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Morphometric Correlates of Dysarthric Deficit in Amyotrophic Lateral Sclerosis

Running title:

Morphometric Correlates of Speech in ALS

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Abstract

Objective: To investigate the volumetric correlates of speech in Amyotrophic Lateral Sclerosis (ALS).

Methods: Twenty-three ALS patients had a structural 3D MRI scan, neuropsychological, linguistic and speech assessments. Twenty-three healthy adults of comparable age, education, white-matter hyperintensities and intracranial volumes were also recruited. Between-group differences in grey matter and white matter (WM) were examined to characterise ALS patients accurately. The association between residual speech and volumetric maps was studied in these patients.

Results: ALS patients showed a pattern of WM reduction, which was located in peri-cortical motor/premotor fibres bilaterally, and in a large volume extending from the pons/midbrain to the cerebellum. A speech composite score was computed, and this was positively associated with premotor/supplementary-motor WM bilaterally, right cerebellar WM, and the posterior-caudal cerebellar cortex, bilaterally.

Conclusion: Variance in residual speech in ALS is associated with variance in premotor WM and cerebellar volumes. Since premotor associations were found in volumes where ALS patients showed WM reduction, this region is believed to be directly involved in speech execution in this group. Since cerebellar associations were instead found in volumes free from shrinkage, this region is interpreted as playing a modulatory role, compensating for the impact of ALS pathology.

Introduction

Amyotrophic Lateral Sclerosis (ALS) disrupts structure and function of motor and non-motor neural pathways (1-2), often triggering cognitive (mostly executive and linguistic) decline (3-4). Deficits in language appear to be independent from any concurrent dysexecutive symptom (5), and, at least in the sub-population with a C9Orf72 mutation, there is evidence of volumetric loss and cortical thinning of the opercular portion of Broca's area (6). On the other hand, the association between ALS's neural disruption and peripheral aspects of linguistic processing has been a largely unexplored research territory. Impaired speech (dysarthria) is a prominent feature of ALS. This normally results from an interplay between diverse factors, such as respiration, phonation, vocal resonation and articulation (7). Linguistic difficulties in ALS may be visible even before the onset of any dysarthric problem (8). Although this indicates that any impairment in the central linguistic components is independent from peripheral aspects of verbal production, it has also been suggested that impoverishment of language might in part reflect an "economy-of-wording" strategy in verbal communication, adopted by patients in response to an increasingly difficult speech (9). To be accomplished accurately, speech is a motor skill that requires precise coordination, and its various contributing factors are believed to be regulated at a neural level (10). At present it is still not completely clear to what extent the factors playing a role in dysarthria are related to the neurodegeneration triggered by ALS. In fact, there are studies which found positive associations between the bulbar sub-score of the revised version of the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R), and the anatomy of the lateral portion of the motor homunculus (11-12). Although this sub-score estimates bulbar function, it incorporates different abilities in one single composite score, in which the assessment of speech is simply quantified with a number ranging from 0 to 4 (13).

This study aimed to explore the structural correlates of speech in ALS more accurately. The association between a proxy derived from clinical assessment of pneumo-phono-articulatory

capacities and regional maps of brain grey-matter (GM) and white-matter (WM) volumes was investigated in a sample of ALS patients experiencing various stages of dysarthric impairment.

Material and methods

Participants

Thirty-one consecutive in-patients diagnosed with probable/possible ALS (all taking Riluzole medication) were enrolled. Diagnoses were made independently by two neurologists, following established criteria (14), and a complete neurological examination was carried out on all patients. Exclusion criteria were set as follows: cerebrovascular disease, hypertension, diabetes, dyspnea, history of head injury or cardiac attacks, prescription of psychoactive medications. The ALSFRS-R scale was administered to evaluate disease severity (13), flanked by the Functional Independence Measure (FIM), to obtain a more detailed picture of functional deterioration (15). No patient relied on artificial devices to support respiration. Registration of onset type (spinal/bulbar) and disease duration served for a better characterisation of each participant. A neuropsychological assessment was carried out to typify the patients' overall cognitive profile. The Mini-Mental State Examination (MMSE), the Raven Progressive Matrices and the Verbal Fluency tasks were used as proxies of overall cognitive abilities, based on their wide use in research on neurodegenerative diseases, and their susceptibility to difficulties in high-order cognitive functions such as abstract reasoning, semantic access and executive management of retrieval (16-18). These tests were included in the procedure to provide a comprehensive description of the sample of patients. The Aachen Aphasie Test (AAT) was administered to assess language skills (19), together with a measurement of phonetic inventory, i.e. the ability to produce the sounds of the Italian language in their various interword positions (20). One patient was excluded because of severe cognitive impairment in

