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Integration of structural and functional magnetic resonance imaging in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis as a system failure is a concept supported by the finding of consistent extramotor as well as motor cerebral pathology. The functional correlates of the structural changes detected using advanced magnetic resonance imaging techniques such as diffusion tensor imaging and voxel-based morphometry have not been extensively studied. A group of 25 patients with amyotrophic lateral sclerosis was compared to healthy control subjects using a multi-modal neuroimaging approach comprising T_1-weighted, diffusion-weighted and resting-state functional magnetic resonance imaging. Using probabilistic tractography, a grey matter connection network was defined based upon the prominent corticospinal tract and corpus callosum involvement demonstrated by white matter tract-based spatial statistics. This 'amyotrophic lateral sclerosis-specific' network included motor, premotor and supplementary motor cortices, pars opercularis and motor-related thalamic nuclei. A novel analysis protocol, using this disease-specific grey matter network as an input for a dual-regression analysis, was then used to assess changes in functional connectivity directly associated with this network. A spatial pattern of increased functional connectivity spanning sensorimotor, premotor, prefrontal and thalamic regions was found. A composite of structural and functional magnetic resonance imaging measures also allowed the qualitative discrimination of patients from controls. An integrated structural and functional connectivity approach therefore identified apparently dichotomous processes characterizing the amyotrophic lateral sclerosis cerebral network failure, in which there was increased functional connectivity within regions of decreased structural connectivity. Patients with slower rates of disease progression showed connectivity measures with values closer to healthy controls, raising the possibility that functional connectivity increases might not simply represent a physiological compensation to reduced structural integrity. One alternative possibility is that increased functional connectivity reflects a progressive loss of inhibitory cortical influence as part of amyotrophic lateral sclerosis pathogenesis, which might then have relevance to future therapeutic strategies.

Keywords: amyotrophic lateral sclerosis; motor neuron disease; neurodegenerative mechanisms; systems neuroscience; MRI/fMRI

Abbreviations: ALS = amyotrophic lateral sclerosis

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Introduction

The adult-onset neurodegenerative syndrome of amyotrophic lateral sclerosis (ALS) is characterized by a catastrophic and usually rapid failure of upper and lower motor neurons. Although the median survival is 3 years from symptom onset, ALS is also characterized by marked prognostic heterogeneity (Kiernan *et al*., 2011). The intense search for much needed diagnostic, prognostic and monitoring biomarkers (Turner *et al*., 2009) may also provide evidence for disease mechanisms and thus novel targets for therapeutic intervention.

Structurally, the motor system components affected as core neuropathological features of ALS are the upper motor neurons of the corticospinal tract and the functionally linked lower motor neurons in the brainstem and anterior horns of the spinal cord. The corpus callosum has been shown to be involved consistently across heterogeneous cases of ALS (Filippini *et al*., 2010). There is a consistent extramotor cerebral lesion in ALS, and an established clinicopathological spectrum with frontotemporal dementia (Phukan *et al*., 2007). Combined clinical and post-mortem analysis suggests a simultaneous upper and lower motor neuron degenerative process (Ravits and La Spada, 2009), explaining some of the characteristic clinical heterogeneity, and further supporting the concept of ALS as a system failure.

A comprehensive model of pathology in ALS must thus simultaneously consider the relationship between structural and functional changes within the degenerating cerebral network. Diffusion tensor imaging has revealed pathology in white matter tracts which faithfully matches historic post-mortem neuropathological studies in ALS (Smith, 1960; Filippini *et al*., 2010). Analysis of resting-state functional MRI reveals the temporal correlation between the low-frequency spontaneous fluctuations in the resting whole-brain that form several, functionally-distinct networks (Smith *et al*., 2009). This technique has been shown to be of potential clinical value as sensitive markers of disease (Greicius, 2008; van den Heuvel and Hulshoff Pol, 2010) and, as it does not require any physical activity, is particularly valuable in the clinical setting of ALS.

With the hypothesis that the characteristic structural white matter damage in ALS may be directly related to abnormal functional connectivity, both measures were integrated using a novel multimodal MRI approach.

Materials and methods

Subjects

Twenty-five patients with sporadic ALS were recruited from the Oxford Motor Neuron Disease Care and Research Centre as part of the Oxford Study for Biomarkers in Motor Neuron Disease ('BioMOx'). All patients were diagnosed by one of two experienced ALS neurologists (K.T., M.R.T.), according to El Escorial criteria, and all were either limb or bulbar in symptom onset. Patients with clinically suspected dementia were excluded from this study as ethical approval required informed consent. Otherwise, all patients with ALS for whom both diffusion tensor imaging and resting-state functional MRI sequence data were available (acquired in the same session) were included. This group overlapped with that used in a previously published MRI study of solely structural changes (Filippini *et al*., 2010).

All participants underwent clinical examination on the day of study (M.R.T.) with active follow-up. Functional status was measured using the revised ALS Functional Rating Scale (maximum score 48, falling with increasing disability). Disease duration was calculated from symptom onset to scan date in months. A rate of disease progression was then calculated as (48-revised ALS Functional Rating Scale)/disease duration (Ellis *et al*., 1999).

