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Resting state functional connectivity alterations in primary lateral sclerosis

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ABSTRACT

Resting state functional connectivity of the sensorimotor and extramotor brain networks was studied in 24 patients with primary lateral sclerosis (PLS) relative to 26 healthy controls. The relationships of RS functional connectivity with patient clinical and cognitive status and white matter tract damage (i.e., corticospinal tracts, corpus callosum, and superior longitudinal fasciculus) were investigated. Compared with controls, PLS patients showed an increased functional connectivity within the sensorimotor, frontal, and left frontoparietal networks spanning the pre- and postcentral, medial and dorsal frontal, insular, and superior temporal regions. Patients with more severe physical disability and a more rapid rate of disease progression had increased sensorimotor connectivity values. The increased functional connectivity within the frontal network was associated with executive dysfunction. In addition, higher functional connectivity correlated with greater structural damage to network-specific white matter tracts. This study shows clinically meaningful increased resting state functional connectivity in PLS.
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1. Introduction

Primary lateral sclerosis (PLS) is a rare disorder characterized by an isolated degeneration of the upper motor neurons (UMN) with a lack of clinical signs of lower motor neuron (LMN) impairment (Pringle et al., 1992). PLS presents usually in the sixth decade of life with an insidious onset of a symmetric, slowly progressive spastic paresis, usually beginning in the lower extremities and eventually evolving into a tetrapyramidal syndrome with marked pseudobulbar features (Pringle et al., 1992). Clinical studies have estimated that 2% to 5% of patients seen in adult neuromuscular clinics are diagnosed with PLS (Le Forestier et al., 2001; Pringle et al., 1992). Early in the disease course, PLS is difficult to distinguish from the predominant UMN amyotrophic lateral sclerosis (ALS) phenotype (Chio et al., 2011). However, PLS has a slower rate of progression and a more benign prognosis than ALS, with a survival of more than 10 years from symptom onset (Gordon et al., 2006; Kuipers-Upmeijer et al., 2001; Le Forestier et al., 2001).

Like ALS, PLS is a clinical diagnosis without established supportive biomarkers. On structural magnetic resonance imaging (MRI), atrophy of the primary motor cortices (PMC) was found in PLS patients (Kuipers-Upmeijer et al., 2001; Tartaglia et al., 2009). Diffusion tensor (DT) MRI shows a more severe white matter (WM) involvement of the corticospinal tracts (CST) and motor transcallosal fibers in PLS compared to those in ALS patients (Agosta et al., 2013b; Ciccarelli et al., 2009; Iwata et al., 2011; Muller et al., 2012; Unrath et al., 2010; van der Graaff et al., 2011).

Analysis of resting state (RS) functional MRI (fMRI) reveals the temporal correlation between the low-frequency spontaneous fluctuations in the resting brain. This allows identification of functionally distinct networks that are related to specific sensory,

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motor, and cognitive brain functions (Smith et al., 2009). RS fMRI alterations have been observed in the motor network of ALS patients (Agosta et al., 2011; Douaud et al., 2011; Jelsone-Swain et al., 2011; Mohammadi et al., 2009; Tedeschi et al., 2010; Verstraete et al., 2010). Previous studies in ALS have also described functional connectivity abnormalities extending well beyond the PMC (Agosta et al., 2013a; Douaud et al., 2011; Luo et al., 2012; Mohammadi et al., 2009; Tedeschi et al., 2010).

In the present study, we investigated RS functional connectivity of the sensorimotor and extramotor brain networks (i.e., default mode network [DMN], frontal network, left and right frontoparietal networks) in patients with PLS, and explored whether the RS fMRI alterations are related to patient clinical and cognitive status and WM structural connectivity breakdown.

2. Methods

2.1. Subjects

Patients with sporadic PLS (Pringle et al., 1992) were recruited at 3 tertiary referral Motor Neuron Disease (MND) Clinics in Northern Italy (San Raffaele Scientific Institute and "Vita-Salute" University, Milan; University of Turin, Turin; Università degli Studi di Milano, Milan). All patients had no evidence of acute or chronic denervation on repeated electromyographical (EMG) examinations and had had their symptoms for at least 3 years. To be eligible, subjects had to meet the following criteria: no family history of MND, no clinical diagnosis of frontotemporal dementia (Rascovsky et al., 2011), age at onset $\geq$40 years, no mutations of major genes related to hereditary spastic paraparesis (i.e., SPG3A, SPG4, SPG7, SPG11) or any other major systemic, psychiatric, or neurological illnesses, no history of substance abuse, and no other causes of focal or diffuse brain damage, including strokes, lacunae, and other evidence of cerebrovascular disease at routine MRI scans. Within 48 hours from MRI, functional status was assessed using the ALS Functional Rating Scale (ALSFRS-r) (Cedarbaum et al., 1999). A quantitative assessment of clinical UMN involvement was based on the UMN score (Turner et al., 2004). The rate of disease progression at study entry was calculated as: (48 − ALSFRS-r score)/ time from symptom onset (Ellis et al., 1999). Healthy controls were recruited among spouses of patients and by word of mouth, underwent a neurological evaluation, and were included in the study only if the results were normal. A total of 24 PLS patients and 26 healthy controls were enrolled consecutively (Table 1). The study was approved by the local ethical committees, and written informed consent from all subjects was obtained before study initiation.

2.2. Neuropsychological assessment

Neuropsychological assessment was performed by an experienced neuropsychologist blinded to the MRI results, and evaluated the following: global cognition with the Mini Mental State Examination (Folstein et al., 1975); executive function with the semantic and phonemic fluency (Novelli et al., 1986) and the fluency indices (controlling for individual variations in motor disabilities) (Abrahams et al., 2000), Stroop test, Wisconsin Card Sorting Test (WCST) (Laiacona et al., 2000), digit span backward (Orsini et al., 1987), and Cognitive Estimation Test (Della Sala et al., 2003); reasoning and abstraction abilities with the Raven coloured progressive matrices and Weigl test (Basso et al., 1987; Spinnler and Tognoni, 1987); verbal memory with the digit span forward (Orsini et al., 1987) and Rey's word list (Carlesimo et al., 1996); and language with the BADA oral naming of objects and actions (Miceli et al., 1994). Scores on neuropsychological tests were age, sex, and education corrected by using normative values.

