Probabilistic diffusion tractography: a potential tool to assess the rate of disease progression in amyotrophic lateral sclerosis

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The goal of probabilistic tractography is to obtain a connectivity index along a white matter pathway that reflects fibre organization and is sensitive to pathological abnormalities contributing to disability. Here, we present the development of voxel-based connectivity measures along the tractography-derived corticospinal tract (CST). We investigated whether these connectivity measures are different in patients with amyotrophic lateral sclerosis (ALS) and correlate with the rate of disease progression. We also investigated whether fractional anisotropy (FA), which reflects directional coherence of fibre tracts, is reduced in the CST of ALS patients and relates to disease progression rate. Thirteen patients with probable or definite ALS and 19 healthy subjects were studied. The probabilistic tractography algorithm segmented the bilateral CST, along which FA and connectivity values were obtained. To take into account the asymmetric distribution of connectivity values, two summary statistic measures that focused on voxels with higher connectivity values were selected and then used in the analysis, together with the mean connectivity and the mean FA. To complete the analysis, the same summary measures for FA were included. Differences in all these indices between patients with moderate or rapid disease progression rate and controls were investigated using linear regression, adjusted for age and white matter fraction. The association between FA or connectivity in the CST and the disease progression rate was assessed using linear regression. Patients with a rapid disease progression rate had significantly lower summary connectivity measures than controls in the left CST, but there was only a borderline statistical difference in mean connectivity. Patients with rapid progression had a significantly lower mean FA, and any other FA measure, in both CSTs than controls. When only patients were considered, strong associations between the rate of disease progression and all the connectivity measures in the left CST were found (P-values between $P < 0.001$ and $P = 0.002$, partial correlation coefficients between $-0.90$ and $-0.82$). However, there was no evidence of an association between disease progression rate and any of the FA measures in the bilateral CST. Our findings suggest that FA and connectivity provide complementary information, since FA is sensitive to the detection of all the group differences, whereas the summary connectivity measures correlate with disease progression rate. The development of such connectivity measures raises their potential as markers of disease progression in ALS, and provides guidance for their use in other neurological diseases.

Keywords: tractography; diffusion; ALS; progression; connectivity

Abbreviations: ALS = amyotrophic lateral sclerosis; CST = corticospinal tract; DTI = diffusion tensor imaging; FA = fractional anisotropy; WMF = white matter fraction

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Introduction

The pathological hallmark of amyotrophic lateral sclerosis (ALS) is the degeneration of both the upper and lower motoneurons (Rowland and Shneider, 2001). Whilst the lower motoneuron involvement is routinely quantified by electromyography, the assessment of upper motoneuron damage is more challenging. New MRI techniques, such as MR spectroscopy and diffusion tensor imaging (DTI), have shown potential in assessing upper motoneuron injury (Ellis et al., 1999; Toosy et al., 2003; Kaufmann et al., 2004).

DTI is able to characterize the diffusion properties of water molecules in vivo (Basser et al., 1994). This diffusion is restricted or hindered by the presence of barriers, such as cellular membranes and subcellular structures, which behave as obstacles to the free motion of water. As a result, the water molecules tend to diffuse preferentially in orientations relatively free of obstruction, such as along axons, leading to a directional bias or anisotropic diffusion (Pierpaoli and Basser, 1996). Therefore, changes in anisotropy, which can be quantified by DTI, reflect changes in tissue microstructure and organization of fibres (Beaulieu, 2002). In particular, it has been suggested that a reduction in anisotropy can reflect axonal fibre degeneration and myelin breakdown in both the peripheral and central nervous system (Beaulieu et al., 1996; Pierpaoli et al., 2001).

Recent publications have reported that reduced anisotropy is seen in the corticospinal tract (CST) in patients with ALS (Ellis et al., 1999; Toosy et al., 2003), occurs early in the course of the disease (Graham et al., 2004; Sach et al., 2004) and relates to disease severity (Ellis et al., 1999). However, not all studies have found a correlation between anisotropy and disease severity (Toosy et al., 2003; Hong et al., 2004), and it is not clear whether an association between anisotropy and rate of disease progression exists.

In general, previous studies have used a region-of-interest approach, which consists of manually defining regions of interest along the CST of each subject, using anatomical knowledge (Ellis et al., 1999; Toosy et al., 2003; Hong et al., 2004). This method has technical limitations and is operator-dependent. An alternative technique has been voxel-based morphometry (VBM) (Abe et al., 2004; Sach et al., 2004), in which anisotropy maps are co-registered into a standard space, allowing comparisons of anisotropy value between groups. However, the spatial transformation of the diffusion tensor images into a standard space may be challenging (Park et al., 2003).

Here, we propose a novel method for investigating CST differences between ALS patients and controls, using probabilistic diffusion tractography. Diffusion tractography relies on the alignment in white matter between the dominant orientation of local water diffusion and the mean orientation of white matter fibres (Beaulieu, 2002). Using this information, tractography methods can infer in vivo continuity of fibres from voxel to voxel, and reconstruct an entire white matter pathway (Mori and van Zijl, 2002). Among all the available tractography algorithms, the probabilistic approaches are particularly attractive, because they generate probabilistic maps of fibre connectivity between brain regions and can trace pathways into grey matter (Behrens et al., 2003b; Parker et al., 2003). A key goal of probabilistic diffusion tractography methods is to develop a voxel-based connectivity index that is sensitive to structural changes contributing to clinical disability and that can be used along with fractional anisotropy (FA) in clinical studies. Indeed, this voxel-based connectivity may reflect not only the integrity and coherence of white matter tracts, as anisotropy does, but also some additional features, such as tract geometry and length.