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comprehension which made speech assessment not possible. Ethical approval was received from the IRCCS Institutional Review Board (Venice, Italy) and following ethical standards as set in the Helsinki Declaration on human experimentation. Informed written consent was obtained from all participants.

Assessment of dysarthric symptoms

Although physiological, kinematic, and acoustic techniques have been designed to measure the physical properties of speech production, clinical assessment of dysarthria in ALS patients is mainly based on the evaluation of perceptual and functional properties of speech. The methodology included in this study reflects routines used in a clinical setting. Pneumo-phono-articulatory features of ALS patients' verbal production were assessed using subtests 1, 2 and 4 from the Italian version of the Robertson's Profile Test of dysarthria (21), adapted by Fussi and Cantagallo (22). These were a quantitative evaluation of maximal Expiratory Duration Rate (EDR), maximal Phonatory Duration Rate (PDR) and Diadochokinetic Rate (DKR). EDR and PDR consisted in the ability to sustain a prolonged "/s/" and "/a/" sound, respectively. EDR has been already used as an indicator of the aerodynamic area of vocal production in previous research (23), while PDR is normally used in clinical practice as a measure to estimate phonatory competence (24). DKR identifies, instead, the ability to repeat sequences of sounds rapidly, and is a test often used in neurological settings to assess articulatory skills (25). The score indicates the correct sound sequences orally generated in 5s for each item (items: "/u-i/", "/pa/", "/ta/", "/ka/", "/kala/", "/ptk/"), and the six performance scores were averaged. One patient could not be examined because their health conditions deteriorated before speech assessment could be performed, and they were, therefore, excluded from the study, giving a sample size of 29 ALS participants.

MRI procedure

All remaining patients were invited to have a brain-scan protocol. Three-dimensional T1-weighted structural scans were acquired on a 1.5T Philips Achieva scanner with a Turbo Field-Echo Sequence, as part of a comprehensive scanning protocol. Acquisition parameters were as follows: voxel dimension: 1.1x1.1x0.6mm; FOV: 250mm; matrix size 256x256x124; TR: 7.4ms, TE: 3.4ms; flip angle: 8°. T2-weighted and FLAIR-weighted scans were also acquired to allow the evaluation of vascular load and the detection of contingent abnormalities. The scanning procedure could not be completed in six patients because of medical incompatibility with the MRI environment, or lack of compliance. Twenty-three ALS patients were thus included in the final analyses. Twenty-three healthy controls (as previously determined by full neuropsychological examination and history), individually matched as closely as possible to the ALS patients for gender, age, and education level were included in the scanning sessions. The inclusion of a control group served to characterise the extent of neural damage in ALS patients.

Data analysis

MRI images were preprocessed with Matlab 7 and Voxel-Based Morphometry (VBM), implemented in the Statistical Parametric Mapping (SPM) 8 software (Wellcome Trust Centre for Neuroimaging, London, UK) (26). This procedure included tissue-class segmentation, spatial transformation to a template, and spatial smoothing, carried out with an 8mm Full-Width at Half Maximum gaussian kernel.