Table 1
Demographic, clinical, and structural MRI features of PLS patients and controls

	Healthy controls	PLS	*p*
N	26	24	
Age at MRI (y)	63.5 ± 10.0 (43–79)	62.8 ± 8.8 (43–79)	0.92
Sex (female)	13 (50%)	12 (50%)	1.00
Disease duration (mo)		102.0 ± 58.4 (38–247)	
ALSFRS-r		36.7 ± 5.7 (22–42)	
UMN score		13.7 ± 1.8 (10–16)	
Disease progression rate		0.15 ± 0.11 (0.03–0.4)	
WM lesion load (mL)	0.9 ± 1.2 (0–5)	0.5 ± 0.8 (0–4)	0.21
R CST FA	0.53 ± 0.04 (0.4–0.6)	0.50 ± 0.04 (0.4–0.6)	0.02
L CST FA	0.53 ± 0.03 (0.5–0.6)	0.51 ± 0.03 (0.4–0.6)	0.004
R SLF FA	0.43 ± 0.03 (0.4–0.5)	0.43 ± 0.02 (0.4–0.5)	0.74
L SLF FA	0.44 ± 0.03 (0.4–0.5)	0.43 ± 0.02 (0.4–0.5)	0.94
CC FA	0.52 ± 0.03 (0.5–0.6)	0.50 ± 0.03 (0.4–0.6)	0.55
CC-genu FA	0.46 ± 0.04 (0.4–0.5)	0.47 ± 0.02 (0.4–0.5)	0.50
CC-body FA	0.48 ± 0.04 (0.4–0.6)	0.46 ± 0.03 (0.4–0.5)	0.06
CC-splenium FA	0.54 ± 0.03 (0.5–0.6)	0.54 ± 0.03 (0.5–0.6)	0.61
CC-PMC FA	0.47 ± 0.03 (0.4–0.5)	0.41 ± 0.03 (0.3–0.5)	<0.001
CC-premotor FA	0.43 ± 0.03 (0.4–0.5)	0.43 ± 0.03 (0.4–0.5)	0.65
CC-SMA FA	0.49 ± 0.03 (0.4–0.5)	0.44 ± 0.04 (0.4–0.5)	<0.001

Values are mean ± standard deviation (range) or number (%). *p* Values refer to Mann–Whitney *U* test or Fisher exact test, as appropriate. Disease progression rate: (48-ALSFRS-r score)/time from symptom onset.
Key: ALSFRS-r, ALS Functional Rating Scale–Revised; CC, corpus callosum; CST, corticospinal tract; FA, fractional anisotropy; L, left; MRI, magnetic resonance imaging; PLS, primary lateral sclerosis; PMC, primary motor cortex; R, right; SLF, superior longitudinal fasciculus; SMA, supplementary motor area; UMN, upper motor neuron.

2.3. MRI acquisition

Brain MRI scans were obtained using a 3.0 T scanner (Intera; Philips Medical Systems, Best, the Netherlands). A T2*-weighted single-shot echo planar imaging (EPI) sequence for RS fMRI was acquired from all subjects (repetition time [TR] = 3000 ms, echo time [TE] = 35 ms, flip angle = 90°, field of view [FOV]=240 × 240 mm^2; matrix = 128 × 128, slice thickness = 4 mm, 200 sets of 30 contiguous axial slices, parallel to the AC-PC plane). Total acquisition time of RS fMRI was approximately 10 minutes. During scanning, subjects were instructed to remain motionless, to keep their eyes closed, and not to think about anything in particular. The following structural MRI sequences were also acquired: (1) T2-weighted spin echo (SE) (TR = 3500 ms, TE = 85 ms, echo train length = 15, flip angle = 90°, 22 contiguous, 5-mm-thick axial slices, FOV = 230 × 184 mm^2, matrix = 512 × 512); (2) fluid-attenuated inversion recovery (FLAIR) (TR = 11000 ms, TE = 120 ms, flip angle = 90°, 22 contiguous, 5-mm-thick axial slices, FOV = 230 × 131 mm^2, matrix size = 512 × 512); (3) 3D T1-weighted fast field echo (TR = 25 ms, TE = 4.6 ms, flip angle = 30°, 220 contiguous axial slices with voxel size = 0.89 × 0.89 × 0.8 mm, FOV = 230 × 182 mm^2, matrix size = 256 × 256); and (4) pulsed-gradient SE EP with sensitivity encoding (acceleration factor = 2.5, TR = 8986 ms, TE = 80 ms, 55 contiguous, 2.5-mm-thick axial slices, number of acquisitions = 2; after SENSE reconstruction, the matrix dimension of each slice was 256 × 256, with an in-plane pixel size of 0.94 × 0.94 mm and a FOV of 240 × 240 mm^2) and with diffusion gradients applied in 32 non-collinear directions, using a gradient scheme that is standard on this system (gradient over-plus) and optimized to reduce echo time as much as possible. The b factor used was 1000 s/mm^2. Fat saturation was performed to avoid chemical shift artifacts. All slices were positioned to run parallel to a line that joins the most infero-anterior and infero-posterior parts of the corpus callosum.

2.4. MRI analysis

MRI analysis was performed by an experienced observer who was blinded to subjects' identity. WM hyperintensities, if any, were identified on T2-weighted and FLAIR scans. WMH load was measured using the Jim software package (version 5.0; Xinapse Systems, Northants, UK, http://www.xinapse.com).