Probabilistic tractography can be employed in the investigation of tissue damage in ALS in two different ways. First, it can be used to segment the CST in vivo, and allow calculation of diffusion indices along this tract in an observer-independent way. Second, it can allow calculation of a voxel-based connectivity index. Whilst the first approach has been shown recently to be helpful in guiding the placement of regions of interest on the CST defined by the tractography methods (Aoki et al., 2005), the second approach has not been applied to ALS. Only a pilot study in patients with optic neuritis has been published, and it has suggested that a voxel-based connectivity index might be sensitive to the reduction of fibre integrity, and might reflect the degeneration of axons (Ciccarelli et al., 2005). Therefore, the development of such an index has important clinical applications in patients with ALS, and it may become a useful marker of disease progression with the potential to measure the response to new treatments.

This study was carried out to test the hypothesis that voxel-based connectivity of the CST is different in patients with ALS, reflecting the upper motoneuron damage, and correlates with the rate of disease progression. We employed a probabilistic tractography algorithm to trace the CST, by first performing a connectivity segmentation of the internal capsule, and then by tracking connections between the internal capsule and the primary motor cortex. We also investigated whether anisotropy is reduced in the tractography-derived CSTs of ALS patients and relates to disease progression rate.

Methods

Subjects

We recruited 13 patients (mean age = 54 years, SD = 13, 12 males and one female) with probable or definite ALS (Brooks et al., 2000), who attended the Motor Neuron Disease clinic at the National Hospital for Neurology and Neurosurgery, in London. All patients underwent a neurological assessment the day of their scan, and were scored on the ALS functional rating scale (ALSFRS) through a questionnaire (1996). This scale is weighted towards limb and bulbar function, and gives a total severity score out of 40. Patients with...
greater disability have a lower score. The rate of disease progression rate was calculated by using the following formula (Ellis et al., 1999):

\[
\text{Disease progression rate} = (40 - \text{ALSFRS score})/\text{disease duration}
\]

The median rate of disease progression in patients was 0.472 units/month, the mean was 0.72, and SD was 0.68. In each patient, the Ashworth spasticity scale and the muscle strength scores were assessed for each limb. The muscle strength score, which has been often used to assess muscle strength in patients with ALS (Bourke et al., 2001; de Carvalho et al., 2003), was obtained in the following way: on each side, seven selected upper limb muscles and six selected lower limb muscles were tested and scored from 0 to 5 according to the Medical Research Council (MRC) muscle strength scale. Modifications to the MRC scale add either a minus or a plus to the score. For example, 4− represents a muscle that is slightly weaker than one with a score of 4. We have made 4− equal to 3.50 and 4+ equal to 4.5. By adding the MRC scored for each limb we obtained a muscle strength score, whose maximum value was 35 for each upper limb and 30 for each lower limb. All patients were on treatment with riluzole. Patients’ clinical characteristics are given in Table 1.

Nineteen healthy controls (mean age = 39 years, SD = 11, 4 females and 15 males) were recruited. The difference in age between patients and controls was taken into account in the statistical analysis.

All subjects gave informed, written consent before the study, which was approved by the local research ethics committee.

MRI protocol

All imaging was performed on a 1.5 T Signa Echospeed MRI system (General Electric, Milwaukee, WI, USA) with a standard quadrature head coil and maximum gradient strength of 22 mT/m. The diffusion protocol consisted of a single-shot diffusion-weighted-echo planar imaging (DW–EPI) sequence. The diffusion acquisition parameters were as follows: field of view (FOV) = 220 × 220, matrix = 96 × 96 reconstructed as 128 × 128, giving a final in-plane resolution of 1.7 × 1.7 mm², 60 contiguous axial slices, 2.3 mm slice thickness. The diffusion gradients were applied along 54 directions (diffusion times of \( b = 32 \) ms and \( \Delta = 40 \) ms, maximum b-factor \( b = 1150 \) s/mm²) (Jones et al., 1999). Six volumes with no diffusion weighting were acquired. An additional six volumes with a low diffusion weighting (\( b_{\text{min}} = 300 \) s/mm²) were collected to give the option of reconstructing the diffusion tensor from the combination of volumes with \( b_{\text{min}} \) and \( b_{\text{max}} \) diffusion weighting. This has the effect of reducing the vascular contribution (perfusion) to the diffusion parameters (Henkelman et al., 1994) and to reduce the signal intensity of CSF, hence reducing partial volume effects (Xing et al., 1997). Cardiac gating was used to reduce motion artefacts due to pulsation of blood and CSF (Wheeler-Kingshott et al., 2002). The diffusion data acquisition time was ~25 min (depending on heart rate). Correction of eddy-current distortions in DW–EPI was performed using a two-dimensional image registration technique (Synms et al., 1997). The signal-to-noise ratio (SNR) was calculated on the b0 images of a randomly chosen subject by multiplying the mean signal intensity of a region of interest inside the brain (located within the subcortical white matter) by 0.66, and dividing the result by the SD of a region of interest outside the brain (and away from any imaging artefacts) (Henkelman, 1985). It was equal to 16. The data were then processed to determine the diffusion behaviour on a voxel-by-voxel basis (Basser et al., 1994), from which FA maps were calculated.

