

Regional callosal integrity and bilaterality of limb weakness in amyotrophic lateral sclerosis

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ABSTRACT

Background & Objectives

The corpus callosum is a site of pathological involvement in the neurodegenerative disorder amyotrophic lateral sclerosis (ALS). The corpus callosum shows widespread cortical connectivity topographically distributed along its length. Initial limb weakness in ALS is typically unilateral, becoming bilateral with disease progression. The precise anatomical substrate for this spread is uncertain. The present study investigated sub-regional variations in corpus callosum integrity in ALS, and whether these reflect a relationship with the development of unilateral or bilateral limb weakness.

Methods

Sporadic ALS patients were categorised into unilateral (n=14) or bilateral (n=25) limb weakness at the time of assessment and underwent diffusion tensor imaging. Probabilistic bundle-specific tracking was carried out using MRtrix and TractSeg to parcellate the corpus callosum into seven anatomical segments (rostrum; genu; rostral body; anterior midbody; posterior midbody; isthmus; splenium). White matter tract integrity was assessed in all segments and compared with MRI data acquired from 25 healthy controls.

Results

In the combined patient group, the most prominent differences in diffusivity metrics were in the rostral body, posterior midbody and isthmus of the corpus callosum ($p < 0.04$). Loss of corpus callosum integrity was most prominent in the sub-group with unilateral limb weakness at the time of scanning ($p < 0.05$).

Conclusions

Corpus callosum involvement in ALS is detectable across multiple segments, in keeping with a widespread cortical distribution of pathology. The association of unilateral limb weakness with greater loss of corpus callosum integrity informs connectivity-based hypotheses of symptom propagation in ALS.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a heterogeneous clinical syndrome characterised by combined degeneration of central and peripheral motor neurons, with broader disintegration of the cerebral motor system connectome that overlaps with frontotemporal dementia(1, 2). Patients typically experience an initial focal loss of motor function, occurring in the upper or lower limb, the bulbar territory or, more rarely, respiratory or axial muscles (3). The spread of symptoms in those with an initial limb involvement in ALS is not random, typically involving a contiguous body region, and involving the contralateral limb at some stage (4). A directionality to the neurodegeneration in ALS remains an issue of intense debate, often distilled into the terms ‘dying back’ versus ‘dying forward’ to reflect a primacy of peripheral versus central motor neuronal loss.

The interhemispheric corpus callosum is the largest white matter tract in the human brain, and is characterised by a distinct developmental profile associated with age and gender (5, 6), cortical topographic representation (7), and functional specialisation along its axis (8). The corpus callosum has been identified as a site of histopathological change in *post mortem* ALS (9, 10). This has since been demonstrated in structural neuroimaging studies *in vivo* (11, 12) and *ex vivo* (13), and shown to be associated with reduced interhemispheric functional connectivity of the motor cortices (14-16). A potential role of the corpus callosum as a pathway for disease propagation has been suggested (17).

With developments in automated parcellation software, we sought to explore any sub-regional variability of corpus callosum involvement in ALS, and to specifically test the hypothesis that the presence of unilateral versus bilateral limb weakness involves differences in corpus callosum integrity, which might support its role in interhemispheric propagation of pathology.

METHODS

All right-handed individuals with apparently sporadic, classical forms of ALS without dementia were included from the Oxford Study for Biomarkers in MND ('BioMOx'), a tertiary referral ALS clinic-based biomarker development cohort. The clinical diagnosis of ALS and determination of regional involvement was made by two experienced neurologists (MRT, KT), in accordance with revised El Escorial criteria. ALS patients were classified as having unilateral or bilateral limb weakness on the day of scanning. Disease duration was calculated from date of first reported weakness. Severity of functional disability was assessed using the revised ALS functional rating scale (ALSFRS-R), a four-domain disability questionnaire with scores ranging from 0 to 48, with a lower score reflecting greater disability. Disease progression rate (DPR), as indicated by increasing disability, was calculated by dividing the change in the ALSFRS-R sum score at baseline and follow-up by the time between assessments. A clinical measure of upper motor neuron (UMN) burden was assessed by totaling the number of pathological reflex signs from 15 sites (18). A demographically matched healthy, right-handed control group largely comprised spouses and friends of ALS patients.

Image acquisition

Participants were scanned at the Oxford Centre for Clinical Magnetic Resonance Research using a 3T Siemens Trio scanner with a 12-channel head coil with clinical assessment on the same day. Whole-brain diffusion-weighted images were acquired using an echo-planar sequence (60 isotropic directions; b-value=1000 s/mm²; echo time/repetition time=94/10 000 ms; 2×2×2 mm³ voxel size; 65 slices). Four additional b0 images without diffusion weighting

were acquired. A field map was acquired using a gradient echo imaging sequence ($2 \times 2 \times 2$ mm³ voxel size; 65 slices; echo time 1/echo time 2/repetition time=5.19/7.65/655 ms) to account for distortions present in diffusion-weighted data caused by field inhomogeneities.

