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Malek Abidi, Giovanni de Marco, Annabelle Couillandre, M. Feron, Eya Mseddi, Nicolas Termoz, G. Querin, P. -f. Pradat, P. Bede

Adaptive functional reorganization in amyotrophic lateral sclerosis: coexisting degenerative and compensatory changes

M. Abidi[a], G.de Marco[a,b], A. Couillandre[a,b], M. Feron[a], E. Mseddi[a], N. Termoz[a,b], G. Querin[c,d], P. -F. Pradat[c,d,*] and P. Bede[c,d,e,*]

[a]*CeRSM Laboratory, Nanterre University,UPL, Paris;* [b]*COMUE Paris Lumières University, Paris;* [c]*Department of Neurology, Pitié-Salpêtrière Hospital, Paris;* [d]*Biomedical Imaging Laboratory, Sorbonne University, Paris, France; and* [e]*Computational Neuroimaging Group, Trinity College Dublin, Dublin, Ireland*

Keywords: amyotrophic lateral sclerosis, fMRI, imaging, motor neuron disease, MRI, neuroplasticity

Received 22 March 2019 Accepted 10 July 2019

European Journal of Neurology 2019, **0:** 1–8

doi:10.1111/ene.14042

Background and purpose: Considerable functional reorganization takes place in amyotrophic lateral sclerosis (ALS) in face of relentless structural degenera-tion. This study evaluates functional adaptation in ALS patients with lower motor neuron predominant (LMNp) and upper motor neuron predominant (UMNp) dysfunction.
Methods: Seventeen LMNp ALS patients, 14 UMNp ALS patients and 14 controls participated in a functional magnetic resonance imaging study. Study-group-specific activation patterns were evaluated during preparation for a motor task. Connectivity analyses were carried out using the supplementary motor area (SMA), cerebellum and striatum as seed regions and correlations were explored with clinical measures.
Results: Increased cerebellar, decreased dorsolateral prefrontal cortex and decreased SMA activation were detected in UMNp patients compared to con-trols. Increased cerebellar activation was also detected in UMNp patients com-pared to LMNp patients. UMNp patients exhibit increased effective connectivity between the cerebellum and caudate, and decreased connectivity between the SMA and caudate and between the SMA and cerebellum when performing self-initiated movement. In UMNp patients, a positive correlation was detected between clinical variables and striato-cerebellar connectivity. **Conclusions:** Our findings indicate that, despite the dysfunction of SMA–striatal and SMA–cerebellar networks, cerebello-striatal connectivity increases in ALS indicative of compensatory processes. The coexistence of circuits with decreased and increased connectivity suggests concomitant neurodegenerative and adaptive changes in ALS.

Introduction

Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive neurodegenerative condition. Whilst extra-motor involvement is increasingly recognized [1], the hallmark feature of ALS is the progressive degeneration of the motor neuron system. One of the key facets of clinical heterogeneity in ALS is the relative proportion of upper and lower motor neuron involvement which defines the clinical phenotype and determines the disability profile and care needs of patients.

Cortical motor neuron loss and motor cortex atrophy is a well-established feature of ALS [2], but the ensuing functional reorganization is relatively poorly characterized. Pre-symptomatic studies of ALS-associated mutation carriers have highlighted that considerable structural changes can be detected long before symptom onset [3,4]. Functional imaging studies of ALS suggest that a number of compensatory processes take place in the face of progressive motor cortex degeneration and activation patterns shift from the

Correspondence: P. Bede, Computational Neuroimaging Group, Trinity Biomedical Sciences Institute,152-160 Pearse Street, Dublin 2, Ireland (tel.: + 353 1 8964497; e-mail: bedep@tcd.ie).
*Contributed equally as senior authors.

precentral gyrus to supplementary motor, pre-motor and cerebellar regions during movement execution [5]. Despite the plethora of imaging studies in ALS [6], most studies admix heterogeneous patient cohorts [7] without stratifying them based on their genotype, cognitive profile or upper motor neuron (UMN) versus lower motor neuron (LMN) predominance [8,9]. Existing studies highlight increased cortical disease burden in UMN predominant (UMNp) cohorts compared to LMN predominant (LMNp) patients [10,11]. In addition to cortical alterations, increased basal ganglia and cerebellar involvement has been noted in UMNp cohorts [12,13]. Structural magnetic resonance imaging (MRI) studies have consistently highlighted focal cortical [14], subcortical [15,16] and cerebellar [17] degeneration. The vast majority of functional MRI (fMRI) studies in ALS have focused on the execution phase of a motor task. Preparation for a motor task is seldom studied specifically despite evidence of impaired preparation for hand movements from event-related potential techniques [18] and blood flow alterations during movement planning in positron emission tomography studies [19]. In healthy populations movement preparation typically involves the premotor area [20], cerebellum [21] and basal ganglia [22].