Limb involvement in ALS is typically asymmetrical in onset. Given the evidence for an active cortical pathological process (Ravits and La Spada, 2009), prior to analysis, patients were classified by the presumed 'dominant' hemisphere for disease onset. This was defined as the contralateral hemisphere to the side of the first limb weakness reported. By standardizing the image orientation according to this 'dominant' hemisphere (see below), any bilateral cerebral changes seen in the overall group results were not confounded by simple differences in laterality of disease onset among individual patients.

Fifteen healthy controls similar to the patients in age, gender and handedness for writing, and with an identical MRI protocol, were available for comparison. This control cohort differed significantly from the previously published structural MRI study allowing more contemporaneously acquired, as well as well age-matched datasets to be used. Ethical approval for all procedures was obtained prior to study (08/H0605/85) and written informed consent was obtained from all participants.

All participant characteristics are shown in Table 1.

Image acquisition

Scans were performed at the Oxford Centre for Clinical Magnetic Resonance Research using a 3T Siemens Trio scanner (Siemens AG) with a 12-channel head coil, and in line with consensus guidelines put forward by the 2010 Neuroimaging Symposium in ALS (NISALS) (Turner *et al*., 2011). The neuroimaging protocol included T_1-weighted, diffusion-weighted and resting-state functional MRI. High-resolution 3D whole-brain T_1-weighted MRI scans were acquired using a magnetization-prepared rapid gradient echo (MPRAGE) sequence (repetition time/echo time = 2040/4.7 ms, flip angle = 8°, 1 mm isotropic resolution, 6 min acquisition time) and whole-brain diffusion weighted imaging was performed using an echo planar sequence (repetition time/echo time = 10 000/94 ms, 2 mm isotropic, *b*-value = 1000 s/mm^2, 60 isotropically distributed orientations for the diffusion-sensitizing gradients, 10 min acquisition time). Whole-brain functional imaging at rest was performed using a gradient echo echo planar imaging sequence (repetition time/echo time = 3000/28 ms, flip angle = 89°, 3 mm isotropic resolution, 6 min acquisition time). For maximum consistency, subjects were instructed to close their eyes throughout this latter sequence, but to remain awake.

Image analysis

Broadly, ALS-specific structural changes were used as a substrate to identify changes in cerebral functional connectivity. The steps may be summarized as follows: (i) identification of the significant white matter tract differences between patients with ALS and healthy controls; (ii) characterization of the grey matter network connected to this abnormal white matter using tractography, to define an ALS-specific cortical network; and (iii) analysis of resting-state functional MRI changes in relation to this ALS-specific cortical network.

Table 1 Participant demographics and clinical features

	Patients with ALS	Healthy controls	*P*-value
n	25	15	
Mean age ± SD (range)	59 ± 12 (31–83)	53 ± 11 (34–70)	0.12
Gender (male : female)	18 : 7	9 : 6	0.49[a]
Handedness (right:left)	23 : 2	14 : 1	1[b]
El Escorial criteria (possible:probable:definite)	10 : 9 : 7	NA	
Disease duration (months) ± SD (range)	44 ± 36 (10–122)	NA	
Rate of disease progression ± SD (range)	0.55 ± 0.48 (0.11–1.91)	NA	
Age of onset (yrs) ± SD (range)	55 ± 12 (30–74)	NA	
Site of initial symptom onset (left limb:right limb:bulbar)	6 : 13 : 6	NA	
ALSFRS-R ± SD (range)	34 ± 4 (26–43)	NA	

a Yates continuity correction.
b Fisher's Exact test.
ALSFRS-R = revised ALS Functional Rating Scale.

Preprocessing and analysis of imaging data was performed using the FMRIB Software Library (FSL) tools (www.fmrib.ox.ac.uk/fsl) (Smith *et al*., 2004).

Structural connectivity analysis using tract-based spatial statistics and probabilistic tractography

First, a map of the ALS group white matter tract damage was created by investigating voxel-wise differences in diffusion tensor imaging indices using tract-based spatial statistics and a permutation-based non-parametric inference within the framework of the general linear model (Nichols and Holmes, 2002; Filippini *et al*., 2010). Those images from patients reporting initially left-sided limb symptoms (presumed right hemisphere disease onset) were flipped along their *x*-axis so that the hemisphere of disease onset was consistent and aligned on the same side of the image grid for all patients. The images from matched control subjects' images were also flipped for consistency between groups. Results were all considered significant for $P < 0.05$ (after initial cluster-forming threshold at *P*-uncorrected = 0.05), fully corrected for multiple comparisons. Using probabilistic tractography, tract-based spatial statistics significant white matter differences were then used as seed regions of interest to reconstruct and identify the tracts damaged in ALS (characterized by reduced fractional anisotropy and increase in the related measure of radial diffusivity, a surrogate for secondary Wallerian-type demyelination, see Filippini *et al*., 2010). A group-level map of the grey matter network related to this white matter damage was then obtained (Behrens *et al*., 2007) (see Supplementary material for more details).

Tractography-derived functional connectivity analysis

Individual preprocessing consisted of motion correction, brain extraction, spatial smoothing using a Gaussian kernel of full-width at half maximum of 6 mm, and high-pass temporal filtering with a cut-off of 150 s (0.007 Hz). Extra care was given to the registration process, using for instance a non-linear normalization derived from a voxel-based morphometry approach (Douaud *et al*., 2007), ensured that the functional images were well aligned across subjects in the standard space (see Supplementary material).

