Motor Network Degeneration in Amyotrophic Lateral Sclerosis: A Structural and Functional Connectivity Study

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Abstract

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterised by motor neuron degeneration. How this disease affects the central motor network is largely unknown. Here, we combined for the first time structural and functional imaging measures on the motor network in patients with ALS and healthy controls.

Methodology/Principal Findings: Structural measures included whole brain cortical thickness and diffusion tensor imaging (DTI) of crucial motor tracts. These structural measures were combined with functional connectivity analysis of the motor network based on resting state fMRI. Focal cortical thinning was observed in the primary motor area in patients with ALS compared to controls and was found to correlate with disease progression. DTI revealed reduced FA values in the corpus callosum and in the rostral part of the corticospinal tract. Overall functional organisation of the motor network was unchanged in patients with ALS compared to healthy controls, however the level of functional connectedness was significantly correlated with disease progression rate. Patients with increased connectedness appear to have a more progressive disease course.

Conclusions/Significance: We demonstrate structural motor network deterioration in ALS with preserved functional connectivity measures. The positive correlation between functional connectedness of the motor network and disease progression rate could suggest spread of disease along functional connections of the motor network.

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating disease characterised by degeneration of motor neurons in the brain and spinal cord leading to progressive muscle weakness. With a prevalence of approximately 6/100,000 it is considered a rare disease. The pathogenesis of ALS is heterogeneous with monogenic causes in familial ALS and environmental and genetic risk factors in sporadic ALS [1–3]. The natural history shows a large variability. With a median survival of three years it is intriguing that a minority of patients survive more than ten years. Currently we are unable to predict the disease course in the individual patient. Regardless a few clinical prognostic indicators it is largely unknown which factors determine the rate of clinical decline. We hypothesized that motor network characteristics could influence its vulnerability to neurodegenerative effects.

Previous structural imaging studies on either gray or white matter in patients with ALS have revealed signs of upper motor neuron degeneration in vivo [4–12]. A limited number of functional imaging studies have reported altered functional neuronal activity of central motor regions [13–16]. These neuroimaging studies have, however, focused on discrete measures and lack the network perspective in combination with clinical markers.

A neural network can be defined as a population of interconnected nodes that perform a specific physiological function, just as the motor network controls muscle tone and movement. The properties and integrity of cortical neural networks can be explored using structural or functional measures. We performed cortical thickness measures to assess the quality of interconnecting white matter tracts (structural connectivity) (Figure 1a) and Diffusion Tensor Imaging (DTI) to explore the integrity of the interconnecting white matter tracts (structural connectivity) (Figure 1b). DTI is a technique that measures the water diffusion profile in brain tissue. This technique enables the reconstruction of white matter tracts and estimates its microstructural integrity, which is typically expressed as the fractional anisotropy (FA). As fibres degenerate, diffusion becomes less directional and the FA decreases [17]. We studied the functional characteristics of the motor network by resting-state functional MRI (rs-fMRI). rs-fMRI
is designed to detect inter-regional correlations in spontaneous neuronal activity as measured by blood oxygen level-dependent (BOLD) signal fluctuations over time. This phenomenon was first described in the motor system as a manifestation of functional connectivity [18]. Interestingly, recent studies have shown that functional connections in neuronal networks have a structural core of white matter [19–22]. Combining these techniques in neurodegenerative diseases which typically affect a specific neural network, as ALS affects the motor network, has not been reported previously but is promising future direction [23].

We hypothesized cortical atrophy to occur mainly in primary motor areas with conjoint loss of motor tract integrity. The degree of structural motor network degeneration was expected to affect the functional connectivity. Finally, both structural and/or functional motor network decline was hypothesized to correlate with the clinical status. Here, we examined the neurodegenerative effects in patients with ALS by investigating the structural and functional motor network characteristics and we explored their link with clinical markers.

**Results**

**Cortical thickness**

Whole vertex-wise brain comparisons revealed cortical thinning in the precentral gyrus in patients with ALS compared to healthy controls as illustrated in Figure 2 (left and right precentral gyrus: \( p < 0.0001 \) and \( p < 0.001 \), respectively). Focussing on the precentral gyrus most pronounced thinning in ALS was found bilaterally in the two subareas overlapping the central motor representations of the hand and leg. Interestingly, thinning of the primary motor regions was found to be specific, as no other cortical regions showed any changes in cortical thickness \( (p<0.01) \). Indeed, post-hoc analysis of whole brain average cortical thickness revealed that ALS patients showed no differences in whole brain average cortical thickness \( (\text{mean } 2.278 \text{ mm; SD } 0.090 \text{ mm}) \) compared to controls \( (\text{mean } 2.271 \text{ mm; SD } 0.0449 \text{ mm}; p = 0.81) \). Furthermore, focussing on the primary motor network, the average cortical thickness of the precentral gyrus as a more broadly defined anatomical region was significantly reduced in patients with ALS compared to healthy controls \( (\text{ALS mean } 2.589 \text{ mm; SD } 0.120 \text{ versus controls mean } 2.680 \text{ mm; SD } 0.087; p = 0.04) \) (Figure 3a).