All subjects had also high-resolution T1-weighted scans obtained with a 3D inversion-recovery prepared spoiled gradient recall (IR–SPGR) sequence of the brain (inversion time (TI) = 450 ms, repetition time (TR) = 2 s, echo time (TE) = 53 ms, FOV = 310 × 155, matrix = 256 × 128, in-plane resolution of 1.2 × 1.2 mm², 156 contiguous axial slices, 1.2 mm slice thickness).

MRI analysis

Probabilistic tractography algorithm

The probabilistic tractography algorithm that we used is described elsewhere (Behrens et al., 2003a, b). Here we briefly summarize its main principles. The posterior probability distribution on the principal diffusion direction is estimated at each voxel. The width in this distribution represents uncertainty in diffusion direction,
which is due to factors such as the potential co-existence of many fibre directions within a voxel, image noise and subject motion in the scanner. The probabilistic tractography algorithm uses these local probability distributions to estimate the probability that a fibre pathway (or streamline) leaving the ‘seed voxel’ will pass through any other voxel.

Probabilistic tractography analysis

The aim of our tractography analysis was to generate summary measures of the CST in each subject, which were (i) measures of FA; and (ii) a voxel-based connectivity index. In order to compute these summary measures, the first step was to use the tractography algorithm to delineate the CST in each subject, as detailed below. The second step included plotting the voxel values of FA and connectivity within the tractography-derived CST in each subject, and then aggregating them over subjects, in order to select the summary statistic that best describes changes in the FA and connectivity distributions.

We assume that a voxel-based summary connectivity measure is sensitive to the integrity of the white matter fibres. Indeed, at each voxel, the local uncertainty in white matter orientation is dependent upon the integrity of the white matter fibres. Hence, when probabilistic tractography passes through a region of low integrity, the width of the estimated connectivity distribution increases, and consequently, the probability, or connectivity, values beyond this region decrease. Therefore, tractography passing through the CST in patients with ALS is expected to provide a lower connectivity value in each voxel within the tract compared with controls.

Segmenting the CST. Thus, in order to delineate the CST we used tools from the Oxford Centre for fMRI of the Brain Software Library www.fmrib.ox.ac.uk/fsl. The following steps were performed:

(i) Definition of the internal capsule and cortical zones. In order to ensure that the internal capsule was drawn in the same way in all subjects, the individual T1-weighted images were co-registered into a standardized space defined by the Montreal Neurological Institute (MNI152), as employed by FMRI\textsubscript{B} Software Library (FSL) (Jenkinson and Smith, 2001). For standardization, affine transformations were used. The whole internal capsule was drawn on the lowest T1 slice where the entire genu of the corpus callosum was visible. Seven bilateral cortical zones were manually outlined in each subject on the standardized T1 images (Fig. 1A). This was done by using, as a guide, regions of interest derived from the Brodmann atlas via MRICro (Rorden and Brett, 2000). The cortical zones were the following: the primary motor cortex [Brodmann area (BA) 4], the premotor cortex (BA 6), the remainder of the frontal cortex (BAs 8, 9, 10, 11, 32, 33, 44, 45, 46, 47), the somatosensory cortices (BAs 1, 2, 3, 5, 7), the posterior parietal cortex (BAs 23, 31, 39, 40, 43), the temporal cortex (BAs = 20, 21, 22, 27, 28, 29, 30, 34, 35, 36, 37, 38, 41, 42, 52) and the occipital cortex (BAs 17, 18, 19). The areas that occupy the entire cingulated gyrus (BAs 23, 24, 26, 30, 33) and the subgenual area 25 were not included the cortical zones.

The internal capsules and the cortical zones were then transferred back to the individual T1 images, and their correct location was confirmed in all cases. Probabilistic tissue-type segmentation and partial volume estimation were then performed on the individual T1-weighted images (Zhang et al., 2001). The output images were thresholded to include only voxels estimated at >40% grey matter, and the results were used to mask the cortical zones, to

Fig. 1 (A) Division of the cerebral cortex in seven bilateral cortical regions, which have been outlined according to anatomical landmarks and the Brodmann atlas (see Methods for details). The primary motor cortex (in orange) was the ‘target mask’ used to track the CST. (B) Region in the posterior limb of the left internal capsule that showed the highest connection probability to the primary motor cortex overlaid onto an individual T1-weighted image. This region was obtained by tracking connections between the entire internal capsule and the different cortical zones, and was used as a ‘seed region’ for tracking the CST. Voxels with high connectivity values are in yellow; those with low connectivity are in red.
obtain the final cortical zones that were used in the following step.

(ii) Connectivity-based segmentation of the internal capsule. After skull-stripping both the diffusion-weighted and the T1-weighted images (Smith, 2002), we performed affine registration (Jenkinson and Smith, 2001) in each subject between the averaged six non-diffusion-weighted volumes and the individual T1-weighted images, to derive the transformation matrix between the two spaces. Then, the subject’s transformation matrix was applied to the internal capsule mask and to the final cortical masks, which were overlaid onto the individual anisotropy maps to confirm their correct location.