The between-subject analysis of the resting-state functional MRI data was then carried out using a novel dual-regression technique (see Supplementary material), an approach that allows voxel-wise comparisons of resting functional connectivity (Beckmann *et al*., 2009; Filippini *et al*., 2009). Here, the aim was to investigate the functional connectivity directly related to the altered structural connectivity in patients with ALS. Consequently, the group-averaged grey matter network associated with the altered structural connectivity was used as an initial map that was then spatially regressed out from the resting-state functional MRI data set of each subject, instead of the traditional group-level independent component analysis maps. This tailored dual-regression protocol therefore allowed the integration of structural and functional connectivity information, and the comparison of the resulting functional connectivity across groups using non-parametric testing. Results were considered significant for $P < 0.05$ (after initial cluster-forming thresholding at *P*-uncorrected = 0.05), fully corrected for multiple comparisons.

Results

The amyotrophic lateral sclerosis-specific white and grey matter structural connectivity network

Tract-based spatial statistic mapping of fractional anisotropy and radial diffusivity differences between the patients with ALS and the healthy controls demonstrated a highly significant bilateral decrease of fractional anisotropy and co-localized increase of radial diffusivity in the body of the corpus callosum and rostral corticospinal tracts (in keeping with the previously published study that included many of the same patients but a substantially different control group; Filippini *et al*., 2010). Tractography from the region of interest defined by the intersection of these significant tract-based spatial statistics results in the fractional anisotropy standard-space of each subject, revealed that the contributing white matter tracts were essentially the motor-related part of the corpus callosum fibres, the corticopontine and corticospinal tracts, the medial lemniscus and the superior thalamic radiations (Fig. 1).

The grey matter regions corresponding to these white matter tracts were mainly composed bilaterally of the primary sensorimotor and premotor cortex (medially and laterally), the supplementary motor area, the superior parietal lobule [medial part

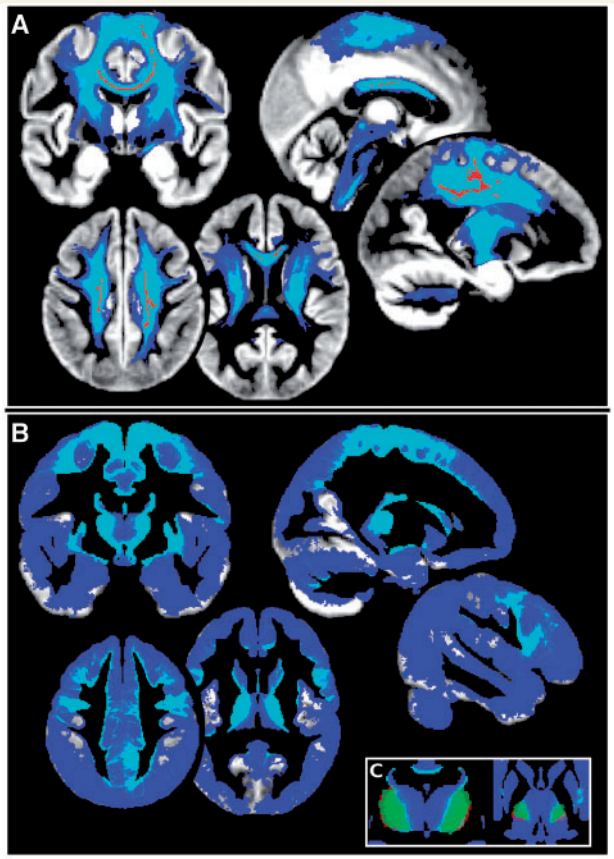

Figure 1 Structural connectivity associated with the ALS-specific white matter damage. (**A**) Average of the tractography results (thresholded at 500 streamlines) obtained from the significant tract-based spatial statistics differences (in red) overlaid on the average grey matter. The tracts reconstructed virtually define the motor part of the corpus callosum, the corticopontine and corticospinal tracts, the medial lemniscus and the superior thalamic radiations. (**B**) Average of the tractography results in the grey matter (unthresholded): the grey matter regions related to the white matter damage are essentially the primary sensorimotor and premotor cortex, the supplementary motor area, the superior parietal, the pars opercularis, the posterior putamen and the thalamus. (**C**) Expanded view of the thalamus: the highest number of streamlines spatially correspond to the premotor (in green) and motor (in red) connections of the thalamus [probabilistic atlas from Behrens *et al*. (2003)]. Radiological convention, left is right.

of Brodmann areas (BA) 5M and 7A], the pars opercularis (Broca's area, BA44), the posterior putamen and the motor parts of the thalamus [according to the Jülich atlas and the probabilistic atlas derived from Behrens *et al*. (2003), both available in FSL] (Fig. 1).

Functional connectivity in relation to the altered structural connectivity in amyotrophic lateral sclersosis

The functional connectivity directly associated with the ALS-specific white matter involvement was found to be significantly increased in patients with ALS compared with the healthy controls. This increase was essentially located within the primary sensorimotor and premotor cortex, more markedly on the left, in the anterior and motor cingulate and paracingulate areas, as well as bilaterally in the frontal and central operculum (essentially in the secondary somatosensory cortex), the left inferior parietal lobule and thalamus, right frontal eye field and dorsolateral prefrontal cortex (BA8 and BA9) (Fig. 2). With the exception of the cingulate and paracingulate areas, the spatial distribution of the increase of functional connectivity corresponded to the grey matter regions where the patients with ALS had the least tractography-built 'virtual tracts' (streamlines) compared with the healthy controls (Fig. 3).