Global native-space volumes of GM, WM, and cerebrospinal fluid were extracted from all scans to compute total intracranial volume and tissue ratios ($Ratio_{GM}$, $Ratio_{WM}$, and $Ratio_{GM-PLUS-WM}$). FLAIR-weighted images were examined, and a scale from 0 to 3 was used in the quantification of periventricular and deep-WM hyperintensities (27). The presence of cortical hyperintensities,

basal-ganglia lesions and cerebral infarcts was also assessed. In the case of one ALS patient, this procedure was carried out on the T2-weighted scan, as no FLAIR scan was available. Numeric variables were analysed with the IBM SPSS 21 software. Demographics were analysed with Independent-Sample t tests or Chi-square tests. Between-group differences in total-brain volumes and ratios were analysed with both Independent-Sample t tests and ANOVAs, correcting for age and education level. Since the quantification of WM lesional burden had limited parametric properties, between-group differences were analysed with a Mann-Whitney U test. *Pearson's r* correlations were run between total-brain volumes/ratios and speech parameters.

All remaining analyses were run with SPM. Independent-Sample t tests were carried out to compute group-dependent regional differences, correcting for age, education level and $Ratio_{GM\text{-}PLUS\text{-}WM}$. The association between speech and brain volumes was instead analysed with Multiple-Regression Basic Models, in which the composite index was analysed in association with GM and WM maps, independently and the ALSFRS-R score and the $Ratio_{GM\text{-}PLUS\text{-}WM}$ were added as nuisance regressors.

A significant $p<0.001$ value (uncorrected) was selected, and the cluster extent was set at 50 contiguous voxels. A cluster-level $pFWE<0.05$ was then chosen as threshold of significance. Given the exploratory nature of the study, the uncorrected findings of the regression models were also examined. Significant MNI-space peak coordinates were converted into Talairach space using a non-linear transform (imaging.mrc-cbu.cam.ac.uk/downloads/MNI2tal/mni2tal-m) and interpreted using the Daemon client (28-29).

Results

Sample characterisation

All variables are reported in Table 1. The score of the distinct ALSFRS-R subsections was consistent with onset type. One patient refused any neuropsychological assessment, but no overt cognitive impairment had been noticed in their initial neurological examination. Overall, cognitive profiles were incomplete, but were equally considered anyway for qualitative description. MMSE scores could be collected for 14 of the remaining 22 ALS patients, and these ranged between 24 and 30, suggesting absence of major cognitive impairment. Only two patients performed below cut-off on the Raven Progressive Matrices test. The Verbal Fluency tests were available for 19 patients. Although ALS patients performed significantly worse than healthy controls on the Category Fluency test (the sole test showing between-group differences), no individual score fell below cut-off. One patient performed below cut-off in the Letter Fluency test. Based on this evidence and on further clinical considerations it was concluded that, for the majority of patients, cognitive skills were, on average, only very mildly impaired. The AAT was available for 21 patients, and these scores were compared with the clinical cut-offs which quantify specific levels of severity (19). Three patients had a moderate deficit in "Comprehension", whereas two other patients had a moderate deficit in "Repetition" and "Naming". The remaining scores were either normal or in the mildly-impaired range. This was also valid for nine patients who did not complete the "Written Language" sub-test in its entirety (their partial scores were compared with the available normative sub-scores). The phonetic inventory was administered to 18 patients, nine of whom did not obtain a full score. In sum, language was mildly affected in ALS patients, both in its receptive and productive components, while phonetics was more substantially impaired.

Individual measurements of speech were compared with reference scores (22). Based on this classification, 15 patients showed impaired performance in all subtests. Of the remaining eight patients, normal EDR, PDR, and DKR sub-scores were achieved by three, six and one patient, respectively, with two patients showing both retained EDR and PDR.

The amount of periventricular and deep-WM lesions did not differ between groups (p=0.597 and p=0.419, respectively). One ALS patient had a single basal ganglionic lesion, whereas three ALS patients had evidence of past cerebral infarcts.

ALS patients had significantly less WM (t_{44}=-2.776, p=0.008), lower $Ratio_{WM}$ (t_{44}=-3.563, p=0.001) and lower $Ratio_{GM-PLUS-WM}$ (t_{44}=-2.386, p=0.021). These findings were confirmed after controlling for age and education ($F_{1,42}$=8.372, p=0.006; $F_{1,42}$=19.086, p<0.001; $F_{1,42}$=8.873, p=0.005, respectively). There were no differences in total intracranial volume, total GM, $Ratio_{GM}$ and, in addition, no difference in regional GM was found. On the other hand, ALS patients had a vast reduction in two separate sections of corticospinal WM: an upper symmetric reduction extending from the corona radiata to the semioval centres of the motor and premotor/supplementary-motor cortices, and a lower midline reduction in pontine/mesencephalic and cerebellar WM (Table 2; Fig.1). There were no areas in which GM or WM volume was larger in the ALS patient group.

