

Flexibility of Fast Brain Dynamics and Disease Severity in Amyotrophic Lateral Sclerosis

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Abstract

Background and Objectives

Amyotrophic lateral sclerosis (ALS) is a multisystem disorder, as supported by clinical, molecular, and neuroimaging evidence. As a consequence, predicting clinical features requires a description of large-scale neuronal dynamics. Normally, brain activity dynamically reconfigures over time, recruiting different brain areas. Brain pathologies induce stereotyped dynamics which, in turn, are linked to clinical impairment. Hence, based on recent evidence showing that brain functional networks become hyperconnected as ALS progresses, we hypothesized that the loss of flexible dynamics in ALS would predict the symptoms severity.

Methods

To test this hypothesis, we quantified flexibility using the “functional repertoire” (i.e., the number of configurations of active brain areas) as measured from source-reconstructed magnetoencephalography (MEG) in patients with ALS and healthy controls. The activity of brain areas was reconstructed in the classic frequency bands, and the functional repertoire was estimated to quantify spatiotemporal fluctuations of brain activity. Finally, we built a k -fold cross-validated multilinear model to predict the individual clinical impairment from the size of the functional repertoire.

Results

Comparing 42 patients with ALS and 42 healthy controls, we found a more stereotyped brain dynamics in patients with ALS ($p < 0.05$), as conveyed by the smaller functional repertoire. The relationship between the size of the functional repertoire and the clinical scores in the ALS group showed significant correlations in both the delta and the theta frequency bands. Furthermore, through a k -fold cross-validated multilinear regression model, we found that the functional repertoire predicted both clinical staging ($p < 0.001$ and $p < 0.01$, in the delta and theta bands, respectively) and symptoms severity ($p < 0.001$, in both the delta and theta bands).

Discussion

Our work shows that (1) ALS pathology reduces the flexibility of large-scale brain dynamics, (2) subcortical regions play a key role in determining brain dynamics, and (3) reduced brain flexibility predicts disease stage and symptoms severity. Our approach provides a noninvasive tool to quantify alterations in brain dynamics in ALS (and, possibly, other neurodegenerative diseases), thus opening new opportunities in disease management and a framework to test, in the near future, the effects of disease-modifying interventions at the whole-brain level.

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Glossary

ALS = amyotrophic lateral sclerosis; **ALSFRS-R** = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; **ECAS** = Edinburgh Cognitive and Behavioural ALS Screen; **LOOCV** = leave-one-out cross-validation; **MEG** = magnetoencephalography; **MiToS** = Milano-Torino Staging; **ROIs** = Regions of Interest.

Amyotrophic lateral sclerosis (ALS) is caused by a combination of pathogenic processes encompassing the whole brain.¹ Up to 50% of patients with ALS develop cognitive and/or behavioral impairment, with about 13% developing the behavioral variant of frontotemporal dementia.²

Accounting for symptoms induced by widespread neurodegeneration requires a precise description of the fine-tuned, large-scale interactions among brain regions.³ The progression of ALS shifts the functional brain networks toward hyperconnectedness (that is, a regime where the activity of each brain region over time is (too) strongly constrained by the rest of the brain).⁴ However, the interactions among regions occur in bursts,⁵ which account for most of the (time-averaged) functional connectivity⁶ and have been linked to behavioral outcomes, underlying their physiologic significance.⁷ Hence, average (i.e., static) functional connectivity might not be enough to account for complex symptoms, and time-resolved analyses might be needed.

The healthy brain quickly recruits the appropriate brain regions, in the correct order, at any moment, to respond to the environment. To this end, the brain does not passively “wait” for stimuli, but, rather, it constantly recruits different groups of regions, each of which might be optimal for a given stimulus and the appropriate response.⁸ The more the brain reconfigures itself (i.e., the more it is flexible), the better it can respond to tasks.⁹ These “reconfigurations” reflect themselves in large-scale bursts of activations in brain signals called “neuronal avalanches.”¹⁰ In fact, although avalanches preferentially spread along the structural connectome,¹¹ they constantly reconfigure, as captured by a large number of patterns over time. Hence, the number of such patterns (i.e., the size of the functional repertoire) provides a measure of brain flexibility, and its reduction relates to clinical impairment.¹²

Hence, we hypothesized that brain dynamics would be more stereotyped (less flexible) in patients with ALS as compared with healthy controls. Furthermore, given that complex brain functions require flexibility, a restriction of the functional repertoire might underpin clinical impairment.