The primary objective of this study is to characterize functional reorganization in a cohort of ALS patients stratified for UMN versus LMN disease burden. Based on recent imaging studies [10–12,23], it was hypothesized that UMNp patients exhibit considerable motor system reorganization compared to LMNp patients.

Methods

This study was approved by the institutional research board of the CPP Ile-de-France Paris VI. Thirty-one patients with ALS and 14 age- and gender-matched healthy controls gave informed consent to participate in this study. Based on standardized clinical evaluation [24,25], ALS patients were divided into two subgroups: a UMNp cohort ($n = 14$) and an LMNp group ($n = 17$) (Table 1). Inclusion criteria included 'definite' or 'probable' ALS according to the revised El Escorial criteria [26], age between 18 and 70 years, right-handedness. Exclusion criteria included frank frontotemporal dementia based on current diagnostic criteria [27], coexisting musculoskeletal conditions that would have interfered with functional evaluation, and contraindications to MRI.

All participants underwent standardized clinical examination on the day of imaging. Functional impairment was evaluated by the revised ALS Functional Rating Scale (ALSFRS-r). Disease progression rate was calculated as (48 − ALSFRS-r)/disease duration (months). All participants underwent a comprehensive neuropsychological evaluation including tests for memory, executive, language and visio-spatial domains (Table 1).

Magnetic resonance imaging

Magnetic resonance imaging data were acquired on a 3-T Siemens Prisma platform. T1-weighted structural images were acquired with a magnetization-prepared rapid acquisition gradient echo sequence with repetition time (TR)/echo time (TE) = 2300/4.2 ms, inversion time 900 ms and isotropic 1 × 1 × 1 mm voxel size. Functional images were obtained using a single-shot echo-planar imaging (EPI) sequence with TR/TE = 2020/27 ms; flip angle = 78°; field of view 198 × 198 mm^2. An event-related motor paradigm was implemented to assess the activation correlates of movement preparation. The instructions were clearly explained before starting the fMRI experiment and subjects were asked to perform simultaneous right/left ankle dorsiflexion. Participants were instructed to prepare mentally for the movement before executing the motor task. The subjects were asked to perform a series of ankle dorsiflexions based on a specific number displayed on the presentation screen (1, 3, 4). The number of movements requested to prepare for was randomly intermixed in the experiment.

Magnetic resonance imaging data processing

Functional MRI analyses were performed using the Statistical Parametric Mapping SPM12 suite (UCL, Wellcome Centre for Human Neuroimaging, London, UK). In each dataset, for T1 equilibrium the first four volumes were discarded. All EPI volumes were corrected to adjust for within-volume time differences and then realigned with the last volume to correct for head movements. Functional scans were subsequently spatially normalized against the standard stereotactic MNI space. Spatial smoothing was performed with an 8 mm full width half maximum Gaussian kernel. Haemodynamic responses to task were modelled with a canonical haemodynamic response function and its first order temporal derivative [28]. The temporal deviation allows the measurement of brain activity prior to movement. It is a good indicator of movement preparation activities [20].

Functional MRI data were analysed for each participant separately on a voxel-by-voxel basis using the general linear model approach for time series. A contrast between motor preparation and rest was