Association between brain morphology and residual pneumo-phono-articulatory capacity

The three speech measures were normally distributed (as indicated by Kolmogorov-Smirnov Tests*'s* p>0.05), were mutually correlated, and were associated with disease severity. EDR and DKR were also correlated with functional independence. Both total WM volume and $Ratio_{WM}$ correlated with EDR and PDR but not with DKR. Aside from the mutual correlations, all r scores indicated moderate associations. GM measures and disease duration were not correlated with any variable (Table 3). To enhance statistical robustness in the correlational analyses (by circumventing the need for correction for multiple comparisons), standardised residuals of EDR, PDR and DKR were calculated and averaged. This average speech composite score characterised the general level of speech production of each patient.

All associations between pneumo-phono-articulatory scores and regional volumetric maps are reported in Table 4 and illustrated in Fig.2. None of these survived a cluster-level pFWE<0.05 but all peaks did survive a less robust small-volume corrected pFWE<0.05.

Speech composite scores were positively associated with a bilateral and symmetric pattern of GM volume located in the posterior and caudal section of the cerebellar cortex. Additionally, significant associations were found in a WM cluster in the right cerebellar hemisphere, and, bilaterally, in WM sections adjacent to both dorsal and ventral parts of Brodmann Area 6. These latter premotor/supplementary-motor volumes were almost entirely contained in the set of voxels were decreased WM density had been seen. No negative associations were found.

Discussion

The association between pneumo-phono-articulatory skills and GM/WM volumes in ALS was investigated. Clinical measures of speech were selected based on their easy measurability in a hospital setting. For the purpose of sample characterisation, regional GM and WM volumetric maps of ALS patients were compared statistically with a group of healthy adults matched for age and education, and having a comparable load of WM hyperintensities.

The group comparison revealed no GM difference between groups. The pattern of GM volumetric alterations observed in ALS has been so far inconsistent (1-2, 30). A cross-sectional meta-analysis identified a sole small significant cluster of GM loss in ALS patients, located in the right precentral gyrus (31), while parallel studies found no precentral reductions (32-33), no volumetric loss in GM at all (34), or mixed pattern of motor and extra-motor GM volumetric decrease (11). On the other hand, less WM volume was found in ALS patients in the corticospinal tract including midbrain, pons and semioval motor/premotor fibres), and in the cerebellum. Notably, this reduction even

survived complete Family-Wise correction. The literature of reference here is even more

heterogeneous, as a number of volumetric studies found no cross-sectional differences in WM

between patients and healthy controls (e.g. 33, 35), while other studies reported WM decrease (36-

37) or even increase (32, 38). As described in a recent review, ALS is a disease characterised by a

remarkable level of clinical heterogeneity (39). In the attempt to maintain a specific focus on brain

volumetric measures, a series of possible reasons might account for these inter-study discrepancies.

Agosta and colleagues commented over the possible impact of gliosis which, activating in response

to neural damage, translates actually into a GM increase, and "may occur at a degree enough to

"mask" tissue loss" (40; page 3). In a similar fashion, Kassubek and colleagues described

volumetric increase in the capsular and periventricular section of corticospinal WM of ALS

patients, and interpreted this piece of evidence as due to the presence of hyperintensities associated

with the disease, which might get somehow misconstrued by the neuroimaging software (38). In

addition to these two biological mechanisms, other, methodological factors contribute to the

variability of findings. These include generally small and heterogeneous samples, and divergences

in methodological aspects (30, 41). There is also evidence that genetic and sporadic ALS differ in

their volumetric signature (6), and this too may be of relevance when samples are of variable

aetiology.