Diffusion preprocessing and corpus callosum parcellation

Diffusion-weighted MRI data was preprocessed using MRtrix 3.0 (<http://www.mrtrix.org>).

Data preprocessing included correction for motion, geometric distortion, and bias field.

Whole-brain fibre orientation distributions (FOD) were estimated using constrained spherical deconvolution(19), resulting in a condensed representation of diffusion along three principal fibre directions per voxel according to tissue type (grey, white, CSF), and then normalised across subjects. FSL-DTIFIT was used to apply a diffusion tensor model at each voxel in the preprocessed diffusion MRI data, resulting in maps of three eigenvalues (L1,L2,L3). Whole-brain maps of fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD) were calculated for each participant's scan.

Probabilistic segmentation based parcellation of the corpus callosum was carried out using TractSeg (20). TractSeg is a robust semi-automated tool for region of interest based bundle-specific tracking using convolutional neural network-based segmentation of white matter bundles modelled on trained features of the raw diffusion signal. Briefly, whole-brain FOD were input into a two stage fully convolutional neural network (FCNN) trained using segmented priors of 72 anatomically well-defined white matter tracts from the Human Connectome Project (21). In the first FCNN, FOD peaks are used to generate one tract probability image, in native space, for each orientation (coronal, axial, sagittal) per tract. The images are concatenated into a 3D image and input into a second FCNN whereby the process is repeated, and the mean used to generate a final probability map of each tract's fibre bundle, thresholded at 50%. This framework has previously been validated and shown to generate

robust and accurate tract segmentations (20). Using this approach, the corpus callosum was parcellated into 7 anatomically consistent volumes of interest (rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus, splenium) (22) based on probabilistic tracking of transcallosal fiber bundles. The accuracy of fiber bundles used to define the corpus callosum parcellations were visually inspected for spurious and truncated fibres. Mean diffusion metrics were extracted from each participant's individual corpus callosum parcellations.

Statistical analyses

Statistical analyses comparing the diffusivity of corpus callosum parcellations between ALS and healthy controls were carried out using SPSS version 21.0. The Shapiro-Wilk's test was used to test normality. Group differences across diffusion metrics were assessed using multivariate analysis of covariance (MANCOVA) followed by two-tailed post hoc comparisons of each dependent variable between cohorts using Bonferroni correction. Age, education, and gender were included as covariates given their significant impact on corpus callosum morphology (23).

RESULTS

Participant data and demographics

ALS (n=39) and healthy control (n=25) participants were all right-handed and well matched demographically (Table 1). ALS patients were segregated into unilateral (n=14) and bilateral (n=25) disease groups based on laterality patterns of limb weakness (Fig. 1). In the unilateral ALS group, incidence of left (n=7) and right (n=7) limb weakness was equally represented. In the bilateral ALS group, a similar incidence of upper (n=6) or lower (n=8) limb weakness was present in patients where weakness had not spread to all 4 limbs.

---INSERT TABLE 1---

---INSERT FIGURE 1---

Corpus callosum white matter abnormality

The corpus callosum was consistently parcellated into 7 anatomical segments across all participants and an average group representation generated, thresholded at 50% (Fig. 2). The extent of lateral truncation of the rostrum and splenium segments are shown in Supplementary Figure 1. Mean cluster volume of parcellated CC segments did not significantly differ between ALS patient groups and healthy controls (Supplementary Table 1).

---INSERT FIGURE 2---

As a combined group, ALS patients showed abnormal diffusivity in the rostral body (MD, AD), posterior midbody (FA, MD, RD), and isthmus (FA) of the corpus callosum, relative to controls (Fig. 2; Table 2; $p < 0.04$). When those with unilateral versus bilateral limb weakness at the time of scanning were considered independently, only those with unilateral limb involvement showed significant and widespread reduction in white matter integrity (MD, RD, AD), throughout all segments of the corpus callosum, excluding the rostrum (Table 3; $p < 0.05$). Patients with bilateral limb weakness showed selective reduced white matter integrity (FA) in the posterior midbody of the corpus callosum (Table 3; $p=0.04$). Within the bilateral limb ALS cohort, no diffusivity differences were observed between those with and without bulbar involvement.

---INSERT TABLE 2---

---INSERT TABLE 3---

DISCUSSION

The present study of sub-regional white matter abnormality in the corpus callosum in ALS has established that the posterior midbody was the most consistently affected, and that the greatest loss of corpus callosum integrity developed in those patients with unilateral limb weakness at the time of assessment.

The presence of regional variability of the corpus callosum in ALS is well supported through pathological approaches. For instance, degenerated nerve fibres have been identified throughout the length of the corpus callosum at post mortem in ALS, but with varying regional involvement, being most prominent in the middle segment, to a lesser extent the posterior segment, and the anterior segment being the least affected (10). Similarly, regional variability in corpus callosum abnormality is reflected in the neuroimaging signature of sporadic ALS with abnormality in the middle segment, extending posteriorly, being consistently reported (12, 15, 24). This pattern can be affected by genotype-specific changes, such as the *C9orf72* mutation, which results in significant anterior corpus callosum involvement (25).