Table 1 The demographic and clinical profile of study participants

	Healthy controls (n = 14)	UMN predominant ALS patients (n = 14)	LMN predominant ALS patients (n = 17)	P value
Age (years)	63.0 (57.0–66.0)	59.0 (20.0–71.0)	62.0 (31.0–74.0)	0.39
Gender (female/male)	5/9	3/10	6/11	0.29
Height (cm)	170 (168–175)	171 (157–187)	176 (154–186)	0.34
Weight (kg)	74.5 (66.0–83.7)	67.2 (53.0–90.0)	66.0 (54.0–86)	0.45
Disease onset				
Upper limb	N/A	3	3	0.37
Lower limb		7	10	0.58
Bulbar		4	4	0.81
ALSFRS-r (max 48)	N/A	37.5 (35.2–41.0)	40.0 (33.0–46.0)	0.07
Disease duration (months)	N/A	23.5 (14.7–37.2)	15.0 (07.0–80.0)	0.73
Disease progression rate	N/A	0.58 (0.12–1.1)	0.46 (0.09–1.0)	0.13
Cognitive assessment				
California verbal learning test II CVLT II				
Immediate recall	N/A	07.0 (04.0–13.0)	06.0 (04.0–10.0)	0.16
Total trial recall (1–5)		57.0 (35.0–68.0)	52.0 (38.0–69.0)	0.32
Short delay free recall		12.0 (09.0–16.0)	12.0 (07.0–16.0)	0.21
Short delay cued recall		01.0 (0–13.0)	02.0 (0–16.0)	0.48
Long delay free recall		14.0 (11.0–16.0)	14.0 (09.0–16.0)	0.41
Long delay cued recall		01.0 (0–13.0)	01.0 (0–16.0)	0.25
Total recognition discrimination		16.0 (13.0–16.0)	16.0 (0–16.0)	0.27
Stroop test				
Reading	N/A	99.5 (46.0–117)	92.0 (41.0–123.0)	0.23
Naming		73.5 (34.0–84.0)	64.0 (40.0–80.0)	0.13
Double task		38.5 (24.0–53.0)	37.0 (17.0–50.0)	0.33
Verbal fluency test				
Phonemic	N/A	25.0 (03.0–40.0)	18.0 (09.0–38.0)	0.46
Semantic		31.0 (16.0–51.0)	32.0 (10.0–45.0)	0.44
Wisconsin card sorting test				
Categories achieved	N/A	06.0 (01.0–06.0)	06.0 (03.0–06.0)	0.36
Perseverative errors		07.0 (0–31.0)	09.5 (05.0–17.0)	0.22
		03.0 (0–11.0)	04.0 (01.0–09.0)	0.38
Digit span				
Forward	N/A	08.0 (04.0–11.0)	07.0 (04.0–13.0)	0.49
Backwards		05.0 (04.0–08.0)	04.0 (02.0–10.0)	0.34

ALS, amyotrophic lateral sclerosis; ALSFRS-r, revised ALS Functional Rating Scale; LMN, lower motor neuron; N/A, not applicable; UMN, upper motor neuron. Values are presented as median (range) for functional scores followed by the minimum and the maximum values. Disease progression = (48 − ALSFRS-r score)/disease duration. All patients were on riluzole therapy.

calculated for each participant. A one-sample t test model was used to identify the activation during movement preparation within each group [$P < 0.05$, family-wise error (FWE) corrected]. Then, a two-sample t test was used to explore differences between the UMNp versus LMNp cohorts and UMNp versus healthy controls during motor preparation ($P < 0.05$, FWE corrected).

Three *a priori* seed regions were defined: the supplementary motor area (SMA), striatum and cerebellum, which are established hubs of movement preparation [21,22]. Anatomical masks of the SMA, striatum and cerebellum were created using the Automated Anatomical Labeling (AAL) atlas of the SPM Wake Forest University (WFU) PickAtlas toolbox. The cerebellar mask included lobule VI and crus I of the cerebellum, and the striatum mask included the caudate and putamen as defined in the AAL atlas in the WFU PickAtlas toolbox (Wake Forest University Health Sciences, Winston-Salem, NC, USA). The cerebellar regions of interest (ROIs) crus I and lobule VI were selected because of their established association with motor planning and preparation [29], and because of their role in self-initiated movement [30].

For each participant, extracted haemodynamic time series from seed regions were deconvoluted and the resulting neuronal time series (physiological variable) were combined with the onset times of each stimulus presented under the movement preparation condition and rest (psychological variables) to derive the interaction term (source signal × experimental context). To test for differences in regression slopes between the two experimental conditions, a general linear model was generated with this interaction term as the explanatory variable. The resulting individual t-contrast images were entered into a random effects group analysis and

tested for statistical significance at $P < 0.05$ (FWE corrected). A one-sample t test analysis was used to identify the connectivity of each seed area (striatum, SMA, cerebellum) for each group ($P < 0.05$, FWE corrected). Then, a two-sample t test analysis was used to explore the difference of connectivity between patients and controls in each index area ($P < 0.05$, FWE corrected). Finally, in order to evaluate the biomarker potential of the network metrics, whether clinical measures such as disability scores, disease duration and progression rates correlate with connectivity measures between cortical regions was explored.

The FreeSurfer image analysis suite (Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Boston, MA, USA) was used for cortical thickness and volume measurements. Study groups were first compared at a 'whole brain' level using FreeSurfer's QDEC application. A general linear model was used with age and gender as covariates and false discovery rate corrections were applied. In a supplementary analysis, ROI volumetric and thickness analyses were carried out on cortical measures from atlas-defined regions. The labels of the Desikan–Killiany and Destrieux atlases were used to define the following cortical regions: primary motor cortex, paracentral gyrus, superior frontal gyrus, caudal middle frontal gyrus, SMA and the primary sensory cortex. Average cortical volume and thickness were retrieved from the above regions and compared in ANCOVAs using age and gender as covariates.

