), the mean score in each neuropsychological test was first calculated in controls and then used as a normative value to derive the number of tests failed by multiple sclerosis patients (i.e. a test was considered failed if the score was lower than the fifth percentile of the distribution in controls). Multiple sclerosis patients who failed 0, 1 or 2 tests were classified as cognitively unimpaired and included in the study. Although none of the participants met the criteria for major depressive episodes or other psychiatric disorders, we further investigated the presence of depressive and anxiety symptoms using the Chicago Multiscale Depression-Inventory (CMDI), and the Hamilton Rating Scale Anxiety (HAM-A), respectively (Hamilton, 1959; Solari *et al*., 2004).

Two-tailed *t*-tests for independent samples were run within SPSS (Statistical Package for the Social Sciences; http://www.spss.it/) to compare all demographic and neuropsychological data between groups.

Structural and functional MRI acquisitions

All MRI scans were performed on a 1.5 T Unit (Signa NV/i, General Electric, Milwaukee, WI, USA) using a standard quadrature head coil. Participants were positioned to lie comfortably in the scanner with a forehead restraining strip and various foam pads to ensure head fixation and minimize motion during scanning.

Proton density and T_2-weighted images were acquired using a conventional dual spin echo sequence (repetition time 3500 ms, echo time 20/85 ms), while T_1-weighted images were obtained with a spin echo sequence (repetition time 550 ms, echo time 13 ms). All two-dimensional images were acquired as axial oblique contiguous 4 mm slices (frequency/phase encoding matrix 256×256, 24 cm field of view) oriented along the anterior–posterior commisure line. A three-dimensional T_1-weighted high-resolution spoiled gradient echo (SPGR) sequence was also acquired (repetition time 15 ms, echo time 6.7 ms, TI 500 ms; flip angle 15°, frequency/phase encoding matrix 256×256) yielding an image volume of 70 slices, 3 mm thick. This last sequence provided an optimal image contrast between GM, WM and cerebrospinal fluid.

For functional imaging, a gradient echo, echo planar (EPI) T_2* weighted sequence (TR 2000 ms, TE 30 ms; flip angle 90°) was employed, with 24 axial slices of 4 mm thickness and 1 mm interslice gap. Slices were prescribed inferior to superior onto a

Table 1 Participant's demographic and neuropsychological data

Demographic and neuropsychological data	Multiple sclerosis	Controls	*P*-values
Age (years)	29.3 ± 8.1	28.7 ± 5.1	0.85
Education (years)	11.6 ± 2.8	12.7 ± 2.6	0.25
Disease duration (years)	4.3 ± 2.8	–	–
EDSS median score and range	1.5 (1–2.5)	–	–
Fatigue (FSS)	30.6 ± 13.3	–	–
Attention			
WAIS-R digit symbol	8.8 ± 3.0	10.5 ± 2.3	0.24
WAIS-R digit span	8.7 ± 2.0	12.7 ± 3.6	0.01
WAIS-R arithmetic	9.5 ± 3.2	11 ± 3.7	0.25
Executive functions			
Modified Card Sorting Test (categories achieved)	5.8 ± 0.3	5.7 ± 0.3	0.96
Word List Generation (WLG)	10.4 ± 3.7	13.1 ± 3.6	0.06
Visuo-spatial processing			
Judgement of Line Orientation (JLO)	27.1 ± 3.6	25.8 ± 2.7	0.59
WAIS-R block design	10.18 ± 3.5	10 ± 2.4	0.92
Verbal memory			
RAVLT Immediate recall	35.8 ± 6.1	43.1 ± 5.3	0.02
RAVLT Delayed recall	7.7 ± 2.3	9 ± 1.6	0.18
Spatial memory			
ROCFT Immediate recall	15.2 ± 5.7	19.2 ± 6.4	0.12
ROCFT Delayed recall	15.3 ± 4.9	18 ± 6	0.28
Depression			
CMDI total score	80.2 ± 7.5	58.9 ± 9.8	0.006
CMDI mood	26.8 ± 6.8	18.9 ± 4.4	0.01
CMDI evaluative	23.2 ± 6	16.3 ± 2.5	0.01
CMDI vegetative	30.2 ± 4.9	23.6 ± 5.1	0.01
Anxiety			
Hamilton Rating Scale (HAM-A)	12.1 ± 1.2	6.2 ± 2.8	0.003

Data are all expressed as mean ± SD (a part for the EDSS which is expressed as median).

midsagittal section. A total of 165 functional brain images were collected in an acquisition time of 5 min and 38 s.