Finally, from each voxel in the internal capsule mask we drew samples from the connectivity distribution to the cortical masks. The probability of connection to a cortical mask was obtained from the proportion of samples that reached each of the cortical zones. Each white matter voxel in the internal capsule was then classified according to the cortical zones with which it had the highest probability of connection. The result was therefore the creation of seven exclusive connectivity-defined regions in the internal capsule mask, including a region that connected to the motor cortex. This connectivity-based segmentation has been extensively used in previous work (Behrens et al., 2003a; Johansen-Berg et al., 2005; Lazar and Alexander, 2005; Ramnani et al., 2005).

(iii) Tracking of the CST. Finally, we used the probabilistic tractography algorithm to trace, in each subject, the CST, from the region in the internal capsule that showed the highest connection probability to the primary motor cortex (or ‘seed region’) (Fig. 1B), to the primary motor cortex itself (or ‘target region’) (Fig. 1A). The tractography algorithm drew 5000 samples from each voxel in the seed region to the target region. The output was a probabilistic map that provided, at each voxel, a connectivity value, corresponding to the total number of samples that passed from the seed region through that voxel to the primary motor cortex. Each connectivity map was then thresholded to include voxels with connectivity values 100 or above, which were then used in the statistical analysis (Fig. 2). The connectivity value of 100 was chosen because voxels with connectivity <100 were widespread across the brain, were not specific for any connections and considered to represent the effect of image noise. A similar approach for choosing a connectivity threshold has been reported previously (Guye et al., 2003). The thresholded connectivity map was then transformed into a binary image, which was used to mask the anisotropy map to delineate the CST. The voxel values of FA within this CST were obtained in all subjects.

Distributions of FA and connectivity values. In order to select the summary measures that best characterize differences in the distributions of FA and connectivity in voxels within subject, we plotted the voxel values of FA and connectivity within each subject, and also aggregated over subjects; the aggregated distribution shape was typical of the within-subject distributions (Supplementary fig. 1), and is shown for the left CST of patients and controls separately in Fig. 3.
(the distributions of voxel values for the right CST were very similar and therefore not shown).

Owing to the symmetric nature of the distribution of FA values within the CST, we were confident that the mean FA was a good summary statistic for the FA in each patient. On the other hand, the distribution of connectivity voxel values was rapidly skewed to the right. Figure 3 shows that the connectivity distributions in controls typically had a small proportion of high connectivity voxels, creating a long right tail to the distribution, extending to values >20 000 in some controls; in patients, however, the range of the right tail appeared to be reduced. Voxels with high connectivity values (>15 000) were located within the core of the CST tract, and, therefore, were considered to be genuine and not related to imaging artefacts (Supplementary fig. 2). For this shape of distribution the mean may be comparatively insensitive to changes occurring mainly in the distribution tail, which includes voxels within the CST with a high connectivity value. We therefore chose the two following summary measures, which focused on the right tail, with higher connectivity values, rather than the whole connectivity distribution:

(i) From the voxels aggregated over all subjects, those below the 75th centile connectivity value were discarded, leaving the top quarter of voxels; then, for each subject, the mean connectivity value was derived and called the 'top quarter mean connectivity'.

(ii) The 95th centile connectivity value in voxels aggregated from all subjects was chosen as a threshold; then, for each subject, the proportion of their voxels exceeding this threshold was derived.

The connectivity values in the 32 subjects came from aggregated totals of 28 530 and 31 829 voxels for left and right CST, respectively.

To complete our analysis, we also computed the within-subject mean connectivity (over all quarters), the top quarter FA and the proportion of voxels with FA higher than 95th centile of the CST.

The within-subject mean FA and mean connectivity, the two summary measures of connectivity described above, and the two summary measures of FA, were used to assess differences between patients and controls, and associations with disease progression rate.

**Brain atrophy**

It is known that patients with ALS show a reduced white matter volume in motor (Ellis et al., 2001) and non-motor regions (Abrahams et al., 2005) compared with controls. Moreover, gender has been reported to have an effect on brain atrophy with
ageing (Xu et al., 2000). Therefore, since the presence of four females in the control group may have an effect on white matter volume, and the presence of white matter atrophy may affect the comparisons of the tractography-derived connectivity and anisotropy between patients and controls, we calculated the white matter fraction (WMF) in each subject in both groups as explained below, and adjusted for it in the statistical analysis.

The high-resolution T₁-weighted images were segmented into white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF) masks, with their associated volumes in millilitres, using SPM99 according to a protocol described previously (Chard et al., 2002). The SPM99 outputs were checked to ensure that the segmentation was successful. Volumes were estimated with a caudal cut-off point, which was defined as the most cranial slice of the cord not containing cerebellum. WMF was calculated as WM volume divided by the total intracranial volume, which was defined as WM + GM + CSF.

Statistical analysis

Differences in muscle strength and spasticity between the left and right limbs

Differences in the muscle strength and spasticity between the left and right upper limbs and between the left and right lower limbs within the whole group of patients and in each patient subgroup were investigated using the Wilcoxon signed ranks test. P-values < 0.05 are reported.