2.4.1. RS functional connectivity

RS fMRI data analysis was carried out using MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) of FMRIB software library (FSL version 4.1.7, http://www.fmrib.ox.ac.uk/fsl/melodic/) (Beckmann et al., 2005). After having removed the first 4 volumes of each image to allow magnet signal stabilization, individual pre-statistical processing consisted of motion correction (http://www.fmrib.ox.ac.uk/analysis/techrep/tr04ss2/tr04ss2/node13.html), removal of non-brain tissue, spatial smoothing using a 6-mm full-width-at-half-maximum Gaussian kernel, and high-pass temporal filtering equivalent to 100 seconds (0.01 Hz). None of the study participants were excluded from analysis because of motion, as the maximum cumulative translation was $<$1.5 mm and the maximum rotation was $<$0.3 degrees for all participants. fMRI volumes were then registered to the individual's 3-dimensional T1-weighted scan and standard space images using FMRIB's Nonlinear Image Registration Tool (FNIRT). Pre-processed fMRI data, containing 196 time-points for each subject, were temporally concatenated across subjects to create a single 4-dimensional dataset. This fMRI dataset was then decomposed into independent components (IC) with a free estimation for the number of components.

2.4.2. DT MRI preprocessing

DT MRI analysis was performed using FSL tools (FSL version 4.1.7, http://www.fmrib.ox.ac.uk/fsl/fdt/index.html) and the JIM5 software. Briefly, the diffusion-weighted data were skull-stripped using the Brain Extraction Tool implemented in FSL. Then, using FMRIB's Linear Image Registration Tool (FLIRT), the 2 acquired diffusion-weighted scans were coregistered by applying the rigid transformation needed to correct for position between the 2 b_0 images (T2-weighted, but not diffusion-weighted), and a rotation component was also applied to diffusion-weighted directions. Eddy current correction was then performed using the JIM5 software, and the 2 acquisitions were concatenated. Finally, the DT was estimated on a voxel-by-voxel basis using DTIfit provided by the FMRIB Diffusion Toolbox; and maps of fractional anisotropy (FA) were obtained.

2.4.3. Tractography: seed definition

Seeds for tractography of the CST, corpus callosum, and superior longitudinal fasciculus (SLF) were defined in the Montreal Neurological Institute (MNI) space on the FA template provided by FSL. Regions of interest (ROI) were defined manually on sagittal or axial slices based on a priori knowledge of the anatomy of the tracts. Seeds were drawn where these tracts pass through a bottleneck to include the highest number of fibers constituting the tract in the starting seed. Tractography was then performed using a single-seed approach. Masks were used to exclude fibers from neighboring tracts. For the CST, an axial ROI was drawn at the top of the bulbar pyramids of each side and included 4 slices. The seed for the corpus callosum was a sagittal ROI that included the 4 median slices on which the corpus callosum was clearly visible. For the SLF, a single coronal seed was drawn on 3 coronal slices posterior to the postcentral gyrus, in the vicinity of the supramarginal gyrus of each side.

The corpus callosum was also partitioned following 2 procedures. First, we divided the sagittal seed ROI used to obtain the entire corpus callosum into 3 parts along its anteroposterior axis, which roughly correspond to the genu, body, and splenium. Second, the corpus callosum was segmented into 3 portions to identify the callosal fibers linking the PMC, lateral premotor cortices, and supplementary motor area (SMA). To this end, the same seed and exclusion masks of the entire corpus callosum were used, and, in addition, each of the following 3 cortical areas were used as target ROI: PMC, corresponding to the bilateral precentral gyrus as defined by the Harvard–Oxford cortical atlas (http://www.cma.mgh.harvard.edu); SMA, corresponding to the bilateral supplementary motor cortex as defined by the Harvard-Oxford cortical atlas; and the premotor cortex, which was drawn manually and included the dorsolateral part of Brodmann area 6 (from MNI y = 5 to MNI y = 29). This ROI corresponds to the posterior parts of the middle and inferior frontal gyri on the Harvard–Oxford cortical atlas.

The seeds, targets, and exclusion masks were first transformed to each subject's native diffusion space, using the inverse of the linear and nonlinear transformations obtained previously to align each subject's FA to the standard space, and then binarized. Transformed seeds, targets, and exclusion masks were evaluated visually by an experienced observer.

2.4.4. Tractography: fiber tracking

Fiber tracking was performed in native DT MRI space using a probabilistic tractography algorithm implemented in FSL (probtrackx), which is based on a Bayesian estimation of diffusion parameters (Bedpostx) (Behrens et al., 2007). Fiber tracking was initiated from all voxels within the seed masks in the diffusion space to generate 5000 streamline samples, with a step length of 0.5 mm and a curvature threshold of 0.2. CST and SLF were run separately for the right and left hemispheres. Tract maps were then normalized, taking into consideration the numbers of voxels in the seed masks. To do so, the number of streamline samples present in the voxels of the tract maps was divided by the way-total, which corresponds to the total number of streamline samples that were not rejected by the exclusion masks (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT/UserGuide) (Behrens, et al., 2007). The tract masks obtained were thresholded at a value equal to 40% of the 95th percentile of the distribution of the intensity values in the voxels included in the tracts (Galantucci et al., 2011). This normalization procedure allowed us to correct for possible differences between tracts due to the different sizes of the starting seeds. In this way, we also excluded background noise and avoided a too-restrictive thresholding when the maximum intensity value was an outlier. Using a "single-seed" approach, the reconstruction of the corpus callosum and bilateral CST and SLF was obtained. Using a "seed-to-target" approach, the partitions of the corpus callosum (namely CC-genu, CC-body, CC-splenium, CC-PMC, CC-premotor, and CC-SMA) were produced. For each tract, the average FA was calculated in the native space.

Group probability maps of each tract were produced to visually check the anatomical accuracy of the tracts of interest across the study subjects. Individual tracts were transformed to MNI space and averaged across subjects. Only voxels present in at least 10% of the subjects were shown (Fig. 1).