Results

The UMNp and LMNp groups are matched in age, ALSFRS-r subscores, disease duration, progression rates and cognitive performance (Table 1).

Brain activation patterns

During self-initiated movement preparation, UMNp patients exhibit decreased activation compared to healthy controls in the right SMA, left SMA, right dorsolateral prefrontal cortex and superior frontal gyrus (Fig. 1). Conversely, UMNp patients activate supplementary cortical regions in contrast to controls in the cerebellum, right crus II, right crus I and left lobule VI (Fig. 2). UMNp patients also present enhanced activation compared to LMNp patients in the left vermis VII, right lobule VI and left precuneus (Fig. 3). No significant differences were identified in activation patterns between healthy controls and LMNp patients.

Effective connectivity

Compared to controls, UMNp patients show significantly decreased SMA–striatal and SMA–cerebellar connectivity. They also exhibit decreased effective connectivity between the SMA and cerebellum compared to LMNp patients, in particular in connection with crus I and crus II.

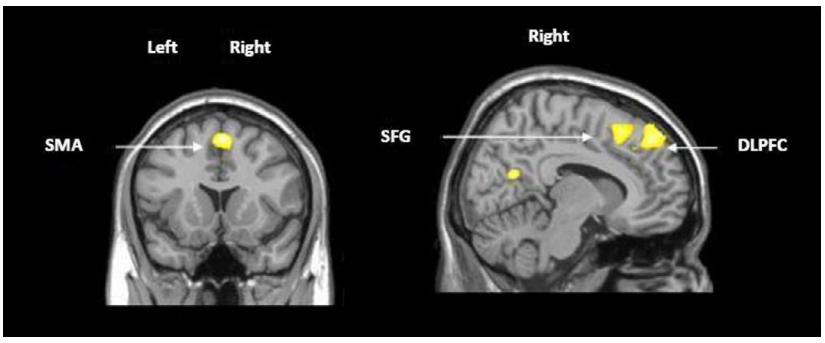

Figure 1 Regional activation in healthy controls compared to UMNp ALS patients during movement preparation. DLPFC, dorsolateral prefrontal cortex; SMA, supplementary motor area; SFG, superior frontal gyrus ($P < 0.05$ FWE).

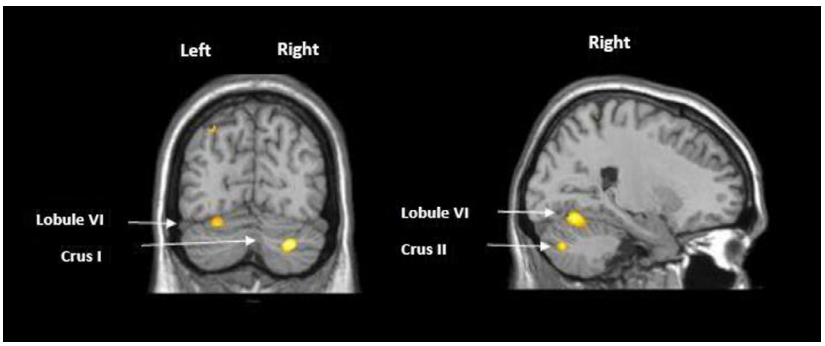

Figure 2 Increased activation in UMNp patients compared to controls during movement preparation ($P < 0.05$ FWE).

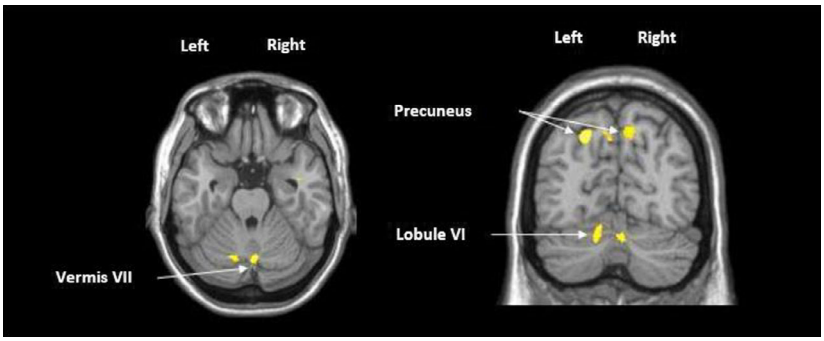

Figure 3 Patterns of increased activation in UMNp patients compared to LMNp patients during movement preparation ($P < 0.05$).