Despite no p value survived a cluster-level FWE correction, a set of uncorrected findings

(interpreted thus following a more liberal approach) indicated that retained speech associates more

with WM than GM volumetric measures. This is consistent with a study in which patients affected

by progressive spastic dysarthria (of various aetiology) had exclusively WM (and no GM) loss (42).

On this note, the pattern of association between residual speech abilities and regional GM observed

in the cerebellum might have been partially artefactual, possibly driven by an inaccurate process of

skull-stripping in the region where the caudal part of the cerebellar hemisphere and the bone tissue

of posterior cranial fossa are in anatomical contiguity.

The overall pattern of association extended to areas involved in motor planning, preparation and execution, including premotor, supplementary-motor, and cerebellar volumes. No association emerged in the lateral portion of the motor homunculus as found in previous studies in which speech abilities were assessed as part of the ALSFRS-R bulbar sub-score. (11-12), or in any portion of the primary motor cortex. In our sample, the correlation between speech composite scores and ALSFRS-R bulbar sub-scores was significant ($p<0.014$), but only moderate in strength ($r=0.505$). Since the construct validity of the composite score supersedes that of the ALSFRS-R sub-score as a proxy of speech, it is suggested that the primary motor cortex might not be primarily involved in the neural control of speech in ALS.

Motor/premotor areas, cerebellum, insula, left operculum and basal ganglia are all reputed to concur in the neural control of verbal execution in the healthy nervous system (10). We found that variability in residual speech was positively associated with volumetric variability of two of the aforementioned regions. The premotor cortex was recently described as a structure playing a compensatory role in sustaining normal speech after acute damage of left putaminal-capsular areas (43). With regard to this study, premotor/supplementary-motor associations were largely located in those regions where a between-group loss of volume had been observed. This "pathological variance in structure"-"loss of function" relation suggests that the role of premotor areas might be a direct involvement of these areas in speech execution. The cerebellum is instead normally considered responsible for creating fine-grained motor models exploited by motor areas to accomplish speech movements (10). Cerebellar activations are visible during tongue and lip movements, and ischemic lesions involving these regions provoke dysarthric symptoms (44). With regard to this study, associations were found in cerebellar regions in which no single voxel of volumetric loss had emerged. This "non-pathological variance in structure"-"loss of function" relation suggests that the cerebellum might act as modulator, rather than a structure involved in the "frontline" of speech execution. In fact, whilst ALS disrupts cortical networks, the nervous system

tries to compensate by inducing parallel increases in connectivity (45). Albeit being based on volumetric evidence, what we observed in this study might have been the outcome of a similar process: circuital disruption involving the set of areas responsible for speech execution, and compensatory involvement of additional, modulatory regions. This entire pattern, however, has to be interpreted with caution, as no finding actually survived a cluster-level FWE correction.

Limitations

Albeit being based on a sample of comparable size as in other publications (46-47), this study was not sufficiently powered to examine the impact of onset type and onset side, both potentially influential in these analyses. Moreover, the rate of disease progression was not measured. There is evidence that this parameter may influence structural properties of WM (47). Additionally, the pattern of association between speech and WM was captured by VBM, which measures macroscopic WM properties, and does not provide any information about fibres' integrity. Although DTI is more sensitive than VBM to WM alterations (48), however, it is also extremely more susceptible to the impact of unspecific short-term stimulation and the possible effect of speech therapy (see 49-50 for a proof of concept). On the other hand, the properties of WM calculated by VBM are more stable, and for this reason the two techniques are methodologically complementary in nature.

Conclusion

Pneumo-phono-articulatory aspects of speech were associated with premotor/supplementary-motor and cerebellar brain volumes. These represented regions believed to support the neural control of speech in healthy adults (10), and are suggested to serve a similar role in ALS. Uncorrected findings suggest that premotor areas might be directly involved in speech production, while cerebellar regions might serve a modulatory role and appear to be recruited to compensate for the neural de-regulation triggered by ALS in motor/premotor regions.