fMRI task

A modified version of a paradigm known to evoke robust responses in cortico-subcortical regions (including PFC and amygdala) was employed (Hariri *et al.*, 2002; Bertolino *et al.*, 2005). The paradigm was composed of alternating blocks (i.e. ABABABABABA) lasting 30 s each and constituted by either neutral (A-blocks) or emotional trials (B-blocks). During neutral trials, simple geometric shapes (i.e. circles, or horizontal and vertical ellipses) were presented in 'trios', such that one was on the top and two were at the bottom of the screen. One of the two shapes presented at the bottom was identical to the top one (i.e. the target) and participants were asked to identify it by pressing a two-choice response button box. A 3-s instruction trial at the beginning of each A-block alerted participants to task demands (i.e. 'match shapes'). During emotional trials, participants viewed three pictures of unfamiliar human faces (either males or females) expressing one of the following negative emotions: sadness, fear or anger. These stimuli were derived from previously standardized sets of photographs (Ekman and Friesen, 1976), and were fully randomized with respect to identity, gender and expression of the face. The 'trios' of emotional stimuli were arranged as well as the neutral stimuli (one on the top and two at the bottom of the screen), and participants had to match one of the two bottom emotional stimuli to the top target one. Again, a 3 s instruction trial at the beginning of each B-block made subjects aware of new task demands (i.e. 'match faces'). There were six A- and five B-blocks in total with six trials *per* block, each lasting 4500 ms. All stimuli were back-projected at the centre of a screen by a computer-controlled system and participants viewed them through a mirror attached to the standard head coil.

Behavioural responses for each trial [i.e. reaction times (RT) and errors] were recorded by an MRI compatible fibreoptic response box (Current Designs, Philadelphia, PA, USA) using a script written in LabView (National Instruments, Austin, Texas, TX, http://www.ni.com/labview/i/). Differences in the mean of RT and number of errors between groups were compared using two-tailed *t*-tests within SPSS.

Structural MRI data analyses

An author (M.L.), unaware of any other result, processed the structural data. The T_2-weighted total lesion load (TLL) quantification was performed on the proton density T_2-weighted and T_1-weighted images using a fully automated threshold technique (EMS, Medical imaging computing, Leuven, Belgium) on a Linux workstation (Dawant *et al.*, 1999). The 3D T_1-weighted images

were also analysed on a Linux workstation by using a previously validated and fully automated method [SIENAX, an adaptation of the SIENA software (Structural Image Evaluation using Normalization of Atrophy) for cross-sectional measurements]. This technique, after deskulling, segmentation and normalization of the whole brain volumes to allow valid statistical inference, calculated an estimate of grey matter and WM volumes. Details on this method, which provides an accuracy of 0.5–1% for single time-point (cross-sectional) evaluations, are extensively described elsewhere (Smith *et al*., 2002; De Stefano *et al*., 2004).

Two-tailed *t*-tests for independent samples within SPSS were used for comparisons of grey matter, WM and total brain volumes (TBV) values between groups. We also tested for correlations between total lesion load, grey matter, WM, TBVs and all neuropsychological measures, including anxiety, depression and fatigue in the multiple sclerosis group (Pearson' *r*).

fMRI preprocessing

Functional data were preprocessed using SPM5 (www.fil.ion.ucl.ac.uk/spm/). The mean EPI was first computed for each participant and visually inspected to ensure that none showed excessive signal dropout in the medial temporal cortex (including the amygdala) and in the medial and orbitofrontal cortices. All EPI were then realigned to the first scan by rigid body transformations to correct for head movements. Next, EPI were normalized to the standard template in the MNI space (Montreal Neurological Institute (MNI)—International Consortium for Brain Mapping) using linear and nonlinear transformations, and smoothed with a Gaussian kernel of full width half maximum (FWHM) 8 mm.

fMRI analysis of regional effects

This analysis aimed to: (i) identify significant differences between multiple sclerosis patients and controls in regional responses of brain areas implicated in emotional behaviour; and (ii) obtain reference coordinates (in each group, independently) to functionally define the amygdala as a 'source' region for connectivity analyses (see next section).

To these ends, a random effects model was implemented using a two-stage process (first and second level). This random-effects analysis allows inferences about the general population from which participants were drawn. For each subject we used a General Linear Model (GLM) to assess regionally specific effects of task parameters on BOLD indices of activation (Friston *et al*., 1994). The model included two experimental factors (neutral and emotional trials), and six realignment parameters as effects of no interest to account for residual motion-related variance. Low-frequency signal drift was removed using a high-pass filter (cut-off 128 s), and an autoregressive modelling [AR(1)] of temporal autocorrelations was applied. At the first level, we generated contrast images displaying the effect of the condition 'emotional versus neutral trials' in each participant. At the second level (group-analysis) these contrast images were entered in a new general linear model to generate statistical images (i.e. SPM-*t*-maps) that explore the main effect of the task in each group, independently (one-sample *t*-tests). Next, a two-sample *t*-test identified significant differences between groups (multiple sclerosis > controls and vice versa) for the same comparison (emotional versus neutral trials). Since multiple sclerosis patients showed greater depression and anxiety scores compared to controls (see Results), the second level model also included these scores as covariates.