**Structural connectivity**

FA values in the corpus callosum and the CST were found to be significantly reduced \( (p<0.05) \) in ALS patients compared to controls (Figure 3b). The FA reduction was more dispersed in the corpus callosum compared to the CST. Tract-based results, analysing the trajectory of FA along the CST tracts, showed that the reduction in FA becomes less pronounced as the tract descends from the cortex to the brainstem corresponding to more loss of integrity in the rostral part compared to the caudal part of the CST (Figure 3b). The FA along the visual tracts as a negative control condition was found to be unchanged in patients with ALS \( (0.583) \) compared to healthy controls \( (0.576; p = 0.07) \). The whole brain average FA was \( 0.453 \) in patients and \( 0.458 \) in controls \( (p = 0.48) \).
Figure 2. Whole brain cortical thickness. Whole brain cortical thickness measurements in patients with ALS compared to healthy controls. Cortical areas with (uncorrected) p-values < 0.01 are marked. The threshold for this illustration was set at 0.2 mm cortical thickness reduction in ALS. doi:10.1371/journal.pone.0013664.g002

Figure 3. Overview of motor network measures. a) Cortical thickness in patients with ALS versus controls in the precentral gyrus in mm (p = 0.04), corrected for age and whole brain cortical thickness; b) Fractional anisotropy (FA) values along the corticospinal tract and the corpus callosum in patients with ALS and controls (** p<0.01; * p<0.05); c) Number of functional connections, corrected for age (threshold 0.40); p = 0.14, CT = cortical thickness. PCG = precentral gyrus. FA = fractional anisotropy. doi:10.1371/journal.pone.0013664.g003
**Discussion**

The present study, based on a network perspective, demonstrates a decline of structural integrity with preserved functional organisation of the motor network in ALS. Firstly, cortical thickness was significantly reduced in the precentral gyrus, known as the primary motor area, in patients with ALS. Secondly, a significant reduction in microstructural organisation was found in the corticospinal tracts (CST) as the major efferent motor conduits to the spinal cord. This involved mainly the rostral part of CST. Furthermore, the corpus callosum, interconnecting the motor network was found to be affected as a whole. Thirdly, exploring the functional connectivity of the motor network in ALS, we found the functional organisation to be intact, however the local connectedness was found to be related with disease progression. This suggests that strong functional connectivity allows for rapid spread of disease.

We observed bilateral cortical thinning of the precentral gyrus in ALS. Interestingly, these effects were most pronounced in two subregions overlapping the somatotopic representation of the hand and leg. These findings are supported by pathological studies which have demonstrated these specific regions to be the areas of the motor cortex with maximal clustering of cortical motor neurons (Betz cells)[24], of which degeneration is considered to be a pathological hallmark of ALS [25]. Indeed, taking the precentral gyrus as a whole, the average cortical thickness in this region was found to be significantly reduced in patients compared to controls. The cortical thickness in the precentral gyrus was shown to be related to disease progression rate, which could suggest increased vulnerability to degenerative effects. Previous studies on gray matter in ALS have shown more widespread atrophy in frontal and temporal regions as well [5,7,26,27]. These studies were exclusively performed using voxel based morphometry, a technique which is known to have an increased signal to noise ratio and is relatively insensitive to cortical atrophy localised in the brain sulci [28]. In other neurodegenerative diseases clinical syndromes were shown to be associated with a specific pattern of cortical thinning which evolves during disease progression marking the clinical relevance of this measure [29-31]. As such, cortical thinning patterns could assist in the differentiation between distinct neurodegenerative diseases or identification of subtypes.

With regard to the connectivity of the motor network, it was primarily the rostral part of the CST that was found to be affected.