Grey matter, white matter and functional connectivity combined

In all participants, the functional connectivity measure obtained in those regions found to be significantly different between the two groups, correlated negatively with fractional anisotropy ($P < 10^{-2}$) and grey matter volume ($P < 10^{-3}$), and positively with radial diffusivity ($P < 10^{-3}$). The combination of results in grey matter volume, white matter fractional anisotropy and functional connectivity measures discriminated the controls from patients well, despite the heterogeneity of the patient group (Fig. 4). The patient with ALS positioned nearest to the control group was noted to have a low level of disability and few upper motor neuron signs on examination.

Disease progression and connectivity

Within the patients with ALS, the relationship between the structural and functional imaging measures and the rate of disease progression as well as disease duration were explored. Both were plotted against the mean of the significantly different fractional anisotropy, radial diffusivity and functional connectivity network values for each patient with ALS. The relationships were non-linear power functions and overall the slower the disease progression and the longer the disease duration, the more the values tended towards those of controls (Fig. 5).

Discussion

Using a novel methodological approach to integrate structural and functional connectivity information, this MRI study demonstrated increased functional connectivity directly associated with an 'ALS-specific' grey matter network, predefined by the consistent regions of white matter damage, and spanning sensorimotor, premotor, prefrontal and thalamic regions. Patients with a slower rate of disease progression (not only longer disease duration) presented connectivity values more comparable to those of healthy controls. Finally, a combination of structural and functional connectivity measures (an ALS 'cerebral signature') appeared to qualitatively discriminate patients from controls, suggesting that the use of a multimodal MRI approach may have greater value as a potential diagnostic biomarker in ALS.

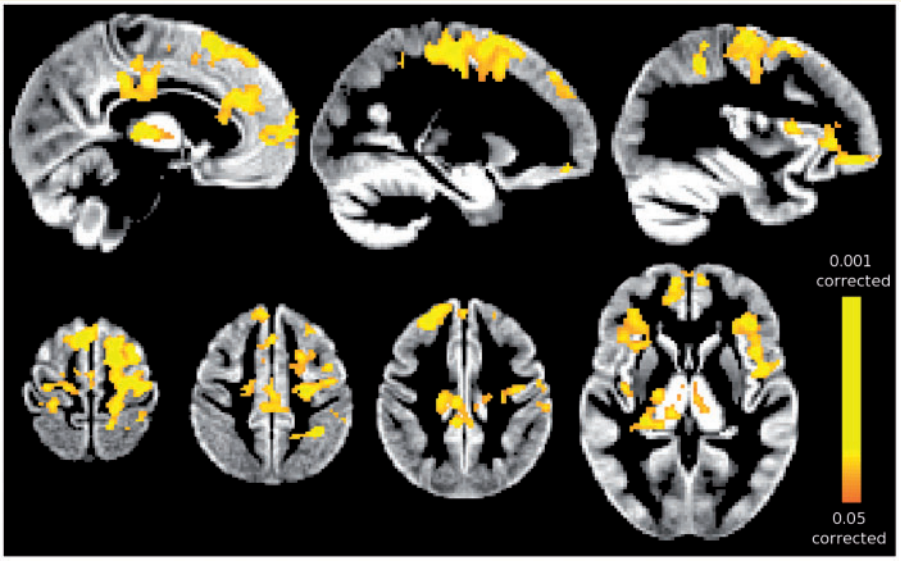

Figure 2 Functional connectivity directly related to the ALS-specific white matter damage. A significant increase of functional connectivity was found in the patients with ALS compared with the healthy controls ($P < 0.05$ corrected) within primary sensorimotor and premotor cortex, anterior and motor cingulate areas, frontal and central operculum, and in the thalamus. Radiological convention, left is right.

Defining and exploring a disease-specific cerebral network

This novel approach initially used a probabilistic crossing-fibre tractography algorithm (Behrens *et al*., 2007) to reconstruct the tracts defining the white matter damage characterized by a decrease of fractional anisotropy and co-localized increase of radial diffusivity. This allowed the determination of a group-level map of the grey matter network related to the tract-based spatial statistics white matter results, and this whole-brain map was then used as a spatial regressor to find the associated temporal dynamics for each subject. Extra care was given to the registration process, to ensure that there was an excellent correspondence between structural, diffusion and functional spaces. The approach combining the boundary-based registration to a non-linear fieldmap correction (Greve and Fischl, 2009), followed by a voxel-based morphometry-style registration of the functional images (Douaud *et al*., 2007), also proved to reduce dramatically the variability across each participant's imaging dataset in the standard space (more details are provided in the Supplementary Material). Following the second step of the dual-regression approach (Beckmann *et al*., 2009), these time-courses were finally used as temporal regressors to find subject-specific functional connectivity maps thus associated with the group-level spatial structural connectivity map. This study therefore defines a protocol that directly relates white matter integrity, structural connectivity and functional connectivity, and is applicable to any well-localized voxel-wise white matter result corresponding to a highly specific grey matter network. There is clear potential for this method to be applied to the study of other neurodegenerative disorders.