Compared to controls, UMNp patients present significantly decreased connectivity between the striatum and SMA and between the striatum and Brodmann area 10. However, UMNp patients show increased connectivity between the striatum and crus I, as well as between the striatum and the superior parietal lobule. Whilst UMNp patients show reduced connectivity between the striatum and middle frontal gyrus compared to LMNp patients, they exhibit increased connectivity between the striatum and the cerebellum (lobule VI) as well as the striatum and the right temporal cortex.

Compared to healthy controls, UMNp patients showed significantly decreased connectivity between the cerebellum and frontopolar prefrontal cortex (Brodmann area 10) and between the cerebellum and posterior cingulate cortex. In contrast, UMNp patients exhibit increased connectivity between the cerebellum and caudate and between the cerebellum and precuneus (Table 2). Compared to the LMNp cohort, the UMNp group shows decreased connectivity between the cerebellum and SMA and increased connectivity between the cerebellum and caudate, and between the cerebellum and thalamus (Tables 2 and 3). No significant differences were detected in ROI effective connectivity between healthy controls and the LMNp group.

Correlation analyses

A positive correlation was identified between clinical variables and the effective connectivity between the striatum and cerebellum in the UMNp cohort. A negative association was detected between clinical scores and the effective connectivity between the cerebellum and superior frontal gyrus and between the cerebellum and middle temporal gyrus. In the LMNp cohort, positive correlations were identified between clinical variables and the SMA to striatum connectivity and disease severity/disease duration and SMA to cerebellum connectivity. Negative correlations were detected between disease severity/disease duration and SMA to anterior cingulate connectivity (Tables S1 and S2).

Table 2 The functional connectivity profile of UMNp patients during movement initiation compared to controls

Seed region	Target region	Side	BA	*x*	*y*	*z*	*t*	pFWE
SMA								
HC > UMNp	Caudate	Left	*	−18	−31	19	4.08	0.01
		Right	*	3	5	13	3.35	0.03
	Cerebellum lobule IV–V	Right	*	12	−40	−11	3.77	0.02
Striatum								
HC > UMNp	SMA	Right	6	21	17	52	2.74	0.04
	Superior frontal gyrus	Right	10	30	65	4	2.9	0.04
UMNp > HC	Crus I	Left	*	−42	−70	−35	3.68	0.02
	Superior parietal lobule	Left	7	−24	−58	61	2.84	0.04
Cerebellum (crus I, lobule VI)								
HC > UMNp	Posterior cingulate	Left	30	−21	−55	10	2.80	0.04
	Middle frontal gyrus	Right	10	27	53	−11	2.73	0.04
UMNp > HC	Caudate	Left	*	−15	5	16	4.00	0.02
	Precuneus	Right	7	3	−61	61	2.84	0.04

Differences in effective connectivity between upper motor neuron predominant (UMNp) ALS patients and healthy controls (HC). BA, Brodmann area; SMA, supplementary motor area. In the Side column, Left and Right refer to left hemisphere and right hemisphere. Coordinates (*x*, *y*, *z*) are provided in MNI space. *t* and pFWE are *P* values corrected for multiple comparisons. *Not available

Table 3 Effective connectivity differences between LMNp and UMNp patients during movement initiation

Seed region	Target region	Side	BA	*x*	*y*	*z*	*t*	pFWE
SMA								
LMNp > UMNp	Crus 2	Left	*	−39	−67	−38	3.84	0.01
	Crus 1	Right	*	33	−64	−44	3.68	0.02
Striatum								
LMNp > UMNp	Middle frontal gyrus	Right	6	36	2	61	2.96	0.04
UMNp > LMNp	Declive (lobule VI)	Left	*	−27	−55	−11	3.85	0.01
	Middle temporal gyrus	Right	37	54	−52	−8	3.22	0.03
Cerebellum (crus I, lobule VI)								
LMNp > UMNp	Postcentral gyrus	Left	3	−30	−28	70	3.78	0.02
	Medial frontal gyrus	Left	6	−3	−16	70	3.51	0.02
UMNp > LMNp	Thalamus	Right	*	9	−28	19	4.73	0.01
	Caudate	Left	*	−12	−28	22	4.69	0.01
		Right	*	12	14	13	4.30	0.01

Differences in effective connectivity between upper motor neuron predominant (UMNp) and lower motor neuron predominant (LMNp) ALS patients. ALS, amyotrophic lateral sclerosis; BA, Brodmann area; SMA, supplementary motor area. In the Side column, Left and Right refer to left hemisphere and right hemisphere. Coordinates (*x*, *y*, *z*) are presented in MNI space. *t* and pFWE are *P* values corrected for multiple comparisons. *Not available