Differences in FA and connectivity between patients and controls

Patients were classified as having a moderate or rapid disease progression rate, depending on whether or not their disease progression rate was less than the disease progression rate median value (0.472). To investigate differences in FA and connectivity values between the whole group of patients and controls, linear regressions were performed with the within-subject summary measure as a response variable on patient indicator, age and WMF as covariates. Similar regressions with indicators for patients with moderate and rapid disease progression rate were used to compare these two patient subtypes with controls. Unless otherwise stated, all differences between subject groups were age- and WMF-adjusted.

We report the non-parametric bias-corrected bootstrap (Carpenter and Bithell, 2000) (1000 replicates) confidence intervals (CIs) and P-values where normality of regression residuals could not confidently be assumed.

Differences in connectivity adjusted for the number of voxels in the CST

A reduction in the number of voxels in the CST, which may be a result of the disease process, may reduce, by indirect association, the patients’ connectivity values. Therefore, in order to take into account the possible effect of the number of voxels on the connectivity value, in the cases of significant differences in the top quarter mean connectivity between groups, we repeated the linear regression analyses by adjusting not only for age and WMF, but also for the total number of voxels in the CST. In the same cases, we also investigated differences in the total number of voxels between patient subtypes and controls using linear regressions adjusted for age and WMF. However, we did not perform this further analysis (i.e. the voxel number adjustment) in the cases of significant differences in the proportion of voxels that have connectivity higher than 95th centile because this summary measure already assumes that subjects with the same value on this measure have a linear relationship between the subject’s total voxel number and their number of high connectivity voxels.

Association between FA and connectivity and disease progression rate

To investigate the association between FA or connectivity summary measures and the disease progression rate, the latter was regressed as a continuous response variable on the FA or connectivity summary measure, with age and WMF as covariates. Age- and WMF-adjusted regression slopes and partial correlation coefficients are reported. Potential confounding by total number of voxels per subject was checked by adjusting for this also in the case of top quarter connectivity. There was no evidence that these regression residuals departed from normality; owing to the small sample, however, regression inference was confirmed by bootstrap.

Statistical significance is taken at \( P < 0.05 \) throughout. Analyses were performed in Stata 8.2 (Stata Corporation, College Station, TX, USA).

Results

Differences in muscle strength and spasticity between the left and right limbs

The whole group of patients presented a similar muscular strength and spasticity on the left and right limbs. Patients with moderate disease progression rate showed a similar level of spasticity but a significantly lower muscle strength in the left upper limb when compared with the right limb [left upper limb: mean = 32 (SD = 2.83) versus right upper limb: mean = 34.7 (SD = 0.82), \( P = 0.04 \)], whereas patients with rapid disease progression had similar involvement of the right and left extremities.

Differences in voxel-based connectivity between patients and controls

On visual inspection of the probabilistic map that represents the CST in each subject, high connectivity voxels appear to be located in the ‘core’ of the tract, whilst low probability values in voxels are located at the edge of the tract, in particular along its lateral aspect.

Differences in the top quarter mean connectivity values

The 75th centile connectivity values were 2517.7 for the left CST and 2351.0 for the right tract, above which there were 7052 and 7767 voxels, respectively. These centile values were located about mid-way in the bulk of the voxel distribution before the start of the tails, and discounted, in each subject, voxels with low connectivity values and concentrated on voxels within the tail of the distributions (Fig. 3). All subjects had at least one voxel above these thresholds. The unadjusted
within-subject top quarter mean connectivity of both CSTs in patients and controls is given in Table 2.

There was no significant difference between patients as a whole and controls in the top quarter mean connectivity of the bilateral CST (patient–control difference for left CST: −540.2, P = 0.19, 95% CI: −1361.9, 281.4; right CST: −319.2, P = 0.51, 95% CI: −1300.8, 662.5). When patients were subdivided by disease progression rate, it was found that (i) patients with rapid disease progression rate had a significantly lower top quarter mean connectivity than controls in the left CST (rapid patient–control difference: −1172.5, P = 0.014, 95% CI: −2083.2, −261.8), but not in the right CST (−398.5, P = 0.51, 95% CI: −1607.8, 810.8); and (ii) there were no significant differences in the top quarter mean connectivity in the bilateral CST between patients with moderate disease progression rate and controls (moderate patient–control difference for the left CST: 47.6, P = 0.91, 95% CI: −843.3, 938.6; right CST: −245.4, P = 0.6, 95% CI: −1428.6, 937.7). The estimated top quarter mean connectivity values adjusted for age and WMF in patient subtypes and controls are shown in Fig. 4B.

Differences in the proportion of voxels with connectivity higher than the 95th centile

The 95th centile connectivity values were, respectively, 6360.3 for the left CST and 5875.3 for the right tract. Figure 3 shows that these values were located towards the start of the tails of the distributions, allowing inclusion of voxels with the highest connectivity values. The unadjusted within-subject mean proportions of voxels with values higher than these 95th centiles are given in Table 2.

There was no significant difference between the whole group of ALS patients and controls in the proportion of voxels that have connectivity higher than 95th centile (patient–control difference for left CST: 0.0214, P = 0.28, 95% CI: −0.0609, 0.0180; right CST: 0.0185, P = 0.44, 95% CI: −0.0671, 0.0301).