Two approaches for thresholding second level maps were applied. First, for *a priori* hypotheses in specific regions of interest (ROI), the threshold was set at $P < 0.05$, Family Wise Error (FWE) correction for multiple comparisons in small volumes [small volume correction (svc)] (Worsley *et al*., 1996; Friston, 1997). The medial PFC also including the anterior cingulate cortex, the orbitofrontal cortex, the ventrolateral PFC and the amygdala were defined as *a priori* ROI given their consolidated role in emotional behaviour (Aggleton *et al*., 1980; Amaral and Price, 1984; Barbas, 2007; Ghashghaei *et al*., 2007). All ROI were defined using Marsbar (http://marsbar.sourceforge.net/) that incorporates the 'aal.02' atlas for automatic_anatomical_labelling (Tzourio-Mazoyer *et al*., 2002). Second, we reported other brain regions which were not predicted *a priori* but met a threshold of $P < 0.001$, uncorrected, 10 contiguous voxels.

fMRI connectivity analysis: psychophysiological interactions

The physiological connectivity between two brain regions can vary as a function of the psychological context (Friston *et al*., 1997). Here, we were interested in the connectivity (i.e. correlation) that is modulated by the psychological context of viewing emotional versus neutral stimuli. This constitutes a psychophysiological interaction (PPI) (Friston *et al*., 1997). We sought to identify 'target' regions that showed differential connectivity according to the context (emotional versus neutral trials) with a 'source' region, specifically the amygdala. This subcortical area was chosen as 'source' given its critical role in processing emotional stimuli and in the generation of negative affect (Davis and Whalen, 2001; Adolphs, 2002; LeDoux, 2003). Using PPI, 'target' regions are not identified because their response is correlated with the activity of the source region or because of the presence of emotional stimuli *per se*, but rather because of the interaction between these two variables. More importantly, we sought to identify 'target' regions for which the *change in connectivity* with the amygdala differed between groups (multiple sclerosis versus controls and vice versa).

According to previous fMRI studies (Hariri *et al*., 2002; Bertolino *et al*., 2005), robust and bilateral amygdala responses were displayed when comparing emotional to neutral stimuli in both controls and multiple sclerosis patients (Table 2 for controls, Table 3 for multiple sclerosis). Hence, for subsequent functional connectivity analyses, the left and right amygdala was used alternatively as 'source' in each group, independently. For each participant, a 10-mm sphere was created around amygdala coordinates derived from multiple sclerosis and control group maps (Tables 2 and 3 for coordinates in each group). The time-series of the BOLD response for each participant was then

Table 2 Main effect of emotional stimuli > neutral stimuli in controls (one sample *t*-test)

Cerebral region	Side	*t*-values	MNI coordinates		
			x	*y*	*z*
vlPFC	R	6.81*	56	32	14
	L	6.58*	−50	22	16
Middle frontal gyrus	L	5.16	−48	16	50
	R	6.30	34	4	38
Superior frontal gyrus	R	4.64	22	28	32
Precentral gyrus	R	9.68	30	−18	76
Postcentral gyrus	R	9.03	48	−22	62
Supplementary motor area	R	7.88	4	−18	60
Insula	R	7.44	36	−16	16
Thalamus	L	6.44	−4	−10	−2
	R	4.55	18	−32	2
Amygdala	L	7.52**	−30	0	−18
	R	7.25**	28	−2	−22
Inferior parietal lobule	L	6.45	−32	−72	44
Middle temporal gyrus	R	7.68	62	−2	−16
Parahippocampal area	L	5.99	−14	−34	−2
Fusiform gyrus	L	12.44	−42	−54	−24
	R	15.67	40	−52	−24
Middle occipital gyrus	R	17.06	34	−82	16
Inferior occipital gyrus	L	13.97	−18	−100	−10
Lingual gyrus	R	21.09	28	−90	−16

*$P<0.03$, **$P<0.002$, FWE svc for *a priori* ROI. Activations in all other regions met the criteria $P<0.001$, uncorrected, 10 contiguous voxels. Coordinates from left and right amygdala were used as references for the 'source' regions in the connectivity analyses (see Methods).

Table 3 Main effect of emotional stimuli > neutral stimuli in multiple sclerosis (one sample *t*-test)

Cerebral region	Side	*t*-values	MNI coordinates		
			x	*y*	*z*
vlPFC	L	7.01*	−56	20	8
	L	6.75*	−56	24	16
	L	6.70*	−56	30	8
	L	6.54*	−40	30	−20
	R	8.28#	52	28	0
mPFC	L	6.32**	−6	56	−18
	L	4.54**	−6	62	−10
Middle frontal gyrus	R	7.11	52	0	54
Amygdala	L	10.03***	−26	−4	−16
	R	8.00#	22	0	−22
Parahippocampal area	R	7.53	30	2	−36
	L	6.45	−24	−34	−10
Thalamus	L	6.30	−8	−22	−2
Precentral gyrus	L	5.59	−58	−4	34
Superior occipital gyrus	R	9.22	22	−102	6
Fusiform gyrus	L	13.60	−44	−54	−24
	R	10.75	40	−50	−24
Precunes	L	6.40	−30	−78	−36
Middle occipital gyrus	L	10.73	−30	−98	0
Inferior occipital gyrus	L	10.69	−38	−84	−6

*$P<0.02$, **$P<0.05$, ***$P<0.001$, #$P<0.005$, FWE, svc for *a priori* ROI. Activations in all other regions met the criteria $P<0.001$, uncorrected, 10 contiguous voxels. Coordinates from left and right amygdala were used as references for the 'source' regions in the connectivity analyses (see Methods).