Structural analyses

Standard whole-brain comparisons of the UMNp cohort versus controls and the UMNp cohort versus the LMNp cohort for cortical thickness and volumes did not reach significance following false discovery rate corrections for multiple comparisons and adjustments for age and gender. ROI analyses, however, revealed group differences in the right hemisphere. The right paracentral gyrus exhibited volumetric ($P = 0.05$) and thickness ($P = 0.049$) reduction in the UMNp cohort compared to the LMNp group. A trend ($P = 0.075$) of cortical thickness reduction was also observed in the right precentral gyrus of UMNp patients compared to LMNp patients following adjustments for age and gender. The UMNp cohort exhibited significant ($P = 0.024$) right precentral gyrus volume reductions compared to healthy controls. No intergroup differences were observed in the other cortical ROIs.

Discussion

Whilst the functional reorganization of cortical and subcortical motor areas in ALS during movement execution is relatively well established [31–33], the functional correlates of movement preparation are poorly characterized in ALS. Our findings confirm that LMNp patients exhibit similar activation patterns to healthy controls and do not show increased activation with reference to UMNp patients. Interestingly, our results indicate that UMNp patients exhibit altered premotor activation during movement preparation not only in comparison to controls but also in contrast to LMNp patients. Reduced activation in premotor regions during motor tasks is consistent with previous reports [23,34]. Imaging [35] and postmortem studies [36] have previously described premotor cortex pathology in ALS, but few of these studies have stratified the patients based on UMN/LMN involvement.

Standard whole-brain structural analyses did not capture differences in cortical thickness or cortical volumes between UMNp and LMNp patients, yet their functional activation patterns and connectivity profiles are strikingly different. The divergence of structural and functional imaging profiles suggests that functional changes may precede frank structural atrophy [37]. Another interpretation is that fMRI has greater detection sensitivity to early motor network changes than structural MRI [38]. The correlations between clinical and connectivity metrics highlight the putative biomarker value of network integrity metrics, which may be superior to structural imaging indices [39]. With the increasing use of machine learning applications in ALS [40–43], the evaluation of connectivity indices is particularly important.

The functional reorganization of motor control has been previously linked to the recruitment of structurally less affected areas in ALS [5,12]. We observed increased striato-cerebellar connectivity in our UMNp cohort during movement preparation. Cerebellar and striatal pathways play a role in the pre-programming of movements [44] and represent anatomical hubs to fine-tune gross motor functions. Both structures are highly interconnected and convey both motor and cognitive functions [45]. Despite the comparable disability and structural profile of the two patient groups, fMRI has successfully captured differing

cortical activation patterns between our UMNp and LMNp cohorts. Supplementary activation of subcortical areas and the increased striato-cerebellar connectivity may represent adaptive mechanisms to compensate for structural degeneration. The close association between striato-cerebellar connectivity and clinical variables may indicate that functional reorganization underpins clinical performance.

Conclusions

This imaging study provides compelling evidence of functional reorganization in ALS. Our findings suggest that structurally less affected brain regions increasingly contribute to the execution and planning of motor tasks. Our results also highlight that stratifying patients based on UMN involvement helps to reduce disease heterogeneity and capture unifying activation patterns. The increased reliance on subcortical structures and increased striato-cerebellar connectivity are likely to represent functional adaptation.

Acknowledgements

The authors are grateful for the generosity of all study participants who participated in this research study. We specifically acknowledge the physicians who assisted with the recruitment of patients: Nadine Le Forestier, Maria del Mar Amador, Timothée Lenglet, Gaëlle Bruneteau and François Salachas. This study was funded by a grant from the Association for Research on ALS (ARSLA) and the Institut National pour la Santé et la Recherche Médicale (INSERM). The research leading to these results has also received funding from the program 'Investissements d'avenir' ANR-10-IAIHU-06. Peter Bede is the medical patron of the Irish MND Association (IMNDA); he is supported by the Health Research Board (HRB – Ireland; HRB EIA-2017-019), the Andrew Lydon scholarship, the Iris O'Brien Foundation, the Irish Institute of Clinical Neuroscience IICN – Novartis Ireland Research Grant, and the Research Motor Neuron (RMN-Ireland) Foundation.

Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Association between connectivity metrics and clinical scores in lower motor neuron predominant ALS patients: correlation analyses.

Table S2. Association between effective connectivity and clinical measures in upper motor neuron predominant ALS patients: correlation analyses.