To estimate flexibility, we used source-reconstructed magnetoencephalographic (MEG) data, which have a millisecond time resolution. MEG measures extremely weak oscillations of the magnetic fields produced by postsynaptic neuronal activity,¹³ using a helmet equipped with ultrasensitive (fT, 10^{-15} Tesla) sensors of the magnetic field (superconducting quantum interference devices). Compared with EEG, MEG

(1) allows for a temporally and spatially accurate reconstruction of the neural signals¹⁴ because magnetic fields are undistorted by the layers surrounding the brain (bones and meningeal sheets) and (2) is reference-free, which helps providing unbiased estimates of functional connectivity.^{4,10}

We filtered the signal in the classic frequency bands and defined a *neuronal avalanche* as an event that begins when at least 1 brain region deviates from its baseline activity and ends when all regions restore their typical level of activity. Given a neuronal avalanche, we defined its corresponding *pattern* as the set of all the brain areas that were recruited. Finally, we defined the *functional repertoire* as the set of the unique patterns that happened over time (i.e., the part of the *state-space* that has been visited) and used its size as a proxy for the flexibility of the brain dynamics. We further hypothesized that the restriction of the functional repertoire would relate to a worsening of the patients’ symptoms. To test this hypothesis, we built a model to predict disease severity and clinical staging based on the size of the functional repertoire.

Methods

Study Participants

Patients with ALS were recruited from the ALS Center of the First Division of Neurology of the University of Campania “Luigi Vanvitelli” (Naples, Italy) from September to December 2016 and from May 2018 to March 2019. Patients were right-handed and native Italian speakers diagnosed with ALS according to the revised El-Escorial criteria.¹⁵ All patients underwent Edinburgh Cognitive and Behavioural ALS Screen (ECAS)¹⁶ to assess global cognitive functioning. None of the patients showed any variation in the screened genes *SOD1*, *TARDBP*, *FUS/TLS*, and *C9ORF72*. Healthy controls were also included.

Inclusion criteria were (1) no use of drugs that could interfere with MEG signals; (2) no other major systemic, psychiatric, or neurologic diseases; and (3) no focal or diffuse brain damage at routine MRI.

Standard Protocol Approvals, Registrations, and Patient Consents

The study protocol was approved by the Local Ethics Committee (University of Campania “Luigi Vanvitelli”) with Protocol No. 591/2018, and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Brain Network Analysis

MRI Acquisition

As previously described,¹⁷ MRI images of patients with ALS and healthy controls were acquired on a 3T scanner equipped with an 8-channel parallel head coil (General Electric Healthcare, Milwaukee, WI). MR scans were acquired either after the MEG recording or a minimum of 21 days earlier (within 1 month). This was performed to minimize the risk of noise due to the MRI affecting the MEG acquisition. In particular, 3 dimensional T1-weighted images (gradient-echo sequence inversion recovery prepared fast spoiled gradient recalled-echo, time repetition = 6,988 ms, inversion time = 1,100 ms, echo time = 3.9 ms, flip angle = 10, voxel size = $1 \times 1 \times 1.2 \text{ mm}^3$) were acquired. Some participants (7 patients and 11 controls) did not complete the MRI because of the difficulty in lying down or because they refused to perform the MRI scan. A standard MRI template was used in these cases.

MEG Acquisition

MEG data were acquired with a 163-magnetometer system placed in a magnetically shielded room (AtB Biomag UG, Ulm, Germany).¹⁸ Data acquisition, preprocessing, and source reconstruction were performed as previously described.⁴

Briefly, before each acquisition, 4 reference positions (nasion, right, and left preauricular and apex) were digitalized. Electrocardiographic and electrooculographic signals were recorded.¹⁹ The brain activity of each participant was recorded in 2 segments, each 3.5 minutes long, during the resting state with eyes closed. Data were acquired with a sampling frequency of 1,024 Hz, and a 4th-order Butterworth IIR band-pass filter was then applied to remove components below 0.5 and above 48.0 Hz.²⁰ The filter was implemented offline using Matlab 2019a and the Fieldtrip toolbox 2014.²¹

Data Preprocessing

Principal component analysis was used to orthogonalize the sensors over the base of the references. Then, after visual selection of the data from an experienced operator, supervised independent component analysis (ICA) was used to remove physiologic artefacts such as electrocardiogram and eye blinks (if present).

Source Reconstruction

Source reconstruction was performed using the Fieldtrip toolbox.²¹ MEG data were coregistered to the MRI, and a beamformer approach²² was used to reconstruct time series related to the centroids of 116 regions of interest (ROIs), derived from the automated anatomical labeling atlas.^{23,24} Singular value decomposition was used to obtain a scalar. We then considered the first 90 ROIs for further analysis, excluding the cerebellum given the low reliability.²⁵ The source-reconstructed signals were filtered in the classic frequency bands: delta (0.5–4.0 Hz), theta (4.0–8.0 Hz), alpha (8.0–13.0 Hz), beta (13.0–30.0 Hz), and gamma (30.0–48.0 Hz).