This finding may suggest that the decrease in axonal integrity originates from the cell body, which is in accordance with the observed cortical thinning. These results are supported by the previous observation that cortical hyperexcitability precedes the development of clinical symptoms in pre-symptomatic carriers of a SOD1 mutation [32], thereby suggesting that the early abnormalities in ALS occur within the corticomotorneurons, with anterograde excitotoxicity (often referred to as ‘dying forward’). This aspect of degeneration has been the subject of ongoing debate [32,33] and longitudinal assessments will need to show whether these effects spread caudally during the course of the disease. In addition, our results showed a severe reduction of white matter integrity in corpus callosum tracts interconnecting the affected left and right cortical regions of the primary motor network. Indeed, callosal dysfunction has been found to be an early phenomenon in ALS [34] and structural involvement of the corpus callosum has been reported in combination with functional impairment [35]. Recently, it has been suggested that the corpus callosum plays an important role in the spread of ALS [33], supporting our functional and structural findings.

We report that patients with stronger connectedness of the motor network show a faster progression of disease, indicating an important role for the functional organisation of the motor network in disease progression. Little is known about how ALS affects the overall functional properties of the motor network. A recent study has suggested reduced connectivity in the default-mode network in ALS, a network linked to high-order cognitive processes and similar to our results no significant overall reduction in inter-hemispheric functional connectivity of the primary motor cortex was reported [16]. As the overall organisation of the motor network was preserved in ALS compared to controls, these results suggest that motor network connectedness may not be implicated in the aetiology of ALS but is involved in the spread of the disease along the structurally and functionally linked primary motor regions [33]. In other neurodegenerative diseases like Alzheimer’s disease this hypothesis is supported by the observation that pathological tau-protein spreads in vivo from one brain region to functionally connected spatially distinct regions [36]. In addition, imaging studies have found cortical degeneration in clinical subtypes of dementia occur in distinct functional neural networks [37]. The actual mode of transmission and the applicability of this finding to other neurodegenerative diseases remain, however, subjects for further research. Since, in our study, functional connectivity was found to be of potential prognostic importance it could become a target for therapeutic intervention. Interestingly riluzole, the only drug which delays disease progression is known to increase intracortical inhibition and therefore reduce functional connectivity [38]. An alternative hypothesis for our findings is that the increased local connectedness in patients with a faster disease progression is due to loss of cortical inhibitory interneurons. This effect has been reported previously in studies on excitability of the motor cortex [32,39]. However, in that case one would expect the average local connectedness to be increased in ALS compared to controls, which is not. Our data did not show a correlation between the functional and structural connectivity in the corpus callosum. This is in accordance with our opposing results regarding structural and functional connectivity as well as previous studies exploring this correlation [40,41].

A possible limitation of our study might include the limited sample size and the absence of longitudinal MRI measures. However, ALS is a rare disease with patients often losing ambulation shortly after diagnosis making it difficult to do this type of research in large cohorts of patients. In addition the objective of the present study was to apply multiple imaging
Figure 4. Correlation of imaging parameters with clinical measures. Correlation of ALSFRS-R score and progression rate with: a) cortical thickness in the precentral gyrus (CT PCG) of the clinically most affected side. Cortical thickness is corrected for average whole brain cortical thickness and age; b) FA in the rostral part of the corticospinal tract (CST) and FA in the corpus callosum (CC); c) Gamma - or local connectedness - corrected for local connectedness.
techniques to a relatively homogeneous group of patients to explore the central motor network characteristics in relation to clinical markers. Previous studies have too been able to pick up significant functional effects in similar small sample sizes [42]. However, future studies should further clarify the relevance of neural network characteristics regarding vulnerability to neurodegenerative effects. Secondly, using tractography, it may be difficult to differentiate FA effects in the corpus callosum from effects in the rostral part of the CST, as both tracts originate from the same cortical region and cross each other at the level of the corona radiata. This phenomenon of ‘crossing fibres’ is a general technical limitation in the DTI field and the differences found in this region can be interpreted in multiple ways, either the corpus callosum degeneration alone is responsible for these effects, or the CST degeneration or both. Thirdly, little is known about the reproducibility of resting-state fMRI data. However networks with a strong structural connectivity show more reliable functional connectivity across scanning sessions [20].

Our findings reporting on cortical thickness, white matter integrity and functional connectivity, strongly suggest a decline of structural motor network integrity in ALS. Functional connectivity was relatively preserved but was found to be related to disease progression, supporting the hypothesis that neurodegeneration in ALS spreads along functional connections.

Materials and Methods

Ethics Statement

All of the subjects gave their informed written consent, in line with the Declaration of Helsinki, and as approved by the local medical ethics committee for research into humans.