However, patients with rapid disease progression rate had a significantly lower proportion than controls of voxels in the left CST (rapid patient–control difference: −0.0447, bootstrap P = 0.025, bootstrap 95% CI: −0.0981, −0.0063), but not in the right CST (−0.0325, P = 0.27, 95%
Differences in mean connectivity

The unadjusted within-subject mean connectivity values are given in Table 2.

There was no significant difference between the whole group of ALS patients and controls in the mean connectivity values in both CSTs (patient–control difference for left CST: −169.2, P = 0.5, 95% CI: −721, 382.5; right CST: −327.4, P = 0.22, 95% CI: −858.2, 203.4).

Furthermore, patients with rapid disease progression rate had a borderline significantly lower mean connectivity than controls in the left CST (rapid patient–control difference: −541.4, P = 0.08, 95% CI: −1169.6, 86.8), but not in the right CST (−469.5, P = 0.15, 95% CI: −1116.5, 177.4). Patients with moderate disease progression rate and controls had a similar mean connectivity in both tracts (moderate patient–control difference for left CST: 176.8, P = 0.56, 95% CI: −437.9, 791.4; right CST: −195.3, P = 0.53, 95% CI: −828.2, 437.7).

Differences in connectivity adjusted for the number of voxels in the CST

When differences in the top quarter mean connectivity were also adjusted for the number of voxels (which is shown in Table 2), patients with rapid progression rate still had substantially and statistically significantly lower top quarter mean connectivity than controls in the left CST (rapid patient–control difference: −909.1, P = 0.042, 95% CI: −1781.5, −36.8). There were no significant differences between the mean total number of voxels per patient in the left CST (rapid patient–control difference: −131.2, P = 0.33, 95% CI: −400.2, 137.8; moderate patient–control difference: 11.8, P = 0.9, 95% CI: −273.1459, 296.7073).

Differences in FA between patients and controls

Differences in mean FA

The unadjusted within-subject mean FA values of both CSTs in controls and patients are given in Table 2.

Patients had a significantly lower mean FA than controls in both the left (patient–control difference: −0.0337, P = 0.007, 95% CI: −0.0574, −0.0101) and right CST (−0.0293, P = 0.019, 95% CI: −0.05345, −0.0051).

When patients were subdivided by disease progression rate it was found that (i) patients with rapid disease progression rate had a significantly lower mean FA than controls in both the left (rapid patient–control difference: −0.0464, P = 0.002, 95% CI: −0.0741, −0.0186) and right CST (−0.0407, P = 0.007, 95% CI: −0.0694, −0.0120); and (ii) patients with moderate disease progression rate showed a lower mean FA in both tracts than controls, although these differences did not reach statistical significance (moderate patient–control difference for the left CST: −0.0220, P = 0.11, 95% CI: −0.0492, −0.0052; right CST: −0.0187, P = 0.18, 95% CI: −0.0468, −0.0094). The estimated mean FA values adjusted for age and WMF in patient subtypes and controls are shown in Fig. 4A.

Differences in top quarter mean FA

The 75th centile FA values were 0.5150 for the left CST and 0.5143 for the right tract, above which there were 7426 and 8250 voxels, respectively. The unadjusted within-subject top quarter mean FA of both CSTs in patients and controls are given in Table 2.

Patients had a significantly lower top quarter mean FA than controls in both the left (patient–control difference: −0.0267, P < 0.001, 95% CI: −0.0406, −0.0129) and right CST (−0.0232, P = 0.018, 95% CI: −0.0421, −0.0043).

When patients were subdivided by disease progression rate it was found that (i) patients with rapid disease progression rate had a significantly lower top quarter mean FA when compared with controls in the left (rapid patient–control difference: −0.0198, P = 0.02, 95% CI: −0.0361, −0.0034), but not in the right CST (−0.0144, P = 0.20, 95% CI: −0.0368, −0.0080); and (ii) patients with moderate disease progression rate showed a lower top quarter mean FA in both the left (moderate patient–control difference: −0.0332, P < 0.001, 95% CI: −0.0492, −0.0172) and right CST (−0.0314, P = 0.007, 95% CI: −0.0533, −0.0094).

Differences in the proportion of voxels with FA higher than the 95th centile

The 95th centile FA values were, respectively, 0.6540 for the left CST and 0.6886 for the right tract. The unadjusted within-subject mean proportions of voxels with FA values higher than these 95th centiles are given in Table 2.

As to the top quarter mean FA results, patients had a significant proportion of voxels with FA higher than the 95th centile in both the left (patient–control difference: −0.0459, P < 0.001, 95% CI: −0.0715, −0.0202) and right CST (−0.0397, P < 0.001, 95% CI: −0.0590, −0.0203).