Other studies of functional connectivity in amyotrophic lateral sclerosis

The observation of increased cortical activation and a 'boundary shift' (an extended pattern of activation beyond standard motor regions), has been consistently observed using both PET and functional MRI (Lule *et al*., 2009) in patients with ALS but during performance of a motor task rather than the resting state. This functional activation has been found to extend to the premotor cortex, sensorimotor cortex and anterior insular cortex, inferior parietal lobule, anterior cingulate areas and basal ganglia even though participants had no overt cognitive impairment (Kew *et al*., 1993; Konrad *et al*., 2002, 2006; Tessitore *et al*., 2006; Lule *et al*., 2007; Stanton *et al*., 2007; Mohammadi *et al*., 2010). Notably, all of these regions exhibited an increase in functional connectivity in the present study, i.e. an increase in the temporal correlation of the spontaneous fluctuations between these regions and the grey matter network defined by the ALS-specific white matter microstructural damage. This 'synchrony coupling' between motor and extramotor regions might then be the substrate for the consistent observation of extended activation during a motor task in patients with ALS.

An initial resting-state functional MRI study in ALS reported significantly decreased functional connectivity within the default-mode and sensorimotor networks (Mohammadi *et al*., 2009). This apparent difference might be explained by the whole-brain approach used, which cannot guarantee correspondence of a given independent component across subjects. A subsequent region of interest-based study found evidence of functional disconnection between corresponding points of the left and right primary motor cortices (Jelsone-Swain *et al*., 2010), suggesting

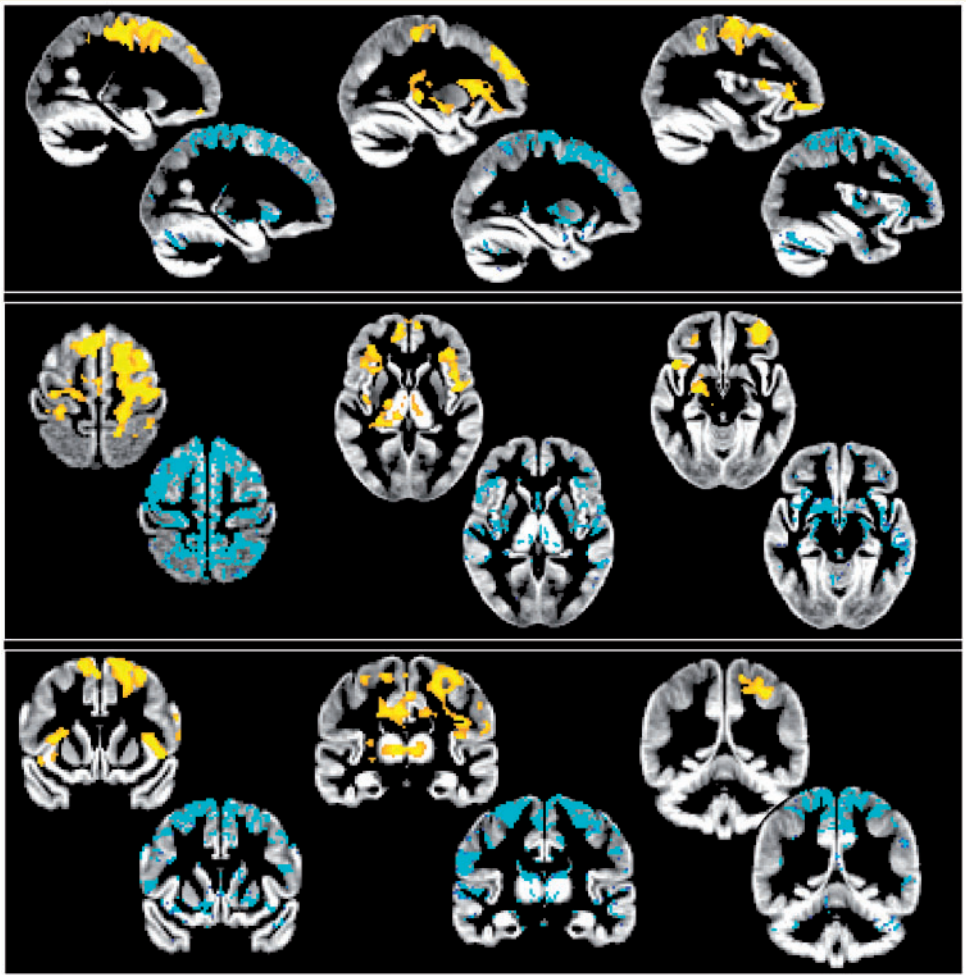

Figure 3 Increase of functional connectivity and lower structural connectivity. The spatial distribution of the significant increase of functional connectivity in the patients with ALS (in red–yellow, colour bar in Fig. 2) corresponded to the areas where the patients with ALS had lower number of streamlines compared with the healthy controls (in blue, thresholded at 10 streamlines of difference on average), with the exception of the cingulate cortex. Radiological convention, left is right.

that interhemispheric integrity was compromised in ALS, a hypothesis supported by both post-mortem (Smith, 1960; Brownell *et al*., 1970) and diffusion tensor imaging (Filippini *et al*., 2010; Iwata *et al*., 2011; van der Graaff *et al*., 2011) observations of consistent corpus callosum involvement. Another study using a region-of-interest approach noted a trend to increased functional connectivity between left primary sensorimotor cortex and motor cingulate cortex (Agosta *et al*., 2011), a region that corresponds to one of the significant clusters reported in the present study (Fig. 2).

What is the explanation for increased functional connectivity in amyotrophic lateral sclerosis?