Analysis of Brain Dynamics

Neuronal Avalanches and Branching Parameter

To quantify spatiotemporal fluctuations of brain activity, we first estimated neuronal avalanches. As previously described,²⁶ an avalanche is defined as an event starting when an unexpected (given a linear process) fluctuation of regional activity is present and ending when all regions are back to normal activity. The number of activations in all ROIs in an avalanche corresponds to its size. Each of the 90 source-reconstructed signals was z -transformed and thresholded according to a cutoff of 3 standard deviations (i.e., $z > |3|$).¹² To confirm that the results are not dependent on the choice of a given threshold, we repeated the analyses setting the threshold to $z > |2.5|$ and $z > |3.5|$.

To select a suitable bin length, we computed the branching ratio σ ²⁷ as follows: for each participant, for each time bin size, and for each avalanche, the geometrically averaged ratio of the number of events (activations) between the subsequent time bin and the current time bin was calculated as

$$\sigma_i = \prod_{j=1}^{N_{bin}-1} \left(\frac{n_{events}(j+1)}{n_{events}(j)} \right)^{\frac{1}{N_{bin}-1}} \quad (1)$$

$$\sigma = \prod_{i=1}^{N_{aval}} (\sigma_i)^{\frac{1}{N_{aval}}} \quad (2)$$

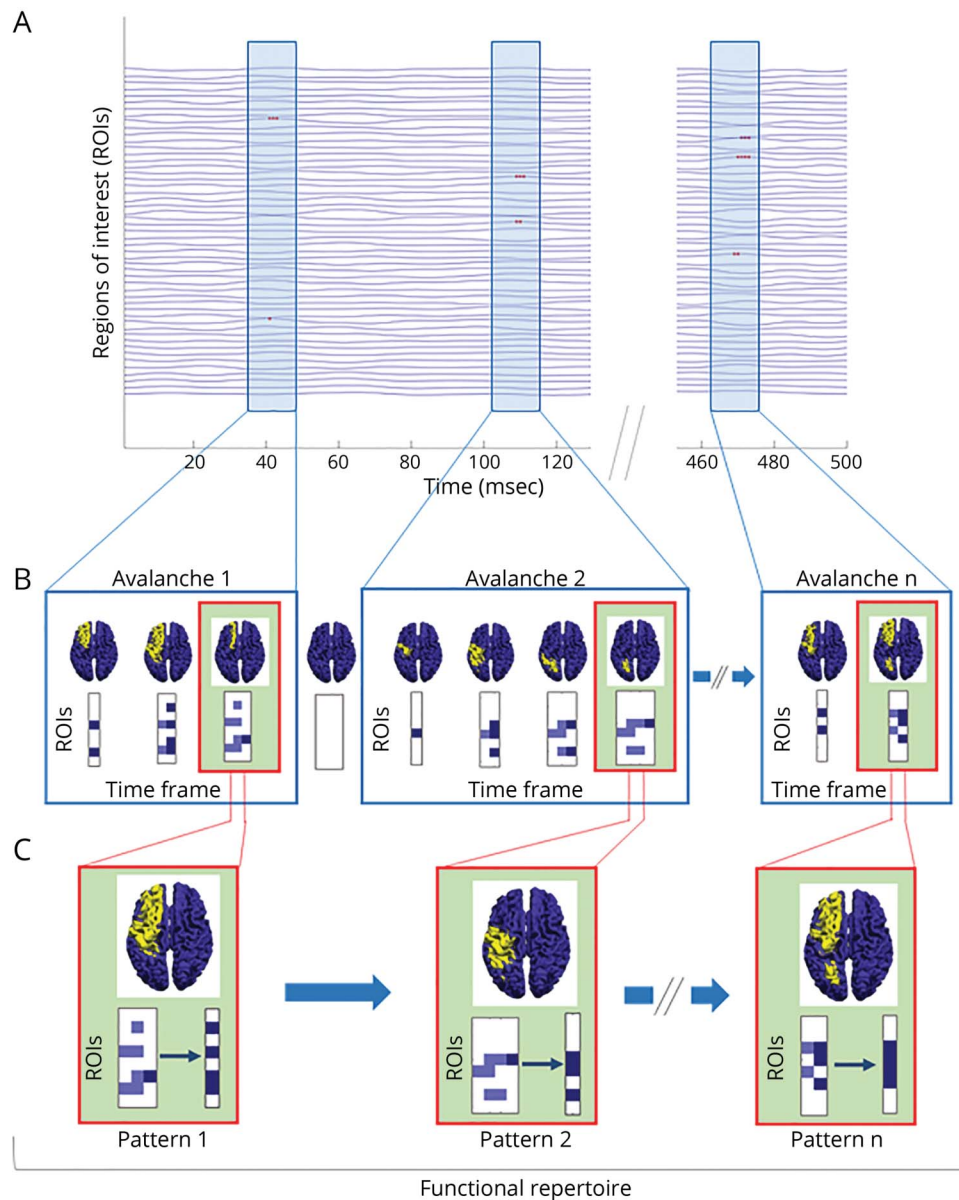
where σ_i is the branching parameter of the i th avalanche in the data set, N_{bin} is the total amount of bins in the i th avalanche, $n_{events}(j)$ is the total number of events active in the j th bin, and N_{aval} is the total number of avalanches in each participant's recording.