Participants

Twelve patients with ALS (mean age and standard deviation: 48.8 ± 10.6 years) and twelve age and sex-matched healthy controls (age: 49.6 ± 10.5) were included. Subject demographics and relevant clinical information are listed in Table 1. Patients were recruited from the ALS outpatient clinic of the University Medical Centre, Utrecht and were diagnosed with probable lab-supported, probable or definite ALS according to the El Escorial criteria [43]. All patients were treated with riluzole. To minimize confounding by non-ALS-related alterations of the brain we excluded subjects older than 65 years of age and subjects with a history of brain injury, epilepsy, vascular risk factors, psychiatric illness and other systemic diseases. Clinical status of the patients was evaluated using the ALS Functional Rating Scale-Revised (ALSFRS-R) and disease progression rate was assessed (48 - ALSFRS-R-score/disease duration (months)). Additional clinical information is provided in the Supplemental Table S1.

Image acquisition

All imaging data were acquired on a 3 Tesla Philips Achieva Medical Scanner. Imaging included the acquisition of an anatomical T1-weighted image; Diffusion Tensor Imaging (DTI) and resting-state functional MRI (rsfMRI).

Cortical thickness: Anatomical T1

A high resolution T1-weighted image was performed for cortical thickness measurements and anatomical reference. Acquisition parameters: 3D FFE using parallel imaging; TR/TE = 10/4.6 ms, flip-angle 8 degrees, slice orientation: sagittal, 0.75 × 0.75 × 0.8 mm voxelsize, FOV = 160 × 240 × 240 mm, reconstruction matrix = 200 × 320 × 320 covering whole brain.

Structural connectivity: Diffusion Tensor Imaging

In the same scanning session, 2 DTI sets each consisting of 30 diffusion-weighted and 5 diffusion-unweighted scans B = 0 scans (b = 0 s/mm²) were acquired to examine structural connectivity of the motor network. Acquisition parameters: DTI-MR using parallel imaging SENSE p-reduction 3; high angular gradient set of 30 different weighted directions [44,45], TR/TE = 7035/68 ms, voxel-size 2 × 2 × 2 mm, FOV = 120 × 120 × 150 mm, reconstruction matrix = 120 × 120 × 75 covering whole brain, b = 0 s/mm² for the diffusion-unweighted scans and b = 1000 s/mm² for the diffusion-weighted scans, second set with reversed k-space read-out [22,46].

Functional connectivity: resting-state functional

Magnetic Resonance Imaging

To examine the functional connections of the motor network, resting-state BOLD signals were recorded for a period of 8 min. Acquisition parameters: 3D PRESTOSENSE p/s-reduction 2/2, TR/TE = 22/32 ms using shifted echo, slice orientation: sagittal, flip-angle 10 degrees, dynamic scan time 0.5 sec, voxel-size 4 × 4 × 4 mm, FOV = 128 × 256 × 256 mm, reconstruction matrix = 40 × 64 × 64 (covering whole brain). A short volume acquisition was used to allow for proper sampling of information in the frequency domain up to 1 Hz, effectively minimising the contribution of respiratory and cardiac oscillations (0.3 and >0.8 Hz, respectively), into the resting-state lower frequencies of interest (0.01–0.1 Hz) [47].

Data analysis and statistics

Structural morphology of motor network: Cortical thickness. Cortical thickness measures were performed using the validated and freely available Freesurfer software package (http://surfer.nmr.mgh.harvard.edu/). First, for each individual dataset (both ALS and healthy controls), the anatomical T1-weighted scan was placed into standard space and grey and white

Table 1. Study population characteristics.

|                | ALS | controls |
|----------------|-----|----------|
| Sex (M/F)      | 10/2| 10/2     |
| Age (years)    | 48.8 (33-65) | 49.6 (33-64) |
| Disease duration (months) | 14.3 (7-30) | 11/1 |
| Time after diagnosis (months) | 5.2 (1-14) | 3/8/1 |
| Site of onset (spinal/bulbar) | 0.75 (0.13-1.38) |

Abbreviations: M = male. F = female. EE = El Escorial. ALSFRS-R = revised ALS functional rating scale.

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matter were segmented. Secondly, for each individual dataset, cortical thickness at every small region of the cortical surfaces was determined by computing the distance between the computed white matter and grey matter surface reconstructions [48]. Thirdly, a group average anatomical image and surface rendering were constructed by normalising all anatomical images to standard space using spherical normalisation. All individual datasets (both of patients with ALS and healthy controls) were normalised to the computed group average anatomical surface, allowing for group comparison between patients with ALS and group matched healthy controls at each small sliver of cortical surface (vertex), covering whole brain.