2.5. Statistical analysis

2.5.1. Demographic, clinical, cognitive, and structural MRI data

Group differences in categorial variables were assessed using the Fisher exact test. Continuous variables were compared using

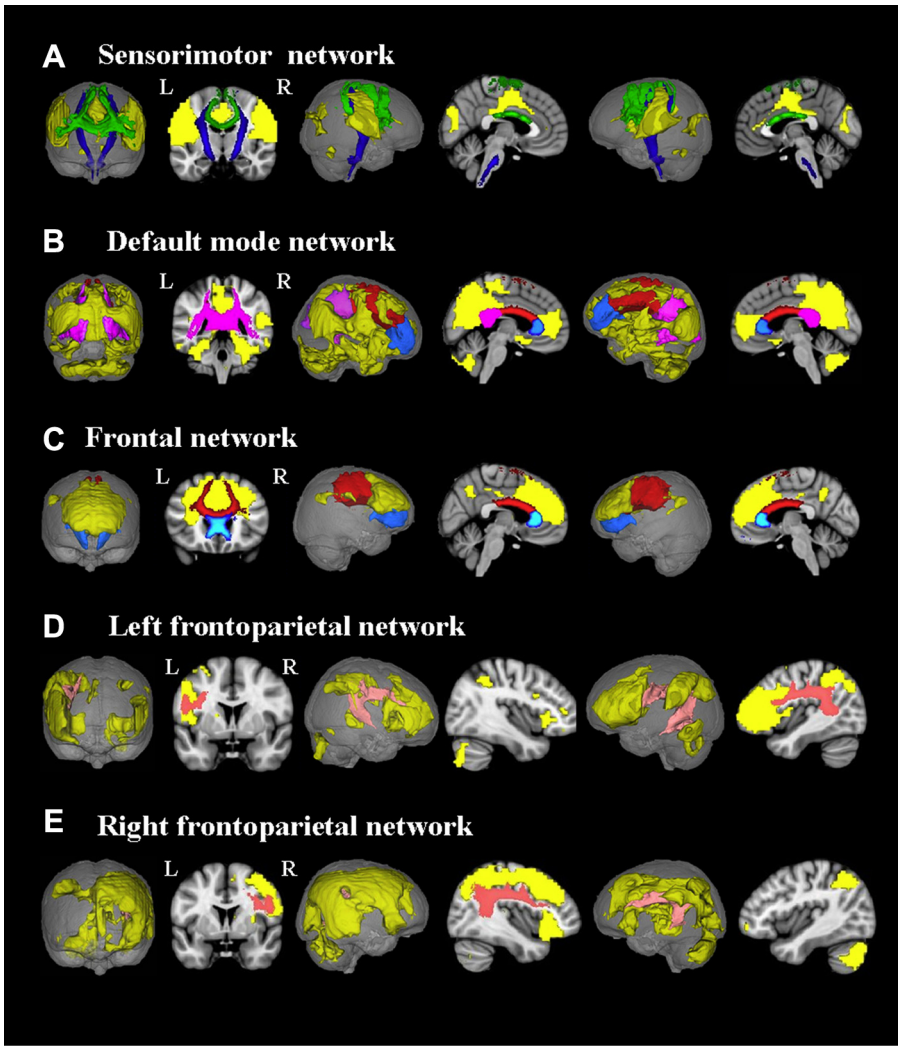

Fig. 1. Spatial maps of the resting state networks and probabilistic maps of the corresponding white matter (WM) tracts. (A) Sensorimotor network (yellow) and probabilistic maps of the corticospinal tracts (blue) and motor callosal fibers (green). (B) Default mode network (yellow) and probabilistic maps of the genu (cyan), body (red), and splenium (violet) of the corpus callosum. (C) Frontal network (yellow) and probabilistic maps of the genu (cyan) and body (red) of the corpus callosum. (D and E) Left and right frontoparietal networks (yellow) and probabilistic maps of the superior longitudinal fasciculi (pink). Spatial maps are Z-statistics. Probabilistic maps of WM tracts were obtained from patients and controls; only voxels present in at least 40% of the subjects are shown. Images are displayed on the 3- and 2-dimensional sections of the Montreal Neurological Institute standard brain in neurological convention.

the Mann–Whitney *U* test (SAS release 9.1; SAS Institute, Cary, NC) ($p < 0.05$).

2.5.2. RS functional connectivity: between-group comparisons

Between-subjects analysis of the RS fMRI data was carried out using a novel dual-regression technique (Filippini et al., 2009), an approach that allows to identify subject-specific temporal dynamics and spatial maps that are associated with each group IC map. Among group-IC spatial maps, IC of interest (sensorimotor network, DMN, frontal network, and left and right frontoparietal networks) were selected by visual inspection based on previous literature (Beckmann et al., 2005; Damoiseaux et al., 2006; Smith et al., 2009) and the frequency spectra of the time courses of the components (i.e., more than 90% of spectral power less than 0.08 Hz). Then, the dual-regression procedure involves the following: the use of selected group-IC spatial maps in a linear model fit (spatial regression) against the single-subject fMRI data sets, resulting in matrices describing temporal dynamics for each IC and subject; and the use of these time-course matrices in a linear model fit (temporal regression) against the associated fMRI data set to estimate subject-specific spatial maps (Filippini et al., 2009). After the dual regression, spatial maps of all subjects were collected into single 4-dimensional files for each original IC. Nonparametric permutation tests (5000 permutations) were used to detect statistically significant differences between groups within the RSN of interest obtained with MELODIC (the single 4-dimensional files for each original IC) (Nichols and Holmes, 2002). The analyses were adjusted for age. Furthermore, analyses were restricted within the spatial RSN of interest using binary masks obtained by thresholding the corresponding Z map image ($Z > 2.3$). A family-wise error (FWE) correction for multiple comparisons was performed, implementing threshold-free cluster enhancement (TFCE) using a significance threshold of $p < 0.05$ and a cluster extent >20 (Smith and Nichols, 2009).