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Disclosures of interests

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Table 1: Characterisation of participants, and neuropsychological and speech scores

	ALS Patients	n	Healthy Adults	n	p
Age (years)	62.70 (11.54)	23	61.91 (13.12)	23	0.83
Education (years)	8.17 (3.72)	23	8.35 (3.77)	23	0.87
Gender (F/M)	10/13	23	10/13	23	1.00
Handedness (left/right)	5/18	23	3/20	23	0.44
MMSE	27.50 (1.99)	14	28.39 (2.04)	23	0.20
Raven Matrices	27.82 (6.57)	22	29.70 (5.39)	23	0.30
Phonemic Fluency	25.53 (9.06)	19	31.57 (12.04)	23	0.07
Semantic Fluency	33.89 (8.39)	19	39.43 (7.72)	23	0.03*
Onset Type (spinal/bulbar)	20/3	23			
Disease Duration (months)	39.26 (12.93)	23			
			Cut-off / Reference score		
ALSFRS-R	31.39 (5.33)	23	≈ 40		
ALSFRS-R (Bulbar Subscore)	9.39 (1.67)	23	≈ 12		
FIM	68 (14.37)	23	≥ 91		
AAT: Token Test	3.71 (4.11)	21	≤ 7		
AAT: Repetition	141.30 (8.00)	20	≥ 142		
AAT: Written Language	65.00 (25.64)	21	≥ 81		
AAT: Naming	111.25 (8.53)	20	≥ 104		
AAT: Comprehension	108 (7.65)	21	≥ 108		
Phonetic Inventory	25.33 (3.97)	18	≈ 28		
EDR	11.39 (6.73)	23	$\geq 15s$		
PDR	12.83 (6.79)	23	$\geq 20s$		
DKR	10.06 (4.16)	23	≥ 17		

Mean raw scores are reported, and standard deviation are included in parentheses. Between-group statistical comparisons were carried out with Independent-Sample t tests (for age, educational levels, and neuropsychological indices), or *Pearson's Chi*-square tests (for gender and handedness).

The ALSFRS-R reference values indicate full scores, which would be expected in those without functional deficits. A FIM ≤91 is indicated by the regional legislation of reference as a cut-off score to entitle patients to be admitted to neurorehabilitative health-care hospitalisation. AAT and speech cut-off scores were indicated in their respective publication.

MMSE: Mini-Mental State Examination; ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised; FIM: Functional Independence Measure; AAT: Aachen Aphasie Test; EDR: Expiratory Duration Rate; PDR: Phonatory Duration Rate; DKR: Diadochokinetic Rate. "*" indicates p<0.05.

Table 2   Between-group differences in regional white matter

Cluster Number	Brain Area	Side	Cluster Size (voxels)	Cluster Level $D_{FWE\ corr}$	Z Score at Local Maximum	Talairach Coordinates		
						x	y	z
1	Precentral Gyrus	R	1320	<0.001	5.62	34	-11	52
	Medial Frontal Gyrus	R			4.64	8	-18	58
	Precentral Gyrus	R			4.55	44	-15	45
2	Pons	L	1831	<0.001	5.46	-16	-26	-22
	Cerebellar Culmen	R			4.17	24	-36	-25
	Midbrain	R			4.01	14	-18	-18
3	Medial Frontal Gyrus	L	1345	<0.001	5.16	-8	-22	60
	Precentral Gyrus	L			4.92	-32	-13	54
	Precentral Gyrus	L			4.84	-32	-21	51

Peak coordinates are reported. L: Left; R: Right; FWE: Family-Wise Error.

Table 3   Correlation between speech parameters, clinical variables, and global brain volumes