Whole brain cortical thinning between ALS patients and healthy participants was assessed using General Linear Model (GLM) by testing differences at all vertices. Furthermore, to examine specifically the hypothesised selective thinning of the precentral gyrus in ALS information of the automatic parcellation of the cortical surface [49,50] was used, parcellating each hemispheric cortical surface in 34 different regions, enabling the computation of an average cortical thickness of each of these region, separately for the two hemispheres. These 68 regions (each hemisphere covering 34 regions) included the left and right precentral gyrus forming the key regions of the motor network. These primary motor regions were selected for further analysis. Group comparison of the cortical thickness in the precentral gyrus was analyzed using a General Linear Model (GLM) included in the used SPSS software package (http://www.spss.com/software/statistics/). Age and whole brain average cortical thickness were included as covariates to adjust for residual confounding despite global matching [51,52]. For singly testing the primary motor regions a statistical threshold of \( \alpha \leq 0.05 \) was considered statistically significant. To examine the role of cortical thinning as a possible marker of disease, the level of cortical thickness of the clinically most affected precentral gyrus was correlated with clinical parameters using linear regression.

**Structural connectivity of motor network: Diffusion Tensor Imaging**

After post-processing of the DTI white matter tracts of the brain were constructed using the Fibre Assignment by Continuous Tracking (FACT) algorithm [53]. The left and right CST and corpus callosum tract were selected by sorting out those white matter tracts that touched the regions of the primary motor network (Figure 5) [21,22,54]. For the tracts of interest, group tracts were constructed to allow group comparison [54] and fractional anisotropy (FA) along the tracts were computed as a measure of white matter integrity of the primary motor tracts [17,55] (see Supplemental Materials S1). Hypothesized decrease of white matter integrity of the left and right CST and corpus callosum in ALS, were examined by comparison of the individual normalized tracts between patients and the matching healthy controls using multiple linear regression analysis. As a control

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**Figure 5. Selection of corticospinal tract (CST) and corpus callosum (CC) fibers.** First, all fibres were tracked. Secondly, the left precentral gyrus was selected as region of interest (ROI 1) and the fibres touching this ROI were selected. Thirdly, the brainstem was selected as second ROI (ROI 2a). The left CST was defined by all fibres touching both ROI 1 and ROI 2a. Finally, a third ROI (ROI 2b) was defined as the right precentral gyrus. The CC was defined by all fibres touching both ROI 1 and ROI 2b. The tracts of the right CST were defined in a similar manner. ROI = region of interest. PCG = precentral gyrus. CST = corticospinal tract. CC = corpus callosum. doi:10.1371/journal.pone.0013664.g005
condition the FA was measured along the visual tracts and the whole brain average FA was computed and compared between patients and controls.

**Functional connectivity of the motor network: resting-state fMRI**

To examine possible alterations in the functional communication in the motor network in ALS, resting-state fMRI was acquired [18,56]. Functional connectivity is defined as the temporal coherence between neuronal signals of anatomically separated brain regions [57,58]. Resting-state fMRI can be used to map functional connectivity by measuring interregional correlations in spontaneous neuronal synchronization reflected by coherency in low-frequency (<0.1 Hz) blood oxygen level-dependent (BOLD) signal fluctuations [10,56,59–63]. Especially regions of the primary motor network are known to show a high level of synchronisation between their spontaneous resting-state time-series, suggesting ongoing functional communication between motor regions [63–65].

To examine the quality of the functional motor network, graph analysis was used, assessing the integrity of functional communication efficiency in the motor network [66,67]. Representing a dynamic system as a network of regions and their interactions as connections allows for the examination of specific properties of the network. These properties include the level of connectedness or cliqueness of the nodes in the network, indicating how close nodes are connected to their direct neighbours [66–68]. This analysis included preprocessing of the resting-state fMRI data and the formation and examination of the organisation of the functional connections between the regions of the motor network (for more details, see Supplemental Materials S1).

In summary, functional connectivity analysis included examining the number of connections providing information on the total level of connectedness of the network, together with the normalised clustering-coefficient gamma providing information about the level of local connectedness and level of local information processing, given by the ratio between the number of connections with the direct neighbours of a node and the total number of possible connections between these neighbours.

**Statistics.** Organisational functional connectivity characteristics (the number of connections and level of local connectedness gamma) were compared between ALS patients and healthy controls using GLM statistics, controlling for possible effects of age.

**Integration of structural, functional and clinical data**

The relation between structural and functional measures was explored. The average FA value of the corpus callosum was compared with the number of functional interhemispheric connections using linear correlation. Finally, the main structural and functional measures were compared with the clinical parameters (ALSFRS-R and progression rate) in a similar manner.