2.5.3. RS functional connectivity: correlations with clinical, cognitive, and DT MRI data

Correlations analyses between functional connectivity maps, clinical and cognitive scores, and FA values of WM tracts were

performed. To minimize the number of comparisons, we defined a priori the variables to be correlated with each RSN (Table 2) on the basis of functional and anatomical knowledge. Based on the results of the between-group comparisons, correlations with clinical, cognitive, and DT MRI variables were then limited to those clusters that showed significant group differences. A TFCE-corrected, permutation-based, non-parametric testing was used to assess such correlations ($p < 0.05$; cluster extent >20). Results that did not survive the correction for multiple comparisons were reported at an uncorrected threshold ($p < 0.05$; cluster extent >20).

3. Results

3.1. Demographic, clinical, cognitive, and structural MRI data

PLS patients and healthy controls did not differ in terms of age, sex, and WM lesion load (Table 1). The neuropsychological features of PLS patients are shown in Table 3. In agreement with the Strong consensus criteria (Strong et al., 2009), 2 PLS patients were found to be cognitively impaired. In addition, 1 PLS patient had abnormal scores in 1 executive and 1 non-executive function test (language), whereas 1 PLS patient showed abnormal scores in 1 executive test only and 4 patients in 1 non-executive test only (3 language, and 1 memory). PLS patients compared with controls showed reduced FA values of the CST bilaterally and the CC-PMC and CC-SMA tracts (Table 1).

3.2. RS functional connectivity: components

The group IC analysis estimated 34 components. Based on their frequency spectra, spatial patterns, and previous literature (Beckmann et al., 2005; Damoiseaux et al., 2006; Smith et al., 2009), the sensorimotor network, DMN, frontal network, and right and left frontoparietal networks were identified (Fig. 1).

3.3. RS functional connectivity: between-group comparisons

3.3.1. Sensorimotor network

Compared with controls, PLS patients showed an increased functional connectivity of the precentral and postcentral gyri, bilaterally ($p < 0.05$, FWE-corrected; Table 4, Fig. 2A). No region of decreased functional connectivity was found in PLS patients relative to controls.

3.3.2. Frontal network

In PLS patients compared with controls, we observed an increased functional connectivity of the anterior cingulate cortex (ACC) and superior medial frontal gyrus bilaterally, left SMA, and right insula ($p < 0.05$, FWE-corrected; Table 4, Fig. 2B). No region of decreased functional connectivity was found in PLS patients relative to controls.

3.3.3. Left frontoparietal network

PLS patients relative to controls showed an increased functional connectivity of the left middle orbitofrontal, inferior frontal, and superior temporal gyri ($p < 0.05$, FWE-corrected; Table 4, Fig. 2C). No region of decreased functional connectivity was found in PLS patients relative to controls.

3.3.4. DMN and right frontoparietal network

No functional connectivity difference was found in the DMN and right frontoparietal network between patients and controls.

3.4. Correlations

3.4.1. Sensorimotor network

Sensorimotor network results are shown in Table 5. In the sensorimotor network, the increased functional connectivity of the right pre- and postcentral gyri was associated with lower ALSFRS-r scores ($p < 0.05$, uncorrected). The increased functional connectivity of the right postcentral gyrus was also related to a more rapid disease progression rate ($p < 0.05$, uncorrected). No correlation was found between the sensorimotor network functional connectivity and the disease duration and UMN score. In the sensorimotor network, the increased functional connectivity of the left pre- and postcentral gyri correlated with reduced FA values of the CC-PMC, whereas the increased functional connectivity of the right precentral gyrus was associated with reduced FA values of the CC-SMA ($p < 0.05$, uncorrected).

3.4.2. Frontal network

Frontal network results also are shown in Table 5. In the frontal network, the increased functional connectivity of the right superior frontal gyrus was associated with a better performance at the WCST global score; whereas the increased functional connectivity of the left superior frontal gyrus and right insula was associated with a worse performance at the phonemic and semantic fluency tests, respectively ($p < 0.05$, uncorrected). The increased functional connectivity of the left SMA and right superior frontal gyrus and insula was associated with reduced FA values of the CC-body ($p < 0.05$, uncorrected). No correlation was found between the frontal network functional connectivity and the FA values of the CC-genu.

3.4.3. Left frontoparietal network

No relationship was found between the functional connectivity of left frontoparietal network, cognitive variables, and DT MRI metrics.

Table 2
Correlation analysis: Clinical, cognitive, and DT MRI variables selected for each RSN of interest

	Sensorimotor network	DMN	Frontal network	Left and right frontoparietal network
Clinical and cognitive variables	ALSFRS-r UMN score Progression rate Disease duration	MMSE Rey's word list	Phonemic fluency Semantic fluency WCST-global score	Phonemic fluency Semantic fluency
WM tracts (FA values)	L CST R CST CC-PMC CC-premotor CC-SMA	CC-genu CC-body CC-splenium	CC-genu CC-body	L SLF R SLF

Key: ALSFRS-r, ALS Functional Rating Scale–Revised; CC, corpus callosum; CST, corticospinal tract; DMN, default mode network; FA, fractional anisotropy; L, left; MMSE, Mini Mental State Examination; MRI, magnetic resonance imaging; PMC, primary motor cortex; R, right; RSN, resting state network; SMA, supplementary motor area; SLF, superior longitudinal fasciculus; WCST, Wisconsin Card Sorting test; WM, white matter.