computed using the first eigenvariate from all voxels' time series in the sphere. Next, the BOLD time series for each individual was deconvolved to estimate a 'neuronal' time series for the 'source', using the PPIs deconvolution parameter defaults in SPM5 (Gitelman *et al*., 2003). The PPI term (PPI regressor) was calculated as the element-by-element product of the amygdala 'neuronal' time series and a vector coding for the main effect of task (1 for emotional trials, and –1 for neutral trials). This product was re-convolved by the canonical haemodynamic response function (HRF). The statistical model also included the main effect of the task convolved by the HRF, the 'source' 'neuronal' time series, and the six movement parameters as effects of no interest. Subject-specific PPI models were run, and contrast images generated such as the identified 'target' regions were those showing a *change* in connectivity with either the left or right amygdala as a function of the psychological context of viewing emotional versus neutral stimuli. The first level contrast images were then entered into second level general linear model analyses and the following contrasts assessed: (i) 'target' regions showing *changes in connectivity* with the 'source' for emotional versus neutral trials in either multiple sclerosis or controls, independently (one-sample *t*-tests); and (ii) 'target' regions for which *changes in connectivity* with the 'source' (for the emotional versus neutral trials comparison) differed between groups (multiple sclerosis versus controls group and vice versa) (two-sample *t*-test). As before, this latter statistical model also included individual's depression and anxiety scores as covariates.

The same statistical approaches previously described were used for thresholding second level connectivity maps (i.e. $P<0.05$, FWE, svc, for ROI, and $P<0.001$, uncorrected, 10 voxels, for other regions).

Results

Neuropsychological assessment

Table 1 summarizes mean/median scores for all demographic, clinical and neuropsychological data in all participants. No significant differences were found between multiple sclerosis patients and controls in mean age and educational level. Overall, cognitive performances of multiple sclerosis patients did not differ from those of controls with the exception of the WAIS-R digit span, and RAVLT immediate recall in which controls responded more accurately than multiple sclerosis. However, these neuropsychological differences did not reach the threshold for cognitive impairment (Amato *et al*., 2001; Cerasa *et al*., 2006; Gioia *et al*., 2007).

None of the participants met the criteria for major depressive episodes or other psychiatric disorders according to the DSM-IV, and although multiple sclerosis patients reported more emotional symptoms than controls, their scores were below the cut-off level

for the Italian population (Solari *et al*., 2004). Nevertheless, these variables were included in all fMRI statistical models exploring differences between groups to account for potential confounding effects (see Methods).

Structural MRI

The mean total lesion load in multiple sclerosis patients was $3.7 \pm 1.1\ cm^3$. As expected, the spatial dissemination of lesions was mainly detected in the periventricular regions. The mean grey matter, WM and TBVs did not significantly differ between multiple sclerosis patients and controls (Multiple sclerosis: GM = 826.8 ± 64.1 ml; Controls: GM = 834.2 ± 33.7 ml; $P = 0.75$; Multiple sclerosis: WM = 689.6 ± 44 ml; Controls: WM = 704.9 ± 31.4 ml; $P = 0.38$; Multiple sclerosis: TBV = 1539.1 ± 51.4 ml; Controls: TBV = 1516.4 ± 91.4 ml; $P = 0.51$). Furthermore, no correlations were found between neuropsychological variables (including anxiety, depression and fatigue) and total lesion load, grey matter, WM or TBVs in multiple sclerosis patients (r's < 0.07, P's > 0.2).

fMRI behaviourals

No significant differences were found between groups in the mean number of errors during matching both emotional (Multiple sclerosis: mean errors = 0.4 ± 1; Controls: mean errors = 0.3 ± 0.7, $P = 0.81$) and neutral stimuli (multiple sclerosis: mean errors = 0.5 ± 0.7; controls: mean errors = 0.8 ± 0.9, $P = 0.32$). In addition, the difference in RT (i.e. RT emotional stimuli–RT neutral stimuli) was comparable between multiple sclerosis patients and controls (RT emotions–RT neutral, multiple sclerosis: mean = 188.1 ± 223.4; RT emotions–RT neutral, controls: mean = 172.5 ± 213.5, $P = 0.86$) demonstrating that there were no significant behavioural effects between groups for processing emotional compared to neutral stimuli.

fMRI regional effects

Several regions, including *a priori* ROI, showed significant activations when comparing emotional versus neutral stimuli in both controls and multiple sclerosis patients, independently (Tables 2 and 3). In particular, robust responses were found in the amygdala, ventrolateral PFC, thalamus and visual cortices in both controls and multiple sclerosis, who also displayed additional foci of activation in the ventrolateral PFC.