		EDR	PDR	DKR	Disease Duration	ALSFRS-R	FIM	Total GM	Total WM	Total Intracranial Volume	Ratio GM	Ratio WM	Ratio GM-PLUS-WM
EDR	Pearson's r	1			-0.318	**0.489**	**0.476**	0.080	**0.417**	0.175	-0.087	**0.435**	0.218
	p				0.140	**0.018**	**0.022**	0.716	**0.048**	0.424	0.693	**0.038**	0.318
PDR	Pearson's r	**0.749**	1		-0.346	**0.472**	0.408	0.215	**0.477**	0.293	-0.057	**0.426**	0.237
	p	**<0.001**			0.106	**0.023**	0.053	0.325	**0.021**	0.175	0.798	**0.043**	0.277
DKR	Pearson's r	**0.728**	**0.504**	1	-0.139	**0.419**	**0.459**	-0.008	0.109	-0.021	-0.006	0.153	0.097
	p	**<0.001**	**0.014**		0.529	**0.047**	**0.028**	0.969	0.619	0.923	0.979	0.485	0.660
Composite Score	Pearson's r	**0.939**	**0.854**	**0.846**	-0.304	**0.523**	**0.509**	0.109	0.380	0.169	-0.057	0.384	0.209
	p	**<0.001**	**<0.001**	**<0.001**	0.159	**0.010**	**0.013**	0.622	0.073	0.440	0.797	0.070	0.338

Significant correlations and p values are indicated in bold. EDR: Expiratory Duration Rate; PDR: Phonatory Duration Rate; DKR: Diadochokinetic Rate; ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised; FIM: Functional Independence Measure; GM: Grey Matter; WM: White Matter.

Table 4 **Positive associations between pneumo-phono-articulatory composite score and brain volumes in ALS patients**

Cluster Number	Brain Area	Side	Cluster Size (voxels)	Cluster Level $D_{FWE\ corr}$	Z Score at Local Maximum	Talairach Coordinates		
						x	y	z
			GM					
1	Cerebellar Inferior Semi-Lunar Lobule	L	93	0.446	4.52	-12	-72	-38
2	Cerebellar Inferior Semi-Lunar Lobule	R	59	0.713	3.70	10	-72	-35
			WM					
1	Frontal Sub-Gyral	R	191	0.102	4.39	22	-13	54
	Precentral Gyrus	R			3.80	38	-2	35
	Precentral Gyrus	R			3.38	34	-9	48
2	Frontal Sub-Gyral	L	109	0.258	4.09	-30	-8	41
	Precentral Gyrus	L			3.76	-36	-2	41
3	Frontal Sub-Gyral	L	99	0.290	3.85	-14	-23	45
	Frontal Sub-Gyral	L			3.62	-14	-15	43
4	Cerebellar Tonsil	R	136	0.188	3.58	22	-68	-32
	Cerebellar Tonsil	R			3.28	26	-48	-31

Peak coordinates are reported. L: Left; R: Right; GM: Grey Matter; WM: White Matter; FWE: Family-Wise Error.

Figure legends

Figure 1.

WM areas in which ALS patients showed lower volumetric values in comparison with the group of healthy controls. Slice coordinates in MNI space are as follows: upper row, y = -39, y = -31, y = -23; lower row, y = -15, y = -7, y = +1.

Figure 2.

Associations between regional WM volumes and performance in the tests measuring speech as indicated by the speech composite score. Z coordinates in MNI space are as follows: upper row, x = -30, x = +39; lower row, y = -17, y = -8, z = -40.
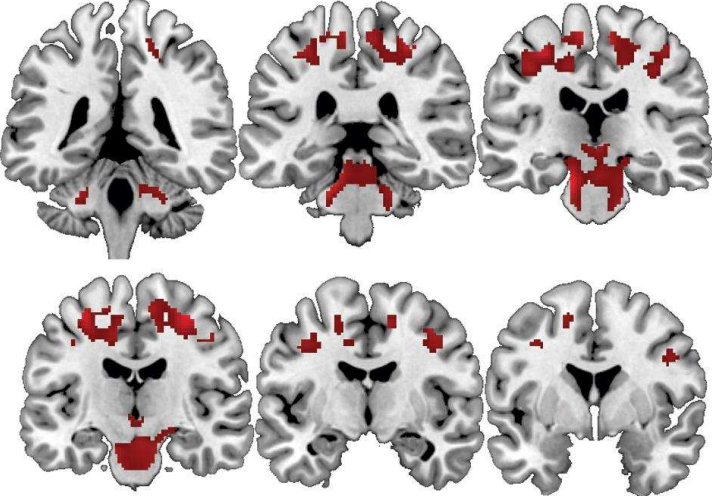
Figure 1

Figure 2