**Supporting Information**

**Materials S1**

Found at: doi:10.1371/journal.pone.0013664.s001 (0.09 MB DOC)

**Table S1**

Found at: doi:10.1371/journal.pone.0013664.s002 (0.04 MB DOC)

**Figure S1**

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**Author Contributions**

Conceived and designed the experiments: EV MvdH LHvdB. Performed the experiments: EV NB. Analyzed the data: EV MvdH JHV. Contributed reagents/materials/analysis tools: MvdH RCWM HEHP LHvdB. Wrote the paper: EV MvdH JHV LHvdB.

References

1. Kunst CB (2004) Complex genetics of amyotrophic lateral sclerosis. Am J Hum Genet 75: 933–947.
2. Rothstein JD (2009) Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. Ann Neurol 65(Suppl 1): S3–9.
3. Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance G, et al. (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319: 1668–1672.
4. Ellis CM, Suckling J, Amaro E Jr., Bullmore ET, Simmons A, et al. (2001) Volumetric analysis reveals corticospinal tract degeneration and extramotor involvement in ALS. Neurology 57: 1571–1578.
5. Chang JL, Lomen-Hoerth C, Murphy, J, Henry RG, Kramer JH, et al. (2005) A voxel-based morphometry study of patterns of brain atrophy in ALS and ALS/FTLD. Neurology 63: 75–80.
6. Turner MR, Hamners A, Allopp J, Al-Chalabi A, Shaw CE, et al. (2007) Volumetric cortical loss in sporadic and familial amyotrophic lateral sclerosis. Amyotroph Lateral Scler 8: 343–347.
7. Thivard L, Pradat PF, Lhericy S, Lacombe L, Dormont D, et al. (2007) Diffusion tensor imaging and voxel based morphometry study in amyotrophic lateral sclerosis: relationships with motor disability. J Neurol Neurosurg Psychiatry 78: 889–892.
8. Sage CA, Peeters RB, Gorner A, Robberecht W, Sunaert S (2007) Quantitative diffusion tensor imaging in amyotrophic lateral sclerosis. NeuroImage 34: 486–499.
9. Ciccarelli O, Behrens TE, Johansen-Berg H, Talbot K, Orell RW, et al. (2009) Investigation of white matter pathology in ALS and PLS using tract-based spatial statistics. Hum Brain Mapp 30: 615–623.
10. Senda J, Ito M, Watanabe H, Atsuta N, Kawai Y, et al. (2009) Correlation

   between pyramidal tract degeneration and widespread white matter involvement in amyotrophic lateral sclerosis: A study with tractography and diffusion-tensor imaging. Amyotroph Lateral Scler 10: 289–294.
11. Graham JM, Papadakis N, Evans J, Wilija A, Romanowski CA, et al. (2004) Diffusion tensor imaging for the assessment of upper motor neuron integrity in ALS. NeuroImage 33: 2113–2119.
12. Sach M, Winkler G, Glauche V, Liepert J, Heinbach B, et al. (2004) Diffusion tensor MRI of early upper motor neuron involvement in amyotrophic lateral sclerosis. Brain 127: 340–350.
13. Tessitore A, Esposito F, Monurrro MR, Graziano S, Panza D, et al. (2006) Subcortical motor plasticity in patients with sporadic ALS: An fMRI study. Brain Res Bull 69: 489–492.
14. Schoenfeld MA, Tempelmann C, Gass C, Kuhnert GR, Duzel E, et al. (2005) Functional motor compensation in amyotrophic lateral sclerosis. J Neurol 252: 944–952.
15. Stanton BR, Williams VC, Leigh PN, Williams SC, Blain CR, et al. (2007) Altered cortical activation during a motor task in ALS. Evidence for involvement of central pathways. J Neurol 254: 1260–1267.
16. Mohammadi B, Kollewe K, Sanii A, Kramplik K, Dengler R, et al. (2009) Changes of resting state brain networks in amyotrophic lateral sclerosis. Exp Neurol 217: 147–153.
17. Kim JH, Loy DN, Liang HF, Trinkaus K, Schmidt RE, et al. (2007) Noninvasive diffusion tensor imaging of evolving white matter pathology in a mouse model of acute spinal cord injury. Magn Reson Med 58: 253–260.
18. Biswal B, Yetkin FZ, Haughton VM, Hyde JS (1995) Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn Reson Med 34: 537–541.
19. Wahl M, Lausserbach-Soon B, Hattingen E, Jung P, Singer O, et al. (2007) Human motor cortex callosus: topography, somatotopy, and link between microstructure and function. J Neurosci 27: 12132–12138.
20. Honey CJ, Sporns O, Cammoun L, Gigandet X, Thiran JP, et al. (2009) Predicting human resting-state functional connectivity from structural connectivity. Proc Natl Acad Sci U S A 106: 2035–2040.
21. van den Heuvel MP, Mandl RC, Kahn RS, Hulshoff Pol HE (2009) Functionally linked resting-state networks reflect the underlying structural connectivity architecture of the human brain. Hum Brain Mapp 30: 3127–3141.
22. van den Heuvel MP, Mandl RC, Laigues J, Hulshoff Pol HE (2008) Microstructural organization of the cingulum tract and the level of default mode functional connectivity. J Neurosci 28: 10844–10851.
23. Agosta F, Chio A, Cozzolino M, De Stefano N, Falini A, et al. (2010) The Present and the Future of Neuroimaging in Amyotrophic Lateral Sclerosis. AJNR Am J Neuroradiol 31: 133–141.