When patients were subdivided by disease progression rate it was found that (i) patients with rapid disease progression rate had a significantly lower proportion of voxels in both the left (rapid patient–control difference: −0.0419, P = 0.011, 95% CI: −0.0734, −0.0104) and right CST (−0.0342, P = 0.006, 95% CI: −0.0577, −0.0107); and (ii) patients with moderate disease progression rate showed a lower proportion of voxels in both the left (moderate patient–control difference: −0.0495, P = 0.003, 95%
Association between FA and connectivity and disease progression rate

The two summary measures of connectivity of the left CST were significantly associated with disease progression rate: disease progression rate is predicted to be lower by 0.07 units/month (95% CI: 0.05, 0.10) per 100 unit rise in top quarter mean connectivity value (Fig. 5) (P < 0.001, partial correlation coefficient = −0.90, with adjustment for mean total number of voxels per subject not substantially altering this: adjusted slope = 0.06, partial correlation = −0.84, P = 0.002), and lower by 0.19 units/month (95% CI: 0.09, 0.28) per 1 percentage point rise in the proportion of voxels with connectivity value above the 95th centile (P = 0.001, partial correlation coefficient = −0.83). We also detected a significant negative association between the within-patient mean connectivity (over all quarters) of the left CST and disease progression rate (disease progression rate is predicted to be lower by 0.1 units/month per 100 rise in mean connectivity, P = 0.002, partial correlation coefficient = −0.82).

However, there was no evidence of association between disease progression rate and any of the connectivity measures of the right CST (for top quarter mean connectivity: P = 0.63, partial correlation coefficient = −0.16; for proportion of voxels >95th centile: P = 0.40, partial correlation coefficient = −0.28; for mean connectivity: P = 0.45, partial correlation coefficient = −0.25).

We did not find a significant association between disease progression rate and mean FA in the bilateral CST: disease progression rate is predicted to be lower by 1.6 units/month (95% CI: −13.3, 16.5) per one unit increase in the left CST mean FA (P = 0.81, partial correlation coefficient = −0.08), and by 5.4 units/month (95% CI: −10.6, 21.4) per one unit increase in the right CST FA (P = 0.47, partial correlation coefficient = −0.25). Similarly to the mean FA results, there was no significant association between disease progression rate and top quarter mean FA of the left (P = 0.77, partial correlation coefficient = 0.10) and right CST (P = 0.43, partial correlation coefficient = 0.27), nor with the proportion of voxels with FA above the 95th centile of the left (P = 0.67, partial correlation coefficient = −0.14) and right CST (P = 0.74, partial correlation coefficient = 0.11).

Discussion

This study demonstrates that there is a strong association between diffusion-derived voxel-based connectivity in the CST of patients with ALS and the rate of disease progression. This voxel-based connectivity was derived by applying a probabilistic tractography algorithm (Behrens et al., 2003b), which tracked the CST from the internal capsule to the primary motor cortex. Recent studies have already applied diffusion tractography methods to segment the motor tracts (Abe et al., 2004; Aoki et al., 2005), but the novelty of our study is that we have developed voxel-based summary connectivity measures that were considered to reflect the white matter fibre organization. This represents
the first step towards the development of a connectivity index that may provide important information about the structure of white matter fibres and be sensitive to pathological changes relevant to clinical disability.

**Differences in connectivity and FA between patients and controls**

On the basis of the distribution of connectivity values over subjects, we selected two measures of connectivity that were expected to be sensitive to changes occurring in the 'tail' of the distribution, which includes voxels with high connectivity values. Since the probabilistic maps representing the CSTs showed that voxels with high connectivity values were located in the 'core' of the tract, it is reasonable to conclude that these measures describe the very centre of the tract. This might not reflect any specific anatomical pathway (Hardy et al., 1979), but might be a result of the higher alignment of the eigenvectors, which reflect the main direction of fibres, in the centre of the tract, than at its edges. We found that patients with rapid disease progression rate had significantly lower connectivity measures than controls in the left CST. On the other hand, only a borderline difference was found in the mean connectivity between patients with a rapid disease progression rate and controls, confirming that the newly developed summary measures were more sensitive than the mean connectivity to pathological changes. However, when the whole group of patients was compared with controls, there was no significant difference in any of the connectivity measures. On the other hand, the mean FA was significantly decreased bilaterally in the corticospinal tract in both the whole group of patients and in those with rapid progression. Similar results were obtained when the top quarter mean FA and the proportion of voxels with FA higher than 95th centile were used to compare patients and controls. These results confirm that the summary FA measures were not more sensitive than the mean FA in detecting differences between groups, except in the case of patients with a moderate disease progression rate, who did not show a lower mean FA when compared with controls.

The finding of reduced anisotropy in the CST of patients with ALS is in agreement with previous papers, which have either placed regions of interest along the tractography-segmented CST (Aoki et al., 2005), or have positioned regions using anatomical landmarks (Ellis et al., 1999; Toosy et al., 2003; Graham et al., 2004; Hong et al., 2004), confirming that FA is a very valuable measure for assessing pathological changes in ALS. Differences in FA between patients and controls remained significant when we adjusted for gender (results not shown), age and WMF, demonstrating that the estimated differences are attributable only to patient versus control status rather than to gender, age and white matter atrophy.