The direct comparison between groups (i.e. multiple sclerosis > controls) for the main effect of the task (i.e. emotional versus neutral stimuli), revealed that the ventrolateral PFC was significantly more activated in multiple sclerosis patients than controls (left ventrolateral PFC: x −52, y 36, z 22; $t = 6.08$, $P < 0.005$, FWE, svc, right ventrolateral PFC: x 46, y 34, z 10; $t = 5.09$, $P < 0.02$, FWE, svc, Fig. 1) (two-sample t-test). Likewise, the left precuneus (x −2, y −60, z 44; $t = 5.57$, $P < 0.001$, uncorrected) and the left superior parietal cortex (x −20, y −58, z 44; $t = 5.49$, $P < 0.001$, uncorrected) showed increased regional responses in multiple sclerosis patients compared to controls. Similar results were obtained when the 'core' affective features of CMDI (i.e. 'mood' and 'evaluative' subscales) rather than the total score were used as covariate of no interest (multiple sclerosis > controls: ventrolateral PFC: t's > 4, P's < 0.05, FWE, svc; precuneus: t's > 4, P's < 0.001, uncorrected; superior parietal cortex: t's > 4, P's < 0.001, uncorrected).

In contrast, the inverse comparison (i.e. controls > multiple sclerosis) did not show any significant BOLD response (no suprathreshold voxels at $P < 0.001$, uncorrected).

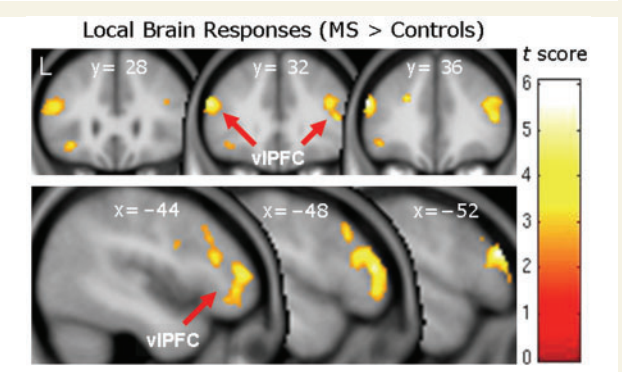

Figure 1 Differences between groups (multiple sclerosis > controls) in regional brain responses for the comparison emotional > neutral stimuli. The ventrolateral prefrontal cortex (vlPFC) showed significantly greater activation in multiple sclerosis patients compared to controls (two-sample t-test). Colour bar represents t-statistics. The x and y coordinates are in the MNI (Montreal Neurological Institute) space. Activations are displayed at $P < 0.001$, uncorrected (MS = multiple sclerosis).

Functional connectivity analyses

Controls showed *changes* in connectivity between the left amygdala (Fig. 2A) and both ventrolateral PFC and medial PFC for the main effect of the task (left ventrolateral PFC: x −54, y 18, z 24; $t = 7.44$, $P < 0.02$, FWE, svc; right ventrolateral PFC: x 44, y 18, z 26; $t = 5.22$, $P < 0.05$, FWE, svc; right medial PFC: x 16, y 52, z −2; $t = 7.58$, $P < 0.004$, FWE, svc; left medial PFC: x −16, y 52, z -6; $t = 5.26$, $P < 0.05$, FWE, svc, Fig. 2B) (one-sample t-test). In addition, they displayed other regions outside *a priori* ROI that showed *changes* in connectivity with the left amygdala for the main effect of emotional condition (i.e. left precuneus: x −22, y −78, z 22, $t = 5.49$, $P < 0.001$, uncorrected; left superior parietal cortex: x −26, y −60, z 64, $t = 6.07$, $P < 0.001$, uncorrected; right middle occipital gyrus: x 40, y −90, z 12, $t = 5.12$, $P < 0.001$, uncorrected). In striking contrast, multiple sclerosis subjects displayed no *changes* in connectivity between the left amygdala (Fig. 3A) and any brain region (*a priori* ROI or other areas) for the same effect of task (emotional versus neutral stimuli) (no suprathreshold voxels at $P < 0.001$, uncorrected, Fig. 3B) (one-sample t-test).

The direct comparison between groups (i.e. controls > multiple sclerosis) confirmed that *changes* in connectivity between the left amygdala and both ventrolateral PFC and medial PFC significantly