The bin length equal to 3 samples yielded a critical process with $\sigma = 1$, hinting at the avalanches as occurring in the context of a dynamical regime near a phase transition. However, we varied time bins from 1 to 5. To compare the dynamics of brain activity among the participants, we took into consideration the same duration for each participant time series (122.79 seconds). Segments of equal duration were randomly selected from the whole recording. For each avalanche, an *avalanche pattern* was defined as the set of all areas that were above the threshold at any point during the avalanche.

Functional Repertoire

For each participant, we estimated the *functional repertoire* defined as the number of unique avalanche patterns which occurred during the recording.¹² *Unique* indicates that each avalanche pattern only counts once toward the size of the functional repertoire (i.e., it does not matter if a given avalanche pattern appears only once or multiple times because the functional repertoire refers to the *kinds* of patterns that occurred, not their number per se). A representation of avalanche patterns and the functional repertoire is shown in Figure 1.

Figure 1 Schematic Representation of Neuronal Avalanches and Functional Repertoire



(A) Source-reconstructed time series. Light blue rectangles represent the time frames in which neuronal avalanches occur; red dots in the rectangles denote activated brain regions (signal above the threshold) in a time interval (msec). (B) In the boxes, avalanche patterns of 3 different neuronal avalanches are illustrated (for clarity, the avalanches reported last up to 4 time frames). An avalanche is defined as an event that begins when at least 1 brain region deviates from its baseline activity (above the threshold) and ends when all regions display a typical level of activity (below the threshold). Given a neuronal avalanche, its corresponding pattern is the set of all the brain areas that were recruited at any time. The brain plots for each time frame of an avalanche show the areas above (yellow) and below (blue) the threshold. Each matrix represents an avalanche pattern: dark blue squares indicate the brain regions (ROIs) activated at a certain time frame, while the light blue ones are all the regions that have been activated up to that moment. (C) In the green boxes, for each of the above avalanches, the brain plot and the set of unique avalanche patterns are illustrated. Unique means that each avalanche pattern only counts once toward the size of the functional repertoire. The number of unique avalanche patterns defines the size of the functional repertoire and is used as a proxy for the flexibility of the brain dynamics.

Switching Between States

A *switch* is defined as crossing the threshold level, in either direction, and, therefore, occurs when an active region becomes inactive, and vice versa, between 2 consecutive time bins. The switch rate (number of switches over duration), averaged over areas, was computed for each participant.

Regional Influence on the Functional Repertoire

At this stage, we split the total functional repertoire into 2 groups: patterns that occurred in both the clinical and control participants (“shared repertoire”) and patterns that were unique to either group (“group-specific repertoire”). Then, using the Kolmogorov-Smirnov test, we compared the distributions of brain region occurrences between shared and group-specific repertoires and performed permutation testing

to identify which brain regions were recruited significantly more in the group-specific repertoire than in the shared repertoire. We then tested if these occurrences were higher in the healthy or the patient group.

Multilinear Model Analysis

We then moved on to test the hypothesis that efficient brain dynamics is linked to the correct functioning of the brain, and hence, the restriction of the functional repertoire is related to disease severity and to clinical staging. To test this hypothesis, we built a multilinear model to predict clinical measures and disease staging, adding brain flexibility (as measured by the size of the functional repertoire)²⁸ to demographic, amnesic, and clinical information. Specifically, we considered the Amyotrophic Lateral Sclerosis (ALS) Functional Rating

Scale-Revised (ALSFRS-R) and the stage of the disease (King's and MiToS clinical staging systems) as dependent variables, while age, education level, sex, disease duration, phenotype, ECAS scores, and size of the functional repertoire were considered as predictors. Multicollinearity was assessed through the variance inflation factor.²⁹ We validated our model using the *k*-fold cross-validation, with *k* = 5.³⁰ Specifically, *k* iterations were performed to train our model, and at each iteration, the *k*th subgroup was used as a test set. The validation was also performed using leave-one-out cross-validation (LOOCV); see the Supplement (links.lww.com/WNL/C297).

Statistical Analysis

To compare age and educational level between patients with ALS and healthy controls, we performed a *t*-test, while Chi-square was used for sex comparison. Permutation testing or Kolmogorov-Smirnov test was performed to compare patients and controls, as appropriate. For permutation testing, the data were permuted 10,000 times, and at each iteration, the absolute value of the difference between the 2 groups was observed, building a null distribution of absolute differences. Finally, the empirical observed difference was rank-ordered against this distribution, yielding a significance value. The relationship between the size of the functional repertoire and the clinical scores was investigated in the ALS group using the Spearman correlation coefficient.