References

1. Christidi F, Karavasilis E, Rentzos M, Kelekis N, Evdokimidis I, Bede P. Clinical and radiological markers of extra-motor deficits in amyotrophic lateral sclerosis. *Front Neurol* 2018; **9:** 1005.
2. Bede P, Bokde A, Elamin M, *et al*. Grey matter correlates of clinical variables in amyotrophic lateral sclerosis (ALS): a neuroimaging study of ALS motor phenotype heterogeneity and cortical focality. *J Neurol Neurosurg Psychiatry* 2013; **84:** 766–773.
3. Schuster C, Elamin M, Hardiman O, Bede P. Presymptomatic and longitudinal neuroimaging in neurodegeneration – from snapshots to motion picture: a systematic review. *J Neurol Neurosurg Psychiatry* 2015; **86:** 1089–1096.
4. Querin G, Bede P, El Mendili MM, *et al*. Presymptomatic spinal cord pathology in c9orf72 mutation carriers: a longitudinal neuroimaging study. *Ann Neurol* 2019; **86:** 158–167.
5. Proudfoot M, Bede P, Turner MR. Imaging cerebral activity in amyotrophic lateral sclerosis. *Front Neurol* 2018; **9:** 1148.
6. Bede P, Querin G, Pradat PF. The changing landscape of motor neuron disease imaging: the transition from descriptive studies to precision clinical tools. *Curr Opin Neurol* 2018; **31:** 431–438.
7. Muller HP, Turner MR, Grosskreutz J, *et al*. A large-scale multicentre cerebral diffusion tensor imaging study in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 2016; **87:** 570–579.
8. Finegan E, Chipika RH, Shing SLH, Hardiman O, Bede P. Primary lateral sclerosis: a distinct entity or part of the ALS spectrum? *Amyotroph Lateral Scler Frontotemporal Degener* 2019; **20:** 133–145.
9. Bede P, Hardiman O. Lessons of ALS imaging: pitfalls and future directions – a critical review. *Neuroimage Clin* 2014; **4:** 436–443.
10. Ellis CM, Suckling J, Amaro E Jr, *et al*. Volumetric analysis reveals corticospinal tract degeneration and extramotor involvement in ALS. *Neurology* 2001; **57:** 1571–1578.
11. Turner MR, Rabiner EA, Hammers A, *et al*. [11C]-WAY100635 PET demonstrates marked 5-HT1A receptor changes in sporadic ALS. *Brain* 2005; **128:** 896–905.
12. Tessitore A, Esposito F, Monsurrò MR, *et al*. Subcortical motor plasticity in patients with sporadic ALS: an fMRI study. *Brain Res Bull* 2006; **69:** 489–494.
13. Konrad C, Jansen A, Henningsen H, *et al*. Subcortical reorganization in amyotrophic lateral sclerosis. *Exp Brain Res* 2006; **172:** 361–369.
14. Bede P, Iyer PM, Schuster C, *et al*. The selective anatomical vulnerability of ALS: 'disease-defining' and 'disease-defying' brain regions. *Amyotroph Lateral Scler Frontotemporal Degener* 2016; **17:** 561–570.
15. Feron M, Couillandre A, Mseddi E, *et al*. Extrapyramidal deficits in ALS: a combined biomechanical and neuroimaging study. *J Neurol* 2018; **265:** 2125–2136.