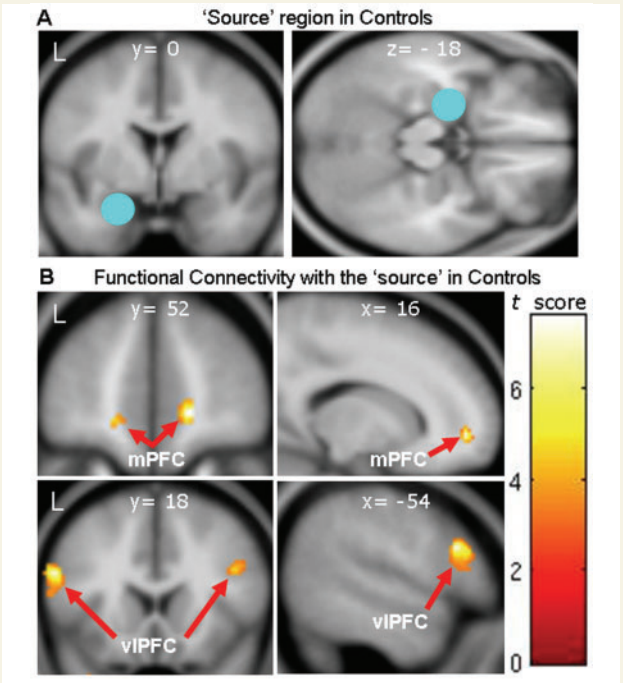

Figure 2 (**A**) 'Source' region for the PPI in healthy controls. The left amygdala was defined as a 10 mm sphere 'source' region (coordinates from Table 2). (**B**) Functional Connectivity (PPI) in healthy controls. The medial prefrontal cortex (mPFC) (top rows) and the ventrolateral prefrontal cortex (vlPFC) (bottom rows) are 'target' regions that showed changes in connectivity with the left amygdala as a function of viewing emotional relative to neutral stimuli. Colour bar represents *t*-statistics. See Results for statistics and Table 4 for comparison between groups (Controls > multiple sclerosis). The *x*, *y* and *z* coordinates are in MNI (Montreal Neurological Institute) space. Activations are displayed at $P < 0.001$, uncorrected.

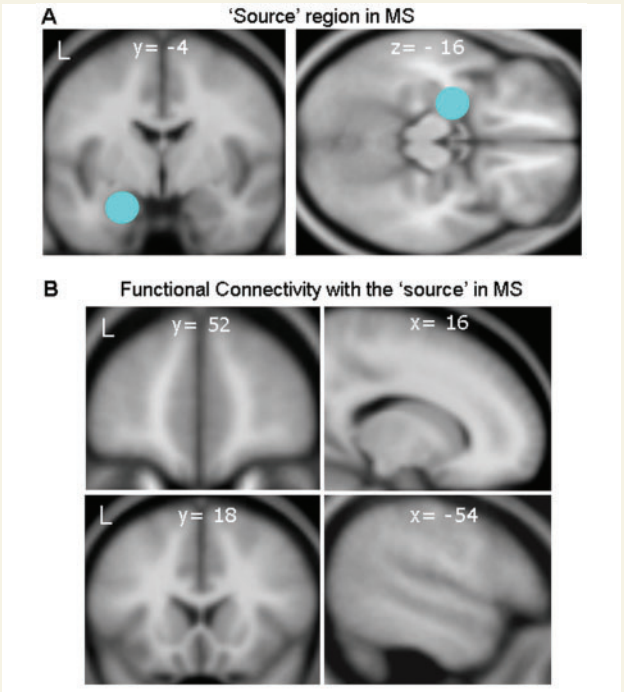

Figure 3 (**A**) 'Source' region for the PPI in multiple sclerosis (MS) patients. The left amygdala was defined as a 10 mm sphere 'source' region (coordinates from Table 3). (**B**) Functional Connectivity (PPI) in multiple sclerosis patients. No brain 'target' regions showed changes in connectivity with left amygdala as a function of viewing emotional relative to neutral stimuli (map thresholded at $P < 0.001$, uncorrected). See Table 4 for comparison between groups (Controls > multiple sclerosis). The *x*, *y* and *z* coordinates are in MNI (Montreal Neurological Institute) space.

differed in controls relatively to multiple sclerosis patients (two-sample *t*-test, Table 4 for coordinates and statistics). The same comparison (i.e. controls > multiple sclerosis) also revealed that other regions outside *a priori* ROI showed differential *changes* in connectivity with the left amygdala in controls relative to multiple sclerosis patients (Table 4). Again, similar results were obtained when the 'mood' and 'evaluative' subscales of CMDI rather than the total score were used as covariate of no interest (Controls > multiple sclerosis: medial PFC: t's > 4, P's < 0.03, FWE, svc, ventrolateral PFC: t's > 5, P's < 0.01, FWE, svc; cuneus: t's > 4.9, P's < 0.001, uncorrected; superior parietal cortex: t's > 4.8, P's < 0.001, uncorrected).

In contrast, the inverse comparison (i.e. multiple sclerosis > controls) did not demonstrate any brain region that presented significantly different *changes* in connectivity with the left amygdala in multiple sclerosis patients relatively to controls (no suprathreshold voxels at $P < 0.001$, uncorrected) (two-sample *t*-test).

Finally, no regions showed significant *changes* in connectivity with the right amygdala either within or between groups (no suprathreshold voxels at $P < 0.001$, uncorrected).

Table 4 Brain regions showing greater connectivity with left amygdala for viewing emotional versus neutral stimuli (Controls > MS)

Cerebral regions	Side	*t*-values	MNI coordinates		
			x	*y*	*z*
vlPFC	R	5.17*	58	22	8
	R	4.68*	40	44	−12
	L	4.63*	−50	14	34
mPFC	R	4.56*	18	52	2
Frontal pole	R	5.43	32	64	4
Superior parietal lobule	R	3.90	34	−68	56
	L	4.91	−24	−74	44
Superior temporal gyrus	R	4.17	62	−14	4
Temporal pole	R	4.18	54	8	−14
Occipital middle gyrus	R	4.21	36	−86	24
Angular gyrus	R	4.15	58	−54	36
	L	4.67	−38	−56	38
Cuneus	R	4.72	16	−84	20
Lingual gyrus	R	4.82	26	−54	−4
	L	4.98	−18	−58	−8
Calcarine cortex	R	4.46	20	−86	0

*$P < 0.05$, FWE, svc for *a priori* ROI. Activations in all other regions met the criteria $P < 0.001$, uncorrected, 10 contiguous voxels.
L = left, R = right.