That the brain forms a central part of ALS pathology is no longer disputed (26). In addition to the clinical, pathological and genetic overlap of ALS with FTD, neuroimaging studies have demonstrated widespread structural and functional changes (27). The potential role of the corpus callosum as a conduit for interhemispheric spread of pathology in ALS has been previously raised in the literature with the view that ALS is a uniquely human disease arising from selective vulnerabilities introduced by the complexity of the neocortex (17). The corpus callosum not only mediates homotopic cortical communication, but functional neuroimaging

also indicates a significant role in heterotopic cortical communication (28), likely serving a dual role of mediating interhemispheric information transfer and hemispheric modulation of central processes (29). This is evident in voluntary motor function, which is predominantly represented by the contralateral motor cortex, but also elicits a significant change in ipsilateral motor cortex excitability that is exacerbated in ischemic stroke patients (30).

The underlying pathological process associated with laterality of limb weakness and subsequent spread in ALS remains unclear, but may involve abnormality in transcallosal inhibitory control (16). Degeneration affects excitatory callosal pyramidal neurons whose primary targets are inhibitory interneurons in the contralateral hemisphere (31) via a corticofugal transsynaptic glutamate excitotoxic process (32, 33). The current findings of a clinical association between reduced corpus callosum integrity and unilateral limb weakness suggests obstruction of the primary interhemispheric commissure of the brain may influence spread of symptoms. There are of course smaller subcortical commissures, which may explain a progressive involvement of deep grey matter structures with disease progression in ALS (34, 35). They have been shown to play a fundamental role in the functional reconfiguration of the brain when interhemispheric transfer is compromised, such as following callosotomy and congenital agenesis of the corpus callosum (28). Our findings would then suggest involvement of extra-motor callosal fibres, as the primary motor connected posterior midbody is selectively impacted across all ALS patients, irrespective of the bilaterality, or not, of limb weakness.

The unilateral group in this study had a higher clinical UMN burden score (mean 12.6 versus 9.6), which has been independently linked to the degree of corpus callosum involvement based on MRI measures (12). UMN-predominant forms of ALS show a consistently slower rate of symptom progression (36), and corpus callosum involvement based on MRI metrics is most pronounced in the pure UMN disorder primary lateral sclerosis (PLS), a clinical

phenotype within the broad spectrum of motor neurone disease, but associated with a median survival 5-10 times that of classical ALS (37, 38). This raises the possibility that disruption to UMN pathways may indirectly influence pathological spread, perhaps specifically to LMN populations. The unilateral and bilateral groups in the present study did not differ significantly in their absolute level of disability, rate of disability progression or disease duration at the time of scan but these are potential confounds that would require larger studies to confidently delineate. Longitudinal study of a larger initially unilateral group before and after symptoms became bilateral would also be valuable, with the caveat that the development of muscle weakness is likely to lag significantly behind the start of motor neuronal loss (39) and seems likely to be similar in MRI measures, which have been shown to be present extensively in pre-symptomatic carriers of the ALS-associated expansion in *C9orf72* (40). While no overt dementia features were present in the current ALS cohort, future inclusion of a comprehensive clinical screening battery, such as the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) across a larger cohort, would permit consideration of callosal integrity in relation to more subtle cognitive impairments, in particular fibres arising from the anterior segment.

The scope of the current study was limited to the CNS. The assessment of LMN burden, in particular the contribution of pathological spread through spinal cord commissures (41), was not considered and the results of this study are not incompatible with spinal pathways being a major conduit associated with the development of bilateral limb symptoms in ALS. The development of robust techniques for quantifying degree of spinal cord pathology in ALS patients (42) is an important step to more fully understand the nature of pathological spread across the entire motor system in ALS.

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DECLARATION OF INTEREST

The authors report no declarations of interest.

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LEGENDS

Table 1. Participant demographic characteristics and clinical profile. ALS patients are reported as a combined group and segregated based on unilateral and bilateral limb involvement. Mean and standard deviation.

Table 2. Diffusion metrics of corpus callosum parcellations in patients with ALS and healthy controls. Mean and standard deviation reported.

Table 3. Diffusion metrics of corpus callosum parcellations in ALS patients with unilateral and bilateral limb involvement, and healthy controls. Mean and standard deviation reported.

Figure 1. Group representation of unilateral and bilateral limb ALS subgroups. Laterality patterns of limb weakness, presence of bulbar symptoms, and incidence in patients. Affected regions are indicated in red.

Figure 2. Group average parcellation of the corpus callosum presented on the MNI152 standard brain shown at the midline (top), and 3D representation (bottom), thresholded at 50%. *Indicates regions of significant diffusivity differences between the combined ALS patient group and healthy controls.