At face value the increase in functional connectivity seen in the ALS group might be considered a physiological, compensatory response to disease-related loss of structural network integrity. However, the positive relationship of functional connectivity with rate of disease progression, (a trend noted in another study of connectivity, Verstraete *et al*., 2010), prompts speculation as to whether it might have a more active role in pathogenesis. One possibility is that increased functional connectivity arises as a result of a loss of cortical inhibitory neuronal influence, with widespread effects as a result of consistent (possibly early) involvement of the corpus callosum in ALS (Innocenti, 2009; Filippini *et al*., 2010).

Evidence of loss of GABA-ergic cortical neuronal influence linked to the pathogenesis of ALS has arisen from a range of research. Paired-stimulus transcranial magnetic stimulation studies in patients with sporadic ALS demonstrate cortical hyperexcitability (Yokota *et al*., 1996) and there appeared to be relative preservation of such inhibitory circuitry in a rare familial form of ALS with consistently slower disease progression (Turner *et al*., 2005*b*). A longitudinal study in unaffected carriers of pathogenic mutations of ALS-related genes also revealed an abrupt increase in cortical hyperexcitability in the months prior to symptom onset (Vucic *et al*., 2008).

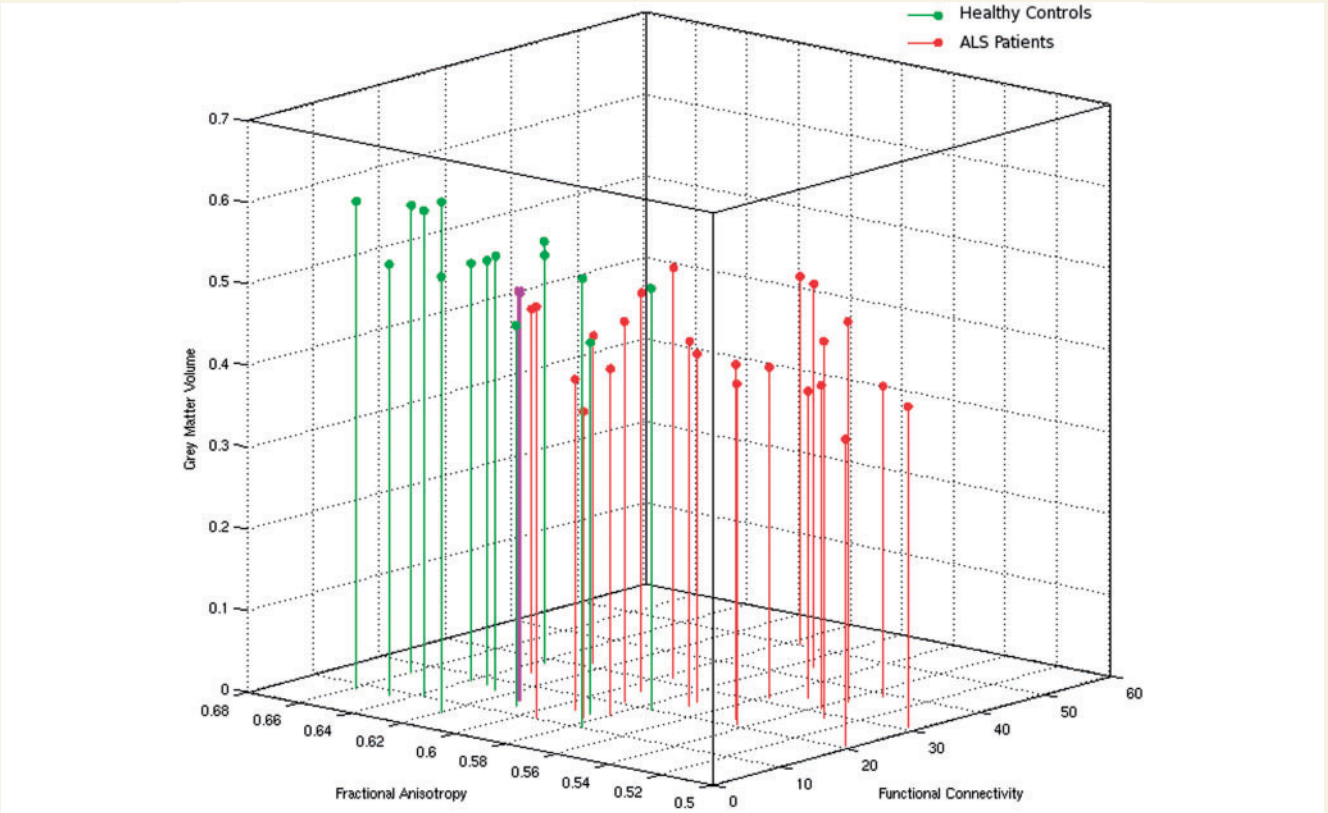

Figure 4 3D stemplot of grey matter, fractional anisotropy and functional connectivity for all participants. The two populations (patients with ALS in red, controls in green) were well discriminated using all three modalities: structural information using grey matter volume, diffusion information with fractional anisotropy values and functional connectivity. The patient with ALS nearest to the control group (shown in purple) had a low level of disability and few clinical upper motoneuron signs. This discrimination representation was qualitative however, circularity inference issues preventing the assessment of sensitivity and specificity based on these imaging results *a posteriori*.