Discussion

Our results clearly demonstrate that when exposed to emotional stimuli, multiple sclerosis patients display enhanced regional activation within the ventrolateral PFC and a lack of functional connectivity between two PFC regions (i.e. the ventrolateral PFC and medial PFC) and the left amygdala, relative to controls (Fig. 4).

It is also important to emphasize that we investigated how emotional stimuli are processed in the brain of multiple sclerosis patients with a benign form of the disease (i.e. EDSS: 1–2.5) and without cognitive impairment. Furthermore, multiple sclerosis patients reported only minor emotional symptoms which were included as covariates of no interest in all fMRI analyses exploring differences between groups. We adopted the approach of studying the neural substrate of emotions in multiple sclerosis in the absence of overt affective disorders because we were primarily interested in exploring adaptive mechanisms that could have taken place in limbic circuits, similar to those described in other networks (Pantano *et al*., 2002; Rocca *et al*., 2008*b*). The rigorous sample selection that we adopted eliminates potential confounders, and helps with interpretation of the results; although it might produce a somewhat artificial situation with respect to the clinical reality. Nevertheless, the importance of studying what emotional expressions are processed in the brain, even in a selected and small sample of patients, is that abnormalities detected could represent an informative marker for affective illnesses in multiple sclerosis. However, further research is necessary; in particular, to investigate other subgroups of multiple sclerosis patients, and to test whether the enhanced ventrolateral PFC response is overridden by the disease's progression, i.e. when emotional disorders are ultimately more likely.

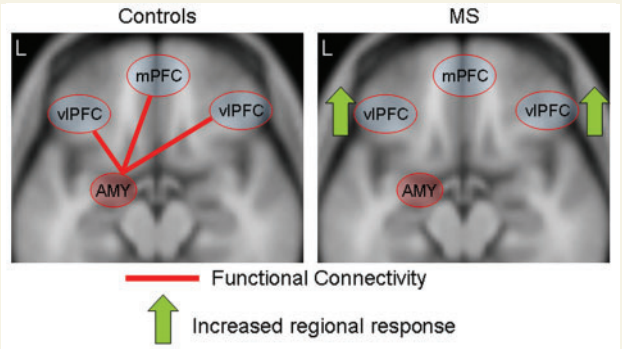

Figure 4 Summary of the results. Multiple sclerosis (MS) patients displayed a lack of functional connectivity between the left amygdala (AMY) and both the ventrolateral and the medial prefrontal cortices (vlPFC and mPFC, respectively); at the same time, they showed increased regional activation in the vlPFC relatively to controls. We hypothesize that the disease's primary factors (e.g. demyelination) alter the axonal conduction between the prefrontal cortex and the amygdala which is reflected in an abnormal pattern of 'communication' between these regions. Local reorganizations in the brain become evident as enhanced regional response of task-dependent regions (i.e. the vlPFC) that might represent a compensatory mechanism aimed to limit the manifestation of emotional symptoms in multiple sclerosis.

The increased activation within the ventrolateral PFC, when processing emotional stimuli, parallels the greater responses of the premotor cortex and inferior frontal gyrus observed in multiple sclerosis patients, relative to controls, when performing sensory-motor and cognitive tasks, respectively (Pantano *et al*., 2002; Prakash *et al*., 2008; Rocca *et al*., 2008*b*). Converging evidence supports the hypothesis that functional reorganizations take place in the brain of subjects with multiple sclerosis to minimize the clinical expression of the brain damage (Filippi and Rocca, 2003). Hence, the increased response of the ventrolateral PFC might reflect adaptive mechanisms limiting the full expression of emotional symptoms in multiple sclerosis and it can be viewed as a compensatory mechanism involving the re-allocation of neuronal resources to preserve affective behaviour. No abnormal amygdala activation to emotional stimuli was found in multiple sclerosis patients and this could also reflect an 'up-regulation' of modulatory functions of the ventrolateral PFC on this subcortical structure. In this respect, it is noteworthy that similar mechanisms (i.e. increased PFC response, and restrained amygdala reactivity) have also been proposed during the recovery from psychiatric disorders such as major depression (DeRubeis *et al*., 2008).