16. Bede P, Elamin M, Byrne S, *et al*. Basal ganglia involvement in amyotrophic lateral sclerosis. *Neurology* 2013; **81:** 2107–2115.
17. Bede P, Elamin M, Byrne S, *et al*. Patterns of cerebral and cerebellar white matter degeneration in ALS. *J Neurol Neurosurg Psychiatry* 2015; **86:** 468–470.
18. Thorns J, Wieringa BM, Mohammadi B, Hammer A, Dengler R, Münte TF. Movement initiation and inhibition are impaired in amyotrophic lateral sclerosis. *Exp Neurol* 2010; **224:** 389–394.
19. Abrahams S, Goldstein LH, Kew JJ, *et al*. Frontal lobe dysfunction in amyotrophic lateral sclerosis. A PET study. *Brain* 1996; **119:** 2105–2120.
20. Cunnington R, Windischberger C, Deecke L, Moser E. The preparation and execution of self-initiated and externally-triggered movement: a study of event-related fMRI. *NeuroImage* 2002; **15:** 373–385.
21. Leiner HC. Solving the mystery of the human cerebellum. *Neuropsychol Rev* 2010; **20:** 229–235.
22. Boecker H, Jankowski J, Ditter P, Scheef L. A role of the basal ganglia and midbrain nuclei for initiation of motor sequences. *NeuroImage* 2008; **39:** 1356–1369.
23. Mohammadi B, Kollewe K, Samii A, Krampfl K, Dengler R, Münte TF. Changes of resting state brain networks in amyotrophic lateral sclerosis. *Exp Neurol* 2009; **217:** 147–153.
24. Simon NG, Lin CS, Lee M, *et al*. Segmental motoneuronal dysfunction is a feature of amyotrophic lateral sclerosis. *Clin Neurophysiol* 2015; **126:** 828–836.
25. Kaufmann P, Pullman SL, Shungu DC, *et al*. Objective tests for upper motor neuron involvement in amyotrophic lateral sclerosis (ALS). *Neurology* 2004; **62:** 1753–1757.
26. Brooks BR, Miller RG, Swash M, *et al*. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000; **1:** 293–299.
27. Rascovsky K, Hodges JR, Knopman D, *et al*. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* 2011; **134:** 2456–2477.
28. Josephs O, Turner R, Friston K. Event-related fMRI. *Hum Brain Mapp* 1997; **5:** 243–248.
29. Koziol LF, Budding D, Andreasen N, *et al*. Consensus paper: the cerebellum's role in movement and cognition. *Cerebellum* 2014; **13:** 151–177.
30. Hoffstaedter F, Grefkes C, Zilles K, Eickhoff SB. The 'what' and 'when' of self-initiated movements. *Cereb Cortex* 2013; **23:** 520–530.
31. Konrad C, Henningsen H, Bremer J, *et al*. Pattern of cortical reorganization in amyotrophic lateral sclerosis: a functional magnetic resonance imaging study. *Exp Brain Res* 2002; **143:** 51–56.
32. Schoenfeld MA, Tempelmann C, Gaul C, *et al*. Functional motor compensation in amyotrophic lateral sclerosis. *J Neurol* 2005; **252:** 944–952.
33. Nasseroleslami B, Dukic S, Broderick M, *et al*. Characteristic increases in EEG connectivity correlate with changes of structural MRI in amyotrophic lateral sclerosis. *Cereb Cortex* 2019; **29:** 27–41.
34. Cosottini M, Pesaresi I, Piazza S, *et al*. Structural and functional evaluation of cortical motor areas in amyotrophic lateral sclerosis. *Exp Neurol* 2012; **234:** 169–180.
35. Bede P, Bokde AL, Byrne S, *et al*. Multiparametric MRI study of ALS stratified for the C9orf72 genotype. *Neurology* 2013; **81:** 361–369.
36. Brettschneider J, Del Tredici K, Toledo JB, *et al*. Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Ann Neurol* 2013; **74:** 20–38.
37. Kawagoe T, Onoda K, Yamaguchi S. Subjective memory complaints are associated with altered resting-state functional connectivity but not structural atrophy. *Neuroimage Clin* 2019; **21:** 101675.
38. Sun Y, Dai Z, Li Y, *et al*. Subjective cognitive decline: mapping functional and structural brain changes – a combined resting-state functional and structural MR imaging study. *Radiology* 2016; **281:** 185–192.
39. Chipika RH, Finegan E, Li Hi Shing S, Hardiman O, Bede P. Tracking a fast-moving disease: longitudinal markers, monitoring, and clinical trial endpoints in ALS. *Front Neurol* 2019; **10:** 229.
40. Grollemund V, Pradat PF, Querin G, *et al*. Machine learning in amyotrophic lateral sclerosis: achievements, pitfalls, and future directions. *Front Neurosci* 2019; **13:** 135.
41. Querin G, El Mendili MM, Bede P, *et al*. Multimodal spinal cord MRI offers accurate diagnostic classification in ALS. *J Neurol Neurosurg Psychiatry* 2018; **89:** 1220–1221.
42. Bede P, Iyer PM, Finegan E, Omer T, Hardiman O. Virtual brain biopsies in amyotrophic lateral sclerosis: diagnostic classification based on *in vivo* pathological patterns. *Neuroimage Clin* 2017; **15:** 653–658.
43. Schuster C, Hardiman O, Bede P. Development of an automated MRI-based diagnostic protocol for amyotrophic lateral sclerosis using disease-specific pathognomonic features: a quantitative disease-state classification study. *PLoS One* 2016; **11:** e0167331.
44. Eccles JC. The initiation of voluntary movements by the supplementary motor area. *Arch Psychiatr Nervenkr (1970)* 1982; **231:** 423–441.
45. Bostan AC, Dum RP, Strick PL. The basal ganglia communicate with the cerebellum. *Proc Natl Acad Sci USA* 2010; **107:** 8452–8456.