The results were corrected by the false discovery rate, across both parameters and frequency bands, and the significance level was set at *p*-value < 0.05. We reported the corrected significances throughout the article. All statistical analyses were performed in Matlab 2019a.

Data Availability

Data not provided in the article because of space limitations may be shared (anonymized) at the request of any qualified investigator, conditional to acceptance by the Ethical Committee. The code used is available in reference 31.

Results

Clinical and Demographic Characteristics of Patients With ALS

Forty-two individuals (32 males, 10 females) diagnosed with ALS according to the revised El-Escorial criteria of ALS¹⁵ and 42 healthy controls (28 males, 14 females) were enrolled in the study. We used the total ALSFRS-R³² and the ALS clinical staging systems to quantify both symptoms' severity and disease staging. Hence, patients were classified according to both the King's³³ and the Milano-Torino Staging (MiToS)³⁴ disease staging systems, which are based on the appearance of sequential clinical milestones during ALS.

According to the strong criteria for cognitive and behavioral assessment in ALS,³⁵ 4 patients had cognitive impairment, 8 patients had behavioral impairment, and 9 patients had both conditions.

Furthermore, 15 patients had a classic phenotype, 16 had lower motor neuron-dominant phenotypes (i.e., 8 patients had a "flail arm" phenotype, 6 had a "flail leg" phenotype, and 2 patients had a "pure lower motor neuron" phenotype), and 11 had a predominant upper motor neuron (UMN) phenotype.

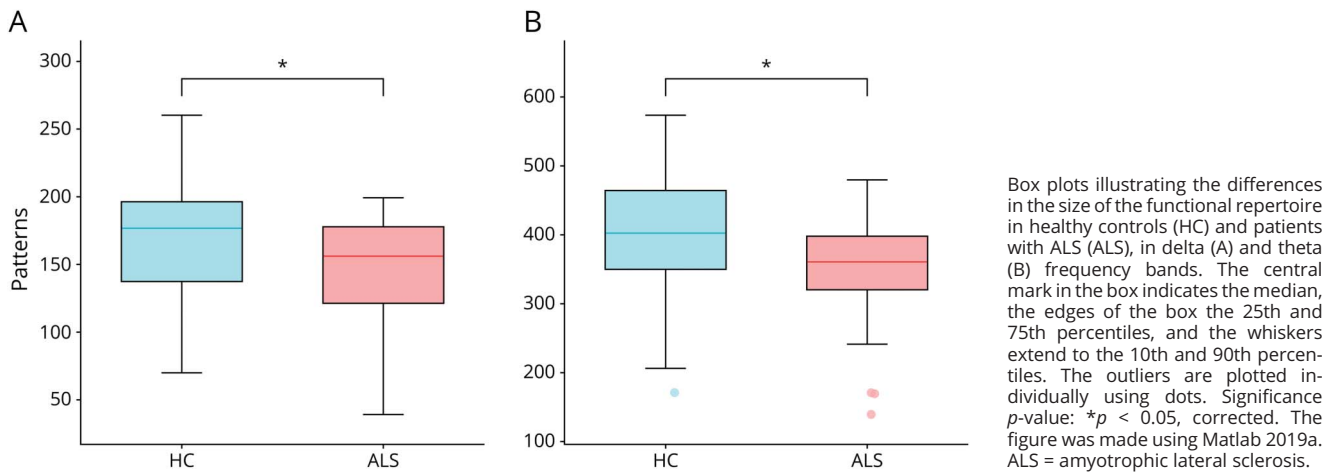
All clinical information about the cohort, such as ALSFRS-R score, stage of the disease, ALS phenotype, site of onset,

Table 1 Demographic and Clinical Features and Neurocognitive Assessment of the Cohort

Parameters	Patients with ALS (n = 42), mean (±SD)	HC (n = 42), mean (±SD)	<i>p</i> Values
Demographic and clinical measures			
Age	64.81 (±12.83)	63.10 (±10.46)	0.50
Male/female	32/10	28/14	0.35
Education (y)	10.21 (±4.61)	11.98 (±4.01)	0.07
Disease duration (mo)	49.21 (±58.07)		
ALSFRS-R	36.08 (±8.24)		
Disease stage	2.56 (±1.04), King's 2.40 (±0.91), MiToS		
Site of onset	8 bulbar 31 spinal 1 respiratory 2 mixed		
Phenotype	15 classic 16 predominant LMN 11 predominant UMN		
Disease progression rate	0.42 (±0.42)		
Neurocognitive assessment			
ECAS total score	87.49 (±25.60)		
Language	20.93 (±5.49)		
Verbal fluency	15.12 (±7.62)		
Executive functions	28.27 (±10.84)		
Memory	12.05 (±4.96)		
Visuospatial abilities	11.10 (±1.95)		
Cognitive/behavioral impairment	4 ALSci 8 ALSbi 9 ALSbci		

Abbreviations: ALS = amyotrophic lateral sclerosis; ALSbi = amyotrophic lateral sclerosis behavioral impairment; ALSbci = amyotrophic lateral sclerosis behavioral and cognitive impairment; ALSci = amyotrophic lateral sclerosis cognitive impairment; ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; ECAS = Edinburgh cognitive and behavioral ALS screen; HC = healthy controls; LMN = lower motor neuron; MiToS = Milano-Torino staging; UMN = upper motor neuron.