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Table 3
Neuropsychological profile of PLS patients

Global cognition		Reasoning and problem solving functions	
MMSE (co: 24)	26.5 ± 2.9 (16–30)	Raven's progressive matrices (co: 18)	29.5 ± 4.7 (18–35)
Executive functions		Weigl test (co: 4.5)	12.4 ± 1.8 (9–16)
Stroop, seconds	42.0 ± 19.3 (20–94)	WCST, global score (co: 90.5)	48.2 ± 34.5 (8–102)
WCST, perseverative responses (co: 42.6)	14.0 ± 11.7 (1–31)	Memory	
Phonemic fluency (co: 17)	27.7 ± 8.9 (16–52)	Digit span forward (co: 3.75)	5.6 ± 1.3 (3–9)
Phonemic fluency index	8.6 ± 5.8 (4–27)	Rey's word list, imm. recall (co: 28.53)	41.7 ± 9.3 (31–62)
Semantic fluency (co: 25)	40.6 ± 9.2 (31–62)	Rey's word list, delay recall (co: 4.69)	8.9 ± 3.0 (4–16)
Semantic fluency index	4.9 ± 1.4 (3–7)	Language	
Digit Span backward	4.0 ± 1.3 (1–6)	BADA, oral object naming, errors (co: 2)	0.7 ± 0.7 (0–2)
Cognitive Estimation Test, total (co: 18)	13.0 ± 2.3 (8–16)	BADA, oral action naming, errors (co: 2)	1.8 ± 1.5 (0–5)

Values are mean ± standard deviation (range). Scores are corrected for age, sex, and education using normative values. WCST, global score calculation = [N used cards − (completed categories*10)]; higher scores mean worse performances (Laiacona et al., 2000). Fluency indices calculation for each letter (P, F, L) or category (animals, fruits, cars) a partial index was calculated: [(60 seconds for reading words previously reported in 1')/N words previously reported in 1']; the total semantic or phonemic index was obtained by averaging the 3 semantic or phonemic partial indices, respectively; higher scores mean worse performances corrected for motor disabilities (Abrahams et al., 2000). Key: BADA, "Batteria per l'Analisi del Deficit Afasico"; co, cut-off; MMSE, Mini Mental State Examination; WCST, Wisconsin Card Sorting test.

4. Discussion

This RS fMRI study demonstrated increased functional connectivity in PLS spanning sensorimotor, medial, and dorsal frontal, insular, and superior temporal regions. Patients with more severe physical disability and more rapid rates of disease progression had increased connectivity values. The level of functional connectivity within the frontal network appeared to be related to executive dysfunction. In addition, the pattern of increased functional connectivity within the sensorimotor and frontal networks was associated with a greater structural damage to network-specific WM tracts. These findings suggest that an abnormal functional connectivity may be another factor characterizing the pathogenesis of PLS. We discuss these results in relation to the previous RS fMRI studies in ALS, taking into account the current literature on the physiopathology of PLS.

In ALS, initial RS fMRI studies confirmed the intuitive expectation of a decrease in functional connectivity in the sensorimotor network and DMN (Mohammadi et al., 2009; Tedeschi et al., 2010), supported by the evidence of an impaired interhemispheric functional connectivity between the PMC (Jelsone-Swain et al., 2011). However, subsequent studies have identified regions of increased functional connectivity, including sensorimotor (Agosta et al. 2011; Douaud et al. 2011; Verstraete et al. 2010) and extra-motor (Agosta et al. 2013a; Douaud et al. 2011; Luo et al. 2012) areas. A possible explanation of such a finding is that increased functional connectivity reflects a compensatory process in relation to the structural breakdown of the motor and frontotemporal systems. The relationship of decreased functional connectivity with clinical and cognitive status in 2 studies of ALS agree with this hypothesis (Agosta et al., 2011, 2013a). However, other studies of ALS found the most increased functional connectivity values in those patients with a faster disease progression (Douaud et al., 2011; Verstraete et al., 2010). In a study using a pre-defined ALS-specific cortical network, increased functional connectivity was found over a large area spanning sensorimotor, premotor, prefrontal, and thalamic regions, which significantly overlapped with areas of structural damage identified using DT MRI (Douaud et al., 2011). These observations lend support to a pathogenic loss of inhibitory neuronal influence on cortical activity, rather than only compensatory recruitment, to explain the increased functional connectivity in ALS (Turner and Kiernan, 2012).

The hypothesis of a central interneuronopathy as a key aspect of ALS pathogenesis has been previously postulated using histopathology, neurophysiological techniques, and positron emission tomography (PET) (Bae et al., 2013; Turner and Kiernan, 2012). In ALS, post mortem studies showed loss of interneuron-specific calcium-binding proteins (Maekawa et al., 2004) and γ-amino butyric acid (GABA) receptor mRNA expression (Petri et al., 2003).

Table 4
Regions of increased functional connectivity of the sensorimotor, frontal and left frontoparietal networks in PLS patients compared with controls*

Sensorimotor network					
No. of voxels	Region	x	y	z	Z
67	R precentral gyrus	58	−8	42	0.989
	R postcentral gyrus	62	−10	40	0.987
		50	−10	36	0.981
38	L postcentral gyrus	−50	−10	30	0.967
	L precentral gyrus	−54	−6	34	0.959
	L postcentral gyrus	−46	−14	36	0.957
Frontal network					
472	L superior medial frontal gyrus	−10	64	10	0.985
434	R insula	34	22	12	0.987
233	R anterior cingulate cortex	6	26	18	0.979
76	L anterior cingulate cortex	−8	42	12	0.961
59	R superior medial frontal gyrus	10	46	40	0.970
21	L SMA	−6	16	60	0.964
Left frontoparietal network					
122	L middle orbitofrontal gyrus	−36	48	−6	0.987
43	L inferior frontal gyrus	−50	40	16	0.987
42	L superior temporal gyrus	−54	−34	18	0.989

The letters x, y, and z are coordinates in the Montreal Neurological Institute space.
Key: L, left; R, right; SMA, supplementary motor area.
* $p < 0.05$, family-wise error-corrected.