A possible limitation of our study is that we have investigated changes in anisotropy only in the CST above the internal capsule, while previous studies have reported that the most significant changes in FA are evident at the level of the cerebral peduncles, where the white matter fibres are coherently oriented (Hong et al., 2004), and of the internal capsule (Ellis et al., 1999; Toosy et al., 2003). However, the observed differences in FA in the CST above the internal capsule, where the fibres are less packed and less anisotropic, up to the precentral gyrus, where the white matter abnormalities are known to be less prominent than at lower levels (Brownell et al., 1970), suggest that the use of tractography to segment the CST may increase the ability to detect subtle changes in fibre coherence and organization. The inclusion of fibres that are less coherent than in other parts of the CST, such as those in the subcortical white matter, together with the tracking of the CST up to the motor cortex, might have led to lower values of FA than those reported previously in the lower parts of the corticospinal tract (Virta et al., 1999) or when restricted to the subcortical white matter (Pierpaoli and Basser, 1996).

Unfortunately, it was not feasible in our study to investigate differences in connectivity or FA between (i) patients with upper and lower motoneuron signs, because of the mixed clinical picture in almost all patients and (ii) patients with bulbar- and limb-onset, because only three patients had a bulbar-onset of the disease.

**Connectivity measures are sensitive to damage in the CST that is associated with disease progression rate**

Although the exact relationship between changes in connectivity and the temporal evolution of the in vivo pathology is unknown, we can interpret the reduction in voxel-based connectivity found in the left CST of patients with ALS to be as a result of motor tract degeneration. The main pathological changes in patients with ALS include loss of pyramidal motoneurons (Betz cells) in the primary motor cortex and axonal degeneration of the CST (Brownell et al., 1970). These abnormalities, together with the proliferation of glial cells, the extracellular matrix expansion, and the intra-neuron abnormalities (Chou, 1995), may contribute to the observed connectivity changes. However, direct comparisons with pathological data and longitudinal studies are needed to clarify the underlying microscopic changes.

We found strong associations between the rate of disease progression and (i) the top quarter mean connectivity ($P < 0.001$, partial correlation coefficient $= 0.90$); (ii) the proportion of voxels with connectivity higher than the 95th centile ($P = 0.001$, partial correlation coefficient $= -0.83$); and (iii) the within-patient mean connectivity ($P = 0.002$, partial correlation coefficient $= -0.82$). These correlations remained strongly significant when we adjusted not only for age and WMF but also for gender (results not shown), demonstrating that the association between disease progression rate and connectivity is independent from gender, age and WMF. Therefore, these findings suggest
that the pathological damage in the left CST detected by connectivity measures is a significant factor contributing to the rapidity of disease progression in patients with ALS.

In contrast to previous publications, which have averaged the water diffusion indices of the left and right side (Ellis et al., 1999; Graham et al., 2004; Aoki et al., 2005), we opted to perform the statistical analyses for the left and right tract separately, and found that the differences in the two measures of connectivity between patients and controls, and their correlations with disease progression rate, were statistically significant on the left side only. This is in agreement with the asymmetric differences in diffusion indices reported previously (Toosy et al., 2003), and supports the descriptions of asymmetry in ALS pathology of the CSTs (Swash et al., 1988). We found that this was not due to an asymmetric involvement of the left and right limbs in patients, because in the whole group of patients and, particularly, in those with rapid disease progression (who showed lower connectivity measures in the left CST), the muscle strength score was similar on both sides. Moreover, patients with moderate disease progression (who, however, did not show significant differences in connectivity measures compared with controls) had a lower muscle strength in the left upper limb compared with the contralateral limb. The lack of correspondence between the side of greater MRI differences and the side of greater muscle strength deficit might be explained by the fact that the muscle strength score is a result of the upper and lower motoneuron involvement, whereas MRI detects only the upper motoneuron damage. Therefore, further studies are needed to understand if the asymmetry in the MRI findings reflects (i) a different degree of alignment and organization in the motor tracts, as has been found in the anterior limb of the internal capsule (Peled et al., 1998), or (ii) the distribution of handedness among the subjects studied.

**FA does not relate to disease progression rate**

Although the mean FA and the newly developed summary FA measures detect significant differences between patients and controls, there was no significant association between any of the FA measures and rate of disease progression. Closer examination of the mean FA results reveals that patients with rapid disease progression rate were similar to moderate patients in respect of their mean FA (Fig. 4). This suggests that, although FA detects pathological abnormalities in the motor tract, it does not appear to be sensitive to changes that correlate with the rapidity of disease progression. In contrast, patients with rapid disease progression rate differed more from moderate patients in respect of their connectivity measures than in respect of their mean FA. This is consistent with the presence of significant association in patients between connectivity and rate of disease progression.

Taking all these findings together, it appears that anisotropy and connectivity provide complementary information, since one can detect pathological changes in all patients with ALS, whilst the other might be a marker of disease progression rate. Furthermore, it is possible that one can capture some features of the fibre organization that cannot be detected by the other, and vice versa.

**Conclusion**

In conclusion, probabilistic tractography may be a useful and non-invasive tool to assess the rate of disease progression in patients with ALS. However, further studies using tractography-derived connectivity in larger patient groups and long-term follow-up are important to confirm whether connectivity is a sufficiently sensitive and specific marker to the disease process, such that it may be used to assess the efficacy of experimental treatments in ALS. Importantly, the technical developments that will result from new developments of a tractography-based connectivity index will not only contribute to future developments in the same field but can also be extended to other neurological diseases to understand the mechanisms of disability progression.

**Supplementary material**

Supplementary data are available at Brain online.

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**References**


Probabilistic diffusion tractography in ALS