Figure 2 Comparison of the Number of Unique Avalanche Patterns



disease progression rate, and neurocognitive assessment, are reported in Table 1.

Functional Repertoire, Avalanche Patterns, and Local Dynamics

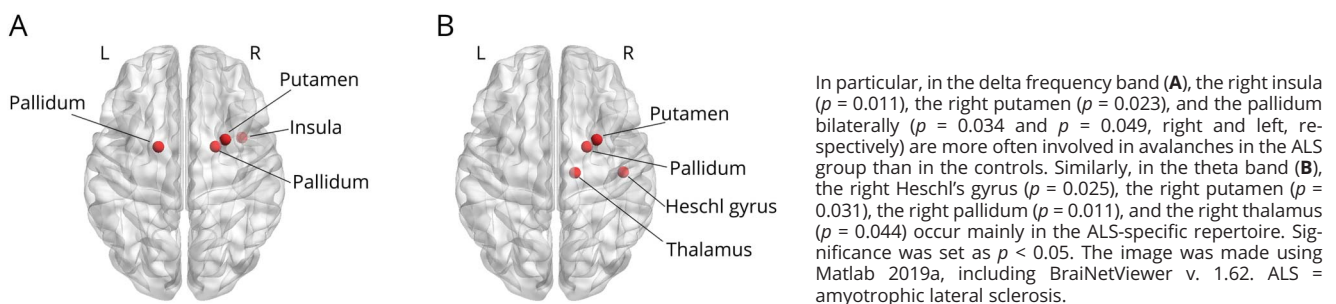
We used source-reconstructed resting-state MEG data acquired from a cohort of patients with ALS and healthy controls to quantify the functional repertoire, which is the total number of unique avalanche patterns occurred in each individual. A comparison between the 2 groups revealed that patients with ALS expressed a restricted functional repertoire, with a lower number of visited patterns. More specifically, we observed these results in both the delta ($p = 0.046$; Figure 2A) and the theta ($p = 0.046$; Figure 2B) frequency bands.

To prove the robustness of our results to specific choices of the avalanche threshold and bin length, we tested these variables across a moderate range of values and repeated the analyses. Specifically, we first used different binnings, ranging from 1 to 5. The results remained unchanged, with patients with ALS displaying a restricted functional repertoire for all the binnings explored (for binning = 2, $p = 0.009$ (delta band)

and $p = 0.010$ (theta band); for binning = 3, $p = 0.010$ (delta) and $p = 0.012$ (theta); for binning = 4, $p = 0.010$ (delta) and $p = 0.015$ (theta); for binning = 5, $p = 0.011$ (delta) and $p = 0.019$ (theta); see eTable 1, [links.lww.com/WNL/C297](https://www.lww.com/WNL/C297)). Furthermore, the avalanche threshold was modified, ranging from 2.5 to 3.5. For both cases, the differences between the groups were confirmed (for $z = |2.5|$, $p = 0.028$ in the delta frequency band and $p = 0.030$ in the theta frequency band, while for $z = |3.5|$, $p = 0.005$ and $p = 0.007$ in the delta and theta bands, respectively; see eTable 1).

We also evaluated how many times each active region became inactive, and vice versa (number of switches), finding no significant differences between the 2 groups (data not shown). These results confirmed that brain dynamics is qualitatively altered in patients with ALS, as compared with controls. In fact, the same number of switches means that the rate at which each region changes its status is similar in the 2 groups. Nonetheless, patients only visit a restricted number of patterns as compared with controls. Hence, the restriction of the functional repertoire is not due to different activation rates but genuinely reflects impaired flexibility.