In addition, multiple sclerosis patients showed, compared to controls, a lack of functional connectivity between the PFC and amygdala, highlighting how abnormal patterns of 'communication' in multiple sclerosis can be evident in limbic circuits as well as in sensory-motor or cognitive networks (Saini *et al*., 2004; Au Duong *et al*., 2005; Rocca *et al*., 2008*a*). It is likely that the disease's pathological mechanisms (e.g. demyelination) alter the axonal conduction between the PFC and amygdala with a resulting dysregulation of their physiological pattern of 'communication'. Perturbations of the neuronal dynamics between cortico-subcortical regions involved in emotional behaviour can in turn trigger compensatory changes in task-specific regions (i.e. ventrolateral PFC). It is also possible that when these initial adaptive mechanisms are overridden by accumulating effects of multiple sclerosis pathology, local brain responses become reduced and emotional symptoms appear; however, this hypothesis remains to be tested in multiple sclerosis patients at different stages of the disease and in presence of affective disorders. Nevertheless, our result of 'functional disconnections' between the PFC and amygdala provides new evidence that the neural substrate of emotional processing in multiple sclerosis resembles the neurobiological mechanisms described for psychiatric conditions such as major depression, aggressive behaviour, generalized anxiety disorder and schizophrenia (Drevets *et al*., 1992; Fakra *et al*., 2008; Monk *et al*., 2008; Passamonti *et al*., 2008). Overall, several studies emphasized the interplay between the PFC (thought to represent emotion regulation) and the amygdala (reflecting generation of negative affect) as a key aspect of emotional behaviour (Davis and Whalen, 2001; LeDoux, 2003; Quirk *et al*., 2003; Quirk and Beer, 2006).

We also found that regional activation in the amygdala for processing emotional stimuli was bilateral in both multiple sclerosis patients and controls; however, significant connectivity effects were only associated with the left amygdala in controls (but not

in multiple sclerosis). It could be that the statistical significance of connectivity effects from the right amygdala are weakened by the greater habituation of the BOLD response to emotional stimuli of this side compared to the left one (Breiter *et al*., 1996; Wright *et al*., 2001; Fischer *et al*., 2003).

In addition, it is interesting that posterior brain regions (e.g. cuneus/precuneus and superior parietal cortex) showed increased regional response and a lack of functional connectivity with the left amygdala in multiple sclerosis patients, relative to controls. These cortices are important in processing visual stimuli and are critical components of the attentional network (Mesulam, 1999). The amygdala receives direct or indirect inputs from them and, in turn, modulates their function via different mechanisms (i.e. directly, through feedback projections, or indirectly, via the basal forebrain 'arousal' system) (Selemon and Goldman-Rakic, 1988; Cavada and Goldman-Rakic, 1989; Davis and Whalen, 2001; Pessoa, 2008). Since interactions between the amygdala and posterior regions are thought to reflect mechanisms by which affective stimuli modulate the attentional network and vice versa (Vuilleumier and Driver, 2007; Pessoa, 2008); impairments of 'communication schemes' between these areas might lead to additional demands to process/attend stimuli that could be otherwise neglected. Similarly, studies investigating adaptive mechanisms in multiple sclerosis patients during attentional tasks found increased activations in analogous posterior 'attentional' regions (Morgen *et al*., 2007).

However, the interpretation of functional reorganization in multiple sclerosis could be problematic. fMRI identified brain activations associated with performances during the task and differences in these brain responses could also reflect alternative strategies for executing the task that do not necessarily imply adaptive processes. In addition, even if fMRI analyses were controlled for variability in depression and anxiety scores, brain responses could still partially reflect differences in emotional symptoms between groups. Another possibility is that tissue damage might affect other neural pathways, such as those more generally involved in perception and action; however, this seems unlikely because we did not find behavioural differences between multiple sclerosis patients and controls for processing emotional relative to neutral stimuli.

It could also be argued that processing and experiencing emotions are distinct processes and that the task we employed only investigated the former. However, previous research suggested that processing emotional stimuli (e.g. facial expressions of fear or disgust) and experiencing the corresponding feelings share similar neurobiological mechanisms, including 'core' regions (Calder *et al*., 2000; Adolphs, 2002; Wicker *et al*., 2003). More importantly, recent behavioural research has shown that multiple sclerosis patients might present, in addition to affective disorders (Feinstein, 2007), deficits in recognizing facial expressions (Henry *et al*., 2009). We were therefore, confident that variations in brain responses between multiple sclerosis patients and controls genuinely reflected differences in the neural substrate common to both processing and experiencing emotions.

Finally, no significant correlations were identified between structural measures (i.e. grey matter, WM, TBVs and total lesion load) and neuropsychological variables, including depression and anxiety. Our inclusion criteria for subjects with a clinically mild form of multiple sclerosis (EDSS: 1–3, no emotional and cognitive symptoms), in which tissue damage tends to be relatively small, could have determined this finding. Furthermore, the present study lacks non-conventional structural MRI data that might be useful to study the potential relationship between functional connectivity abnormalities and diffuse damage of the normal appearing with matter (NAWM).

In conclusion, an additional point should be highlighted. Two prefrontal regions (i.e. the ventrolateral PFC and the medial PFC) showed changes in connectivity with the left amygdala as a function of processing emotional relative to neutral stimuli in controls (but not in multiple sclerosis); however, only the ventrolateral PFC displayed enhanced BOLD response in multiple sclerosis patients compared to controls, and this is thought to reflect compensatory mechanisms. Although the range of areas within which adaptive changes might take place could be limited by the amount of multiple sclerosis pathology; an intriguing possibility could be that substantial differences in the neuronal plasticity exist amongst distinct regions, with some more 'adaptable' than others. Studies designed to investigate the effects of interventions that modulate brain plasticity might provide new insights on this issue and might lead to an improvement of the therapy targeting emotional symptoms in multiple sclerosis.

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