Most of the regions of increased functional connectivity demonstrated in this study have been reported to show a considerable decrease of the density of GABA-ergic interneurons (Maekawa *et al*., 2004), and $GABA_A$ receptor messenger RNA subunit expression in post-mortem immunohistochemical studies (Petri *et al*., 2003, 2006). Furthermore, the network of increased functional connectivity found in the present study is remarkably similar to the pattern of significant decreased binding of the $GABA_A$ receptor PET ligand [^{11}C]-flumazenil in a group of patients with sporadic ALS [Fig. 6, adapted from Turner *et al*. (2005*a*)], although [^{11}C]-flumazenil PET data were not available in the participants of the present study for more certain comparison.

Speculatively therefore, if the increased functional connectivity demonstrated here within the disintegrating ALS cerebral network does indeed reflect a wider failure of inhibitory influences, this adds to the evidence that a central interneuronopathy may be a key aspect of pathogenesis (whether primary or secondary). A fuller understanding of the genetic basis, development and molecular biology of interneuronal function is then a priority, along with consideration of therapeutic strategies that support cortical inhibitory function.

Caveats and limitations

The distribution of the ALS-specific network presented in this study was entirely data-driven based upon whole-brain analysis. The abnormal pattern observed in the functional cortical network was directly obtained from the consistent white matter disruption in the patients. Abnormalities extended beyond the primary motor regions to premotor, sensorimotor and somatosensory networks. ALS is now understood as a clinicopathological spectrum with frontotemporal dementia (Phukan *et al*., 2007). This study was not able to explore extramotor changes in relation to the estimated 30% of patients with ALS with detectable mild cognitive impairment, and so occult changes in cognitive cerebral networks across patients might in theory confound the results. Resolving this issue fully will require detailed neuropsychological study across a wide range of impairment levels (including those with frontotemporal dementia).

Furthermore, this study was not powered for sub-group analysis by regional involvement e.g. bulbar versus limb or upper motor neuron versus lower motor neuron-predominant. A large diffusion tensor imaging-based study demonstrated consistent structural changes across all such phenotypes (van der Graaff *et al*., 2011), supporting the view of ALS as a syndrome. However, a