24. Rivara CB, Sherwood CC, Bouras C, Hof PR. (2003) Stereologic characterization and spatial distribution patterns of Betz cells in the human primary motor cortex. Anat Rec A Discov Mol Cell Evol Biol 270: 137–151.

25. Kierman JA, Hudson AJ (1991) Changes in sizes of cortical and lower motor neurons in amyotrophic lateral sclerosis. Brain 114(2 Pt 2): 843–853.

26. Agosta F, Pagani E, Rocca MA, Caputo D, Pernini M, et al. (2007) Voxel-based morphometry study of brain volumetry and diffusivity in amyotrophic lateral sclerosis patients with mild disability. Hum Brain Mapp 28: 1430–1438.

27. Grossman M, Anderson C, Khan A, Avants B, Elman L, et al. (2008) Impaired action knowledge in amyotrophic lateral sclerosis. Neurology 71: 1396–1401.

28. Hutton C, Draganu S, Ashburner J, Weiskopf N (2009) A comparison between voxel-based cortical thickness and voxel-based morphometry in normal aging. NeuroImage 48: 371–380.

29. Bakour A, Morini JC, Dickerson BC (2009) The cortical signature of prodromal AD: regional thinning predicts mild AD dementia. Neurology 72: 1045–1053.

30. Rohrer JD, Warren JD, Modat M, Ridgway GR, Doshi A, et al. (2008) Patterns of cortical thinning in the language variants of frontotemporal lobar degeneration. Neurology 72: 1562–1569.

31. Rosas HD, Salat DH, Lee SY, Zaleta AK, Pappu V, et al. (2008) Cerebral cortex and the clinical expression of Huntington’s disease: complexity and heterogeneity. Brain 131: 1057–1068.

32. Vucic S, Nicholson GA, Kierman MC (2008) Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. Brain 131: 1540–1550.

33. Eisen A (2009) Amyotrophic lateral sclerosis-Evolutionary and other perspectives. Muscle Nerve 40: 297–304.

34. Wittstock M, Wolters A, Benecke R (2007) Transcallosal inhibition in amyotrophic lateral sclerosis. Clin Neurophysiol 118: 301–307.

35. Bartels C, Mertens N, Hofer S, Merboldt KD, Dietrich J, et al. (2008) Callosal dysfunction in amyotrophic lateral sclerosis correlates with diffusion tensor imaging of the central motor system. Neuromuscular Disord 18: 390–397.

36. Clavaguera F, Bolmont T, Grouiller RA, Abramowski D, Frank S, et al. (2009) Transmission and spreading of tauopathy in transgenic mouse brain.1 Nat Cell Biol 11: 909–913.

37. Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD (2009) Neurodegenerative diseases target large-scale human brain networks. Neuron 60: 42–52.

38. Kakkonen S, Ilmoniemi RJ (2004) Transcranial magnetic stimulation: applications for neurophysiopharmacology. J Psychopharmacol 18: 257–261.

39. Vucic S, Cheah BC, Kierman MC (2009) Defining the mechanisms that underlie cortical hyperexcitability in amyotrophic lateral sclerosis. Exp Neurol 220: 177–182.

40. Lowe MJ, Beall EB, Sakae KE, Koenig KA, Stone L, et al. (2008) Resting state sensorimotor functional connectivity in multiple sclerosis inversely correlates with transcallosal motor pathway transverse diffusivity. Hum Brain Mapp 29: 819–827.

41. Morgan VL, Mishra A, Newton AT, Gore JC, Ding Z (2008) Integrating the thickness of cortical and lower motor neurons in amyotrophic lateral sclerosis from magnetic resonance images. Prog Neuropsychopharmacol Biol Psychiatry 32: 1384–1393.

42. Zhou J, Greicius MD, Gennatas ED, Growdon ME, Jiang JY, et al. (2010) Divergent network connectivity changes in behavioural variant frontotemporal dementia and Alzheimer’s disease. Brain 133: 1352–1367.

43. Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology (2009) Integrating the thickness of cortical and lower motor neurons in amyotrophic lateral sclerosis from magnetic resonance images. Prog Neuropsychopharmacol Biol Psychiatry 32: 1384–1393.

44. Fischl B, Dale AM (2000) Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proc Natl Acad Sci U S A 97: 11050–11055.

45. Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, et al. (2006) An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. NeuroImage 31: 968–980.

46. Fischl B, van der Kouwe A, Destrieux C, Halgren E, Segonne F, et al. (2004) Automatically parcellating the human cerebral cortex. Cereb Cortex 14: 11–22.

47. Rogers J, Kuchanov P, Lancaster J, Shelledy W, Glahn D, et al. (2007) Heritability of brain volume, surface area and shape: an MRI study in an extended pedigree of baboons. Hum Brain Mapp 28: 576–583.

48. van der Schot AC, Vonk R, Brains RG, van Haren NE, KooiChij P, et al. (2009) Influence of genes and environment on brain volumes in twin pairs concordant and discordant for bipolar disorder. Arch Gen Psychiatry 66: 142–151.

49. Mori S, Kaufmann WE, Davatzikos C, Stieltjes B, Amoedi L, et al. (2002) Imaging cerebral association tracts in the human brain using diffusion-tensor based axonal tracing. Magn Reson Med 47: 213–223.

50. Mancl RC, Schnack HG, Louiges J, van den Heuvel MP, Cahn W, et al. (2008) Tract-Based Analysis of Magnization Transfer Ratio and Diffusion Tensor Imaging of the Frontal and Frontotemporal Connections in Schizophrenia. Schizophr Bull 36: 776–787.

51. Crandall RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, et al. (2005) Automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. NeuroImage 31: 968–980.

52. Beckmann CF, DeLuca M, Devlin JT, Mathews VP, Phillips MD (2000) Correlations in low-frequency BOLD fluctuations reflect cortico-cortical connections. NeuroImage 12: 382–387.

53. Aertsen AM, Gerstner GL, Habib MK, Palm G (1989) Dynamics of neuronal firing correlation: modulation of “effective connectivity”. Journal of Neurophysiology 61: 900–917.

54. Friston KJ, Frith CD, Liddle PF, Frackowiak RS (1995) Functional connectivity: the principal-component analysis of large (PET) data sets. J Cereb Blood Flow Metab 15: 3–14.

55. Forsyth J, Chiu A, Droit CR, Zilles K, Zilles K, et al. (2004) Mapping functionally related regions of brain with functional connectivity MR imaging. AJNR Am J Neuroradiol 21: 1363–1344.

56. Salvador R, Martinez A, Pomarol-Clotet E, Sarro S, Suckling J, et al. (2007) Reduced resting-state brain activity in the “default network” in normal aging. Cereb Cortex 18: 1836–1846.

57. Bakekamp CF, DeLuca M, Devlin JT, Smith SM (2005) Investigations into resting-state connectivity using independent component analysis. Philos Trans R Soc Lond B Biol Sci 360: 1001–1013.

58. Akshar S, Salvador R, Whitter B, Suckling J, Bullmore E (2006) A resilience, low-frequency, small-world human brain functional network with highly connected association cortical hubs. J Neurosci 26: 63–72.

59. Danoiseus JS, Rombouts SA, Barkhof F, Scheffens P, Stam CJ, et al. (2006) Consistent resting-state networks across healthy subjects. Proc Natl Acad Sci U S A 103: 13848–13853.

60. van den Heuvel MP, Stam CJ, Boersma M, Hulshoff Pol HE (2008) Small-world and scale-free organization of voxel-based resting-state functional connectivity in the human brain. NeuroImage 43: 528–539.

61. Danoiseus JS, Beckmann CF, Arista EJ, Barkhof F, Scheffens P, et al. (2008) Reduced resting-state brain activity in the “default network” in normal aging. Cereb Cortex 18: 1836–1846.

62. Salvador R, Martínez A, Pomarol-Clotet E, Sarro S, Suckling J, et al. (2007) Frequency based mutual information measures between clusters of brain regions in functional magnetic resonance imaging. NeuroImage 35: 83–98.

63. Stam CJ, Reijneveld JC (2007) Graph theoretical analysis of complex networks in the brain. Nonlinear Biomedical Physics 1: 3.

64. Bullmore E, Sporns O (2009) Complex brain networks: graph theoretical analysis of structural and functional systems. Nature Reviews 10: 186–194.

65. Reijneveld JC, Ponten SC, Berendse HW, Stam CJ (2007) The application of graph theoretical analysis to complex networks in the brain. Clin Neurophysiol 118: 2317–2331.