Figure 3 Mapping of Brain Regions Occurring Significantly More in the ALS-Specific Unique Avalanche Patterns



Subsequently, we investigated the influence of specific regions on avalanche patterns. First, we compared the distributions of brain region occurrences between shared and group-specific patterns. The Kolmogorov-Smirnov test confirmed that the 2 distributions were significantly different ($p < 0.001$), meaning that there was an uneven involvement of brain regions in the avalanches of the 2 groups. Hence, we conducted a post hoc analysis through a permutation test to identify which brain areas occurred more often in the group-specific patterns. The analysis highlighted several brain regions occurring significantly more in the group-specific repertoire than in the shared repertoire (Figure 3). In particular, in the delta frequency band, the right insula ($p = 0.011$), the right putamen ($p = 0.023$), and the pallidum bilaterally ($p = 0.034$ and $p = 0.049$, right and left, respectively) were more often involved in

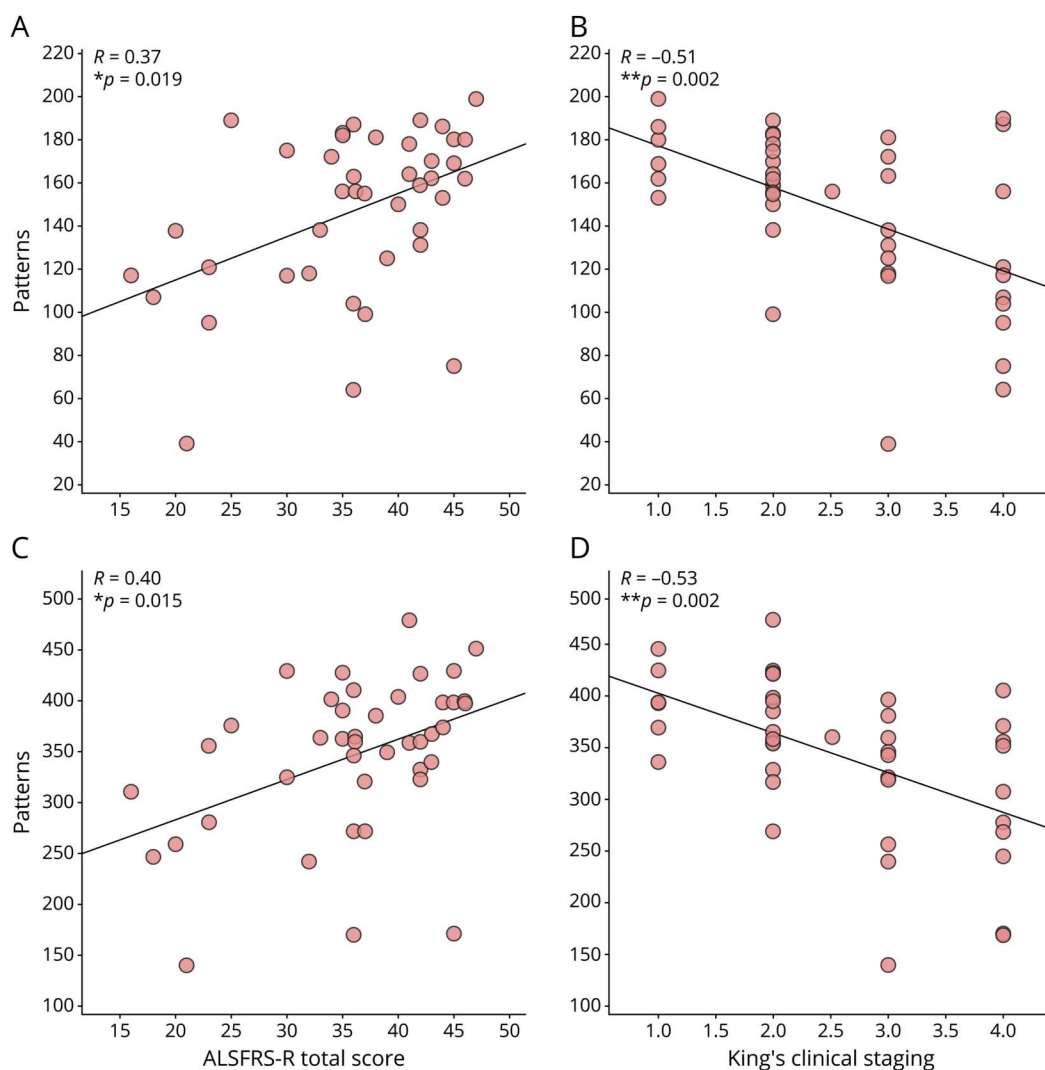
avalanches in the ALS group than in the controls (Figure 3A). Similarly, in the theta band, the right Heschl's gyrus ($p = 0.025$), the right putamen ($p = 0.031$), the right pallidum ($p = 0.011$), and the right thalamus ($p = 0.045$) occurred mainly in the ALS-specific repertoire (Figure 3B).

Multilinear Model Analysis

Subsequently, to understand the clinical significance of the restricted functional repertoire observed in patients with ALS, we correlated the size of the functional repertoire with the ECAS total score, the ALSFRS-R, and both the King's and the MiToS clinical staging systems.