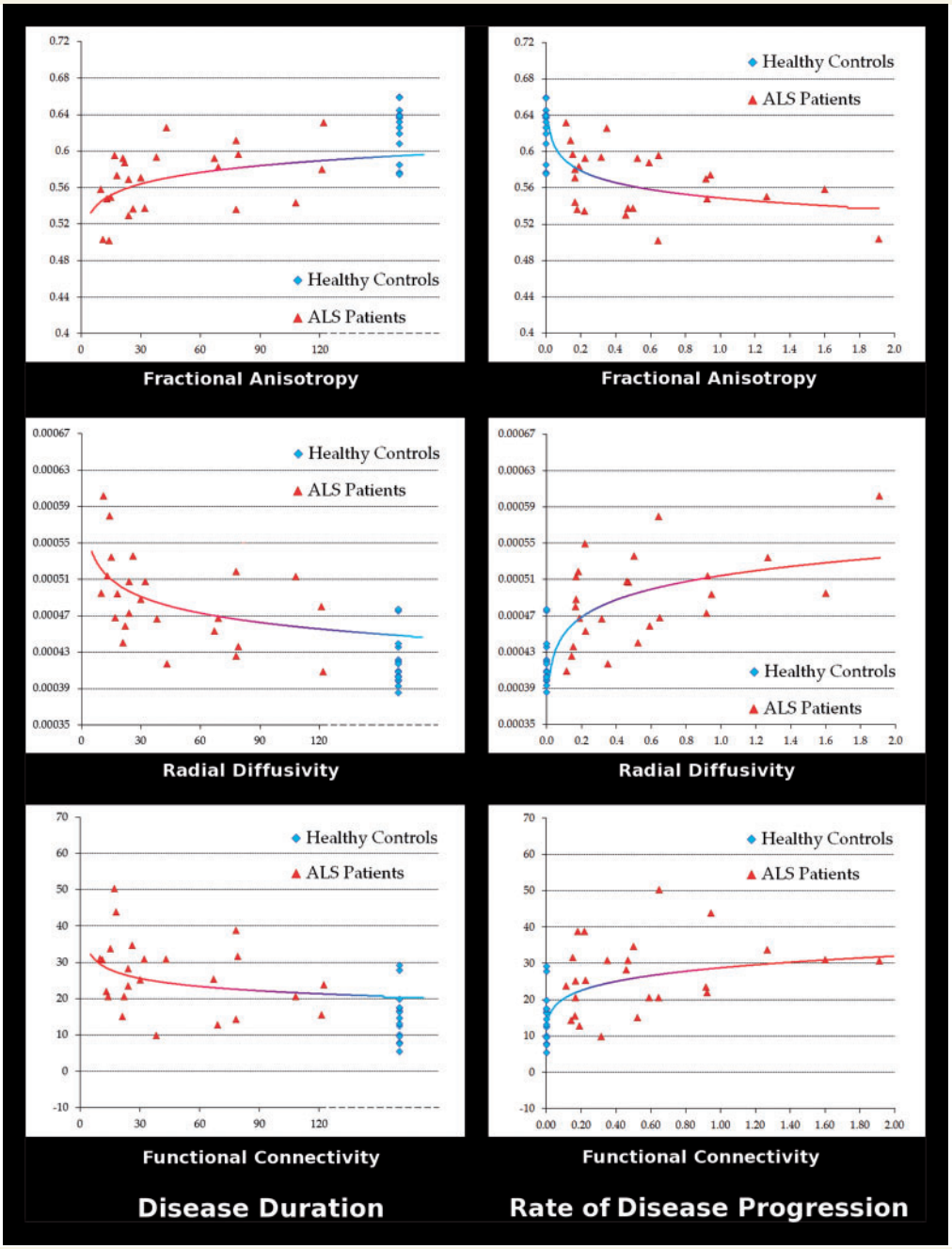

Figure 5 Fractional anisotropy, radial diffusivity and functional connectivity as function of disease duration and rate of disease progression. Plots representing fractional anisotropy, radial diffusivity and functional connectivity values for the patients with ALS (in red) obtained from the corresponding group-difference significant region of interest mean (y-axis), depending on the disease duration or rate of disease progression of the patients (x-axis). Qualitatively, a power model was best in describing all the patients' trajectories (disease duration: fractional anisotropy: $y = 0.51 \times x^{0.032}$, radial diffusivity: $y = 0.0006 \times x^{-0.054}$, functional connectivity: $y = 40 \times x^{-0.13}$; rate of disease progression: fractional anisotropy: $y = 0.55 \times x^{-0.033}$, radial diffusivity: $y = 0.0005 \times x^{0.057}$, functional connectivity: $y = 29 \times x^{0.15}$). Remarkably, each trajectory determined only from the patients' values passed through the control group values (in blue). This suggests that functional connectivity measures tend to normality in those with more benign disease progression.

longitudinal combined structural and functional study in relation to the spread of symptoms among initially focally affected phenotypes might provide further insight.

Some of the patients in this study were of relatively long disease duration, which is an unwanted bias resulting in part from the population attending specialist clinics and those willing to undergo non-therapeutic research. Therefore, the longitudinal study of those seen soon after symptom onset remains an important experiment, although individuals diagnosed very soon after symptom onset typically have an aggressive disease course (the 'referral

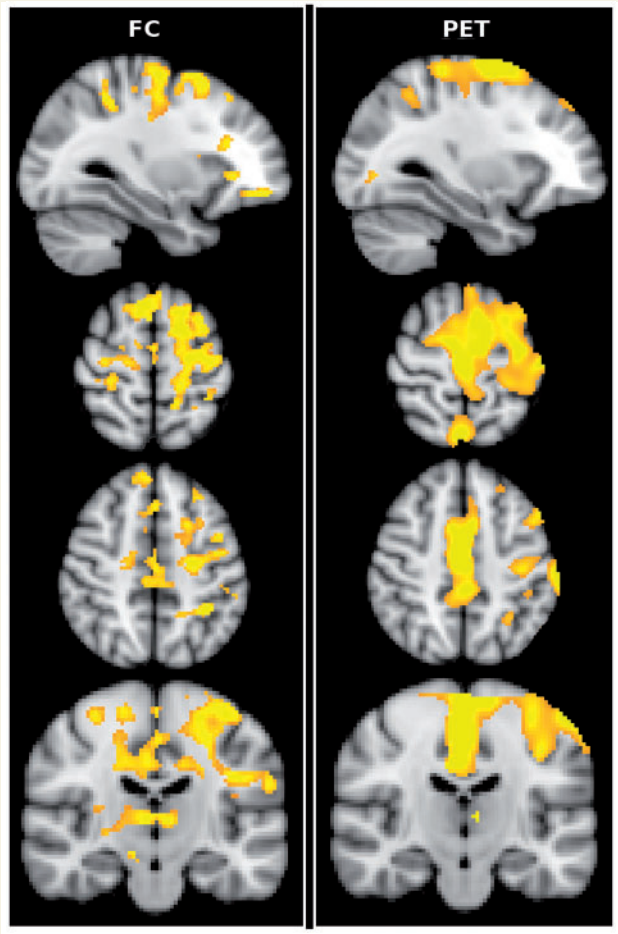

Figure 6 Regions of significant increase of functional connectivity and reduced $GABA_A$ receptor ligand binding in two separate studies of patients with sporadic ALS. There is strong overlap in the spatial pattern between MRI and PET analyses although they were carried out on two different ALS patient populations {resting-state functional MRI data from this study shown on the left, and reductions in PET [11C]-flumazenil binding on the right adapted from Turner *et al*. (2005*a*)}. Radiological convention, left is right. FC = functional connectivity.

delay paradox'; Turner and Al-Chalabi, 2002) that may make serial MRI challenging.

Finally, the important requirement for age and gender matching of controls and availability of all MRI sequences meant that only 15 healthy participants were available for comparison. However, the use of permutation-testing non-parametric inference throughout this study greatly reduces the potential for statistical errors as a result of the smaller number of subjects in relation to the patient group.

Conclusion

The system failure in ALS appears to be characterized by increased functional connectivity in close relation to a disintegrating and widespread structural network. The study of patients with ALS in very early disease, and perhaps also pre-symptomatic carriers of genetic mutations associated with familial ALS, may help to resolve issues of causality, which are currently uncertain. If structural changes are found to be 'driven' by functional alterations, which might then reflect loss of inhibitory influences, then this offers new potential for therapeutic intervention.

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Supplementary material

Supplementary material is available at *Brain* online.

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