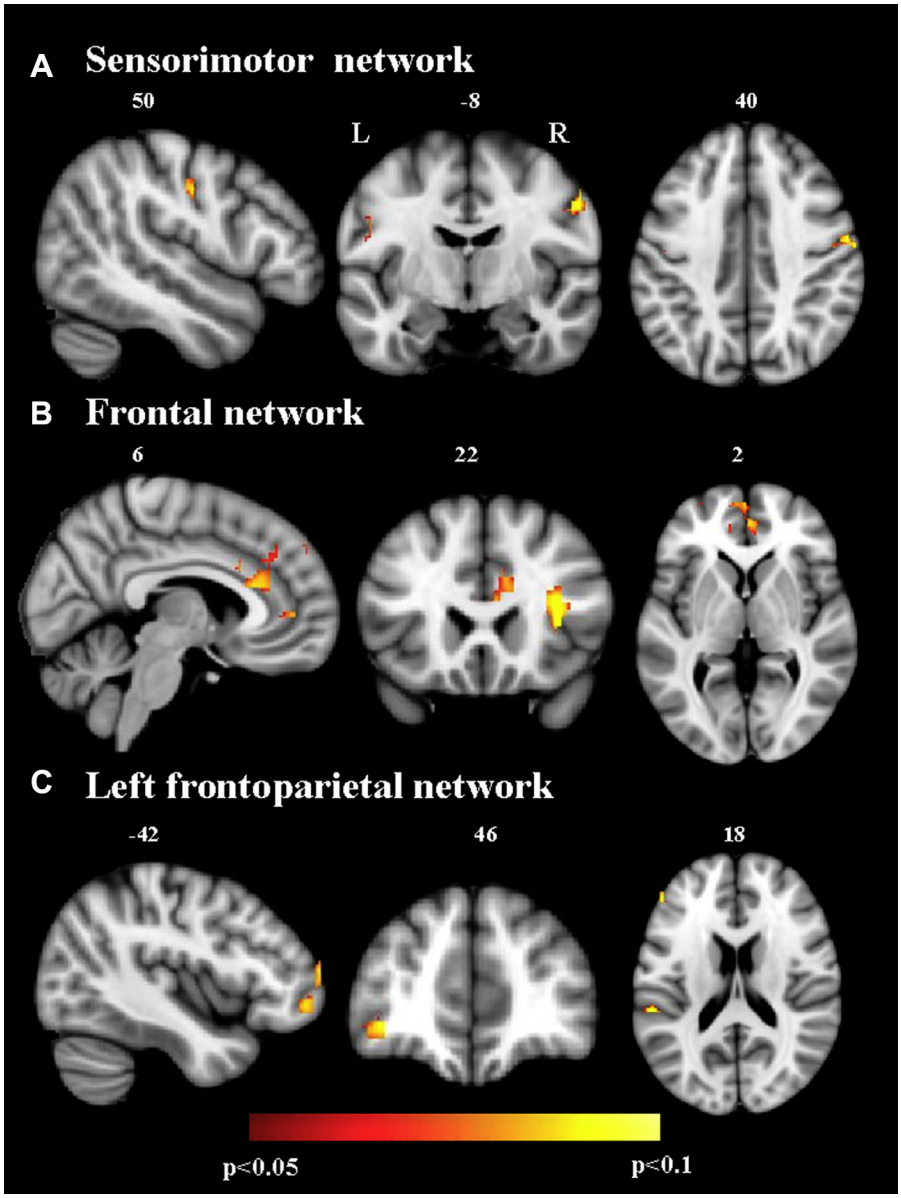

Fig. 2. Regions of increased functional connectivity of the sensorimotor (A), frontal (B), and left frontoparietal (C) networks in PLS patients compared with controls. Results are shown at $p < 0.05$, family-wise error corrected, on the Montreal Neurological Institute standard brain in neurological convention.

Transcranial magnetic stimulation studies of ALS patients showed a deficient transcallosal inhibition and increased cortical excitability (Riva, et al., 2012; Wittstock et al., 2007). Cortical hyperexcitability was found to precede the development of clinical symptoms in presymptomatic *SOD1* mutation carriers (Vucic et al., 2008). In ALS, PET detected a distributed loss of flumazenil binding (Lloyd et al., 2000), a benzodiazepine receptor antagonist that can be used to visualize the distribution of $GABA_A$ receptors in the human brain. Furthermore, investigators in a recent proton magnetic resonance spectroscopy study observed that, compared with healthy controls, patients with ALS had lower levels of GABA in the left motor cortex (Foerster et al., 2012). Little is known about inhibitory circuitry involvement in PLS except that PET flumazenil binding is reduced in the motor cortex, ACC, and parietal and temporal lobes of these patients (Turner et al., 2007).

The positive relationship of sensorimotor functional connectivity with disease severity and rate of disease progression, and the DT MRI data observed in our study, support the notion that an increased functional connectivity is likely to have a role in the pathogenesis of PLS. Indeed, we found a correlation between increased sensorimotor functional connectivity and damage to the transcallosal motor fibers. In addition to CST involvement, PLS patients also showed a marked damage to the motor fibers of the corpus callosum, in keeping with pathological reports (Konagaya et al., 1998; Tan et al., 2003) and previous DT MRI studies (Ciccarelli et al., 2009; Iwata et al., 2011; Muller et al., 2012; Unrath et al., 2010; van der Graaff et al., 2011). Since callosal neurons are excitatory and their major targets are the inhibitory interneurons of the contralateral hemisphere (Li and Pleasure, 2011), our findings are in accordance with the interneuronopathy theory suggesting that

Table 5
Correlation analysis: relationships of sensorimotor and frontal network functional connectivity with clinical, cognitive, and DT MRI variables*

Sensorimotor network					
No. of voxels	Region	x	y	z	Z
ALSFRS-r (negative relationship)					
87	R precentral gyrus	58	6	36	0.993
		54	−2	42	0.977
		62	8	26	0.951
35	R postcentral gyrus	66	−12	36	0.988
Disease progression rate (positive relationship)					
192	R postcentral gyrus	70	−12	32	0.991
CC-PMC FA (negative relationship)					
54	L postcentral gyrus	−62	4	14	0.984
	L precentral gyrus	−52	8	16	0.971
29	L postcentral gyrus	−58	−6	48	0.976
CC-SMA FA (negative relationship)					
26	R precentral gyrus	58	8	38	0.975
Frontal network					
Phonemic fluency (negative relationship)					
33	L superior frontal gyrus	−20	14	54	0.982
Semantic fluency (negative relationship)					
45	R insula	30	26	8	0.982
WCST global score (negative relationship)					
241	R superior frontal gyrus	18	34	32	0.991
CC-body FA (negative relationship)					
332	R superior frontal gyrus	18	38	48	0.991
74	R insula	34	22	12	0.968
31	L SMA	−6	10	62	0.991

The letters x, y, and z are coordinates in the Montreal Neurological Institute space. Key: ALSFRS-r, ALS Functional Rating Scale–Revised; CC, corpus callosum; CST, corticospinal tract; FA, fractional anisotropy; L, left; MRI, magnetic resonance imaging; PMC, primary motor cortex; R, right; SMA, supplementary motor area; WCST, Wisconsin Card Sorting test.
* $p < 0.05$ uncorrected.

severe microstructural damage to the corpus callosum of PLS patients contributes to their altered cortical excitability.

PLS patients showed an increased functional connectivity compared with controls not only in the sensorimotor areas but also in the frontal and left frontoparietal networks, supporting the concept that PLS, like ALS, is a multisystem disorder. Frontal and frontoparietal networks sustain executive functions, problem solving, attentional processes, and emotional salience processing, and overlap largely with regions recruited during the performance of cognitively demanding tasks (Smith et al., 2009). Although PLS seems to hit mainly the PMC, disease-related degeneration with ubiquitin inclusions is known to extend anteriorly to the non-motor frontal cortices and ventrally to the temporal cortices (Konagaya et al., 1998; Kosaka et al., 2011; Mochizuki et al., 2004; Tan et al., 2003). Phosphorylated TDP-43 immunohistochemistry in PLS revealed the presence of many positively stained neuronal cytoplasmic inclusions and dystrophic neuritis/neuropil threads in the affected frontotemporal cortex and subcortical nuclei (Kosaka et al., 2011). DT MRI study of PLS patients found WM abnormalities in extramotor areas (Agosta et al., 2013b; Iwata et al., 2011; van der Graaff et al., 2011). Cognition has received much lower attention in PLS than ALS. In early studies, cognition was reported to be normal in PLS (Konagaya et al., 1998; Tan et al., 2003). However, other investigators have reported a frontal lobe dysfunction syndrome in PLS cases (Caselli et al. 1995; Le Forestier et al., 2001; Piquard et al., 2006; Zago et al., 2008). A prospective study (Grace et al., 2005) found similar proportions of PLS and ALS patients who had deficits of verbal fluency and memory. In our study, roughly 15% of PLS patients showed frontal lobe dysfunction characterized by deficits in set-shifting and/or verbal fluency. The lower proportion of PLS patients with cognitive impairment in our cohort compared with previous ones (Grace et al., 2005; Piquard et al., 2006) may result from patient selection and different criteria used to define cognitive impairment. In addition, although the MRI protocol did not require active tasks, our patients should have been able to lie quietly in the scanner for about 1 hour. We believe that this may have selected higher-functioning subjects relative to those in previous studies that did not use strict inclusion/exclusion criteria.

An altered recruitment of the prefrontal cortex has been previously observed in ALS patients during letter fluency and confrontation naming tasks (Abrahams et al., 1996, 2004). Therefore, a disorganization of the frontal networks may contribute to the appearance of cognitive impairment also in PLS. However, the interpretation of the correlations that we found among frontal functional connectivity, cognitive variables, and WM tract damage is challenging. The functional connectivity of the left frontal and right insular regions of PLS patients was higher, and their verbal fluency deficits and damage to the CC-body were more severe. Conversely, the increased functional connectivity of the right superior frontal gyrus was associated with a better performance at the WCST test. These findings are counterintuitive, as the increased functional connectivity of the frontal cortex could be interpreted as a pathogenetic factor in the first case (as described above for the sensorimotor areas) and as a physiological compensation in an attempt to maintain an efficient cognitive performance in the second case. A possible explanation of such a complex constellation of findings could be a more heterogeneous spatial and temporal progression of cortical degeneration in extramotor compared with motor regions in MND patients (Bae et al., 2013). Longitudinal studies assessing the evolution of cortical involvement, WM tract damage, and cognitive deficits in PLS are now warranted to clarify this issue.

Previous studies investigating DMN connectivity in ALS reported conflicting results, with some authors detecting a reduced DMN connectivity in both the ACC and medial prefrontal cortices and the posterior cingulate and inferior parietal cortices (Mohammadi et al., 2009), and others finding a divergent connectivity pattern with a decreased connectivity of the frontal cortex and an increased connectivity of the precuneus (Agosta et al., 2013a), or no abnormalities (Tedeschi et al., 2010). Discordance across studies may be due to methodological differences in the fMRI analysis or inclusion of patients with different clinical and cognitive features. The low prevalence of cognitive deficits in our study may explain the DMN integrity in PLS patients.

A possible limitation of our study is that, as in the majority of previous reports, PLS was diagnosed according to the criteria of Pringle et al. (Pringle et al., 1992), which have a low specificity, as they do not allow exclusion of patients with subtle EMG abnormalities. Nevertheless, although we selected patients using the criterion of minimum 3 years from symptom onset (Pringle et al., 1992), only 2 subjects had a disease duration of less than 48 months at the time of MRI scan (38 and 46 months, respectively). Both patients have now been followed up clinically and with EMG for at least 1 additional year and did not develop LMN signs. In addition, correlation results did not survive the correction for multiple comparisons. However, the a priori definition of the variables to be correlated with each RSN of interest, as well as the fact that correlation analysis was limited to those clusters that showed significant group differences, should have minimized false-positive results. Finally, the objective of the present study was to apply a multimodal imaging approach to PLS patients to explore RSN characteristics in relation to clinical and cognitive features. Future studies should compare functional and structural connectivity in early PLS and ALS patients to identify diagnostic and prognostic markers in these conditions.

Disclosure statement

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