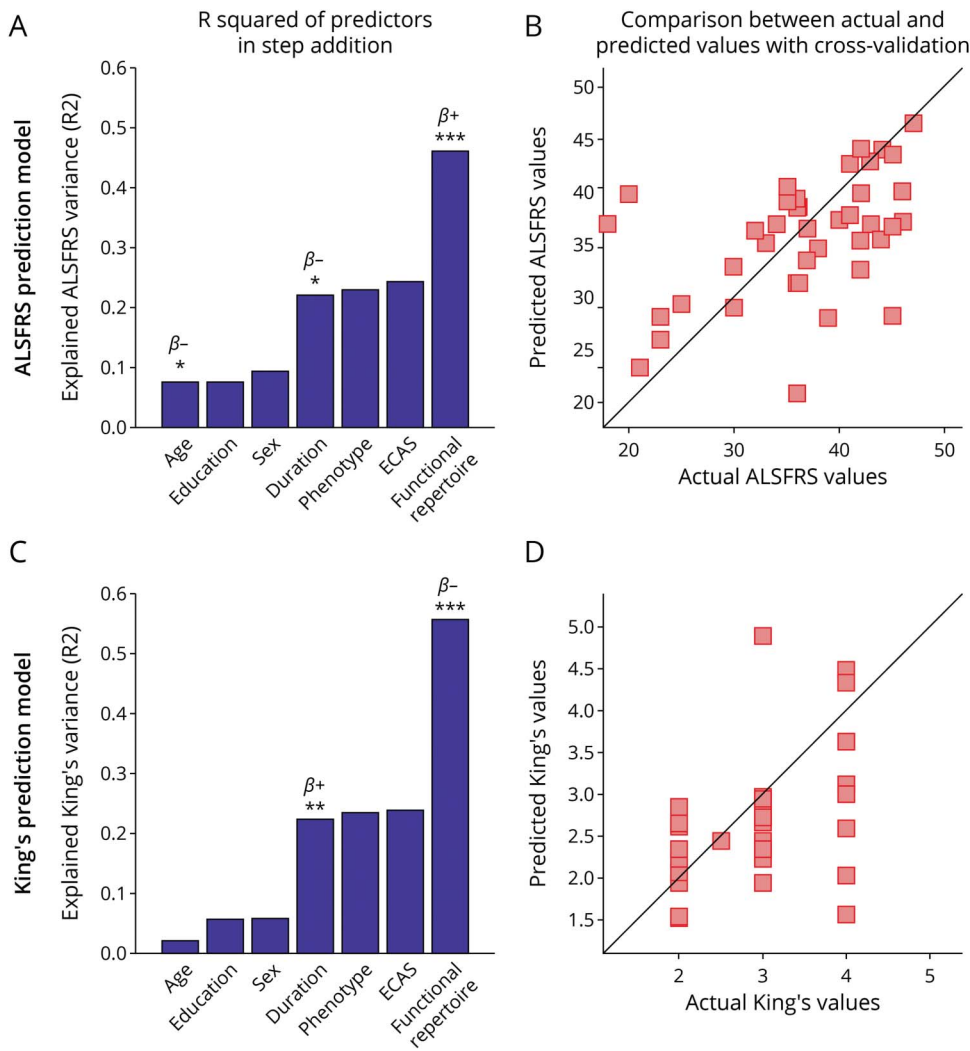
We observed significant correlations between the size of the functional repertoire and the clinical features in both the delta

Figure 4 Relationship Between Brain Dynamics and Clinical Features in the ALS Group in the Delta (A and B) and the Theta (C and D) Frequency Bands



(A) Positive correlation between the number of unique avalanche patterns and the ALSFRS-R ($R = 0.37$, $p = 0.019$) and (B) negative correlation between the number of patterns and the King's clinical staging system ($R = -0.51$, $p = 0.002$) in the delta band; (C) Positive correlation between the number of unique avalanche patterns and the ALSFRS-R ($R = 0.40$, $p = 0.015$) and (D) negative correlation between the number of patterns and the King's clinical staging system ($R = -0.53$, $p = 0.002$) in the theta band. The Spearman correlation coefficient was used, and the results were corrected by false discovery rate (FDR) correction. Significance p -values: $*p < 0.05$, $**p < 0.01$. The figure was made using Matlab 2019a. ALS = amyotrophic lateral sclerosis.

Figure 5 Multilinear Model With *k*-Fold Cross-validation in the Delta Frequency Band



Using as predictors age, education, sex, disease duration, ALS phenotype, ECAS, and the size of the functional repertoire, the model predicts (A and B) the ALSFRS-R (age: $p = 0.038$, $\beta = -0.23$; disease duration: $p = 0.020$, $\beta = -0.05$; functional repertoire: $p < 0.001$, $\beta = 0.11$); (C and D) the King's clinical staging system (disease duration: $p < 0.01$, $\beta = 0.01$; functional repertoire: $p < 0.001$, $\beta = -0.02$). In (A and C), the explained variance of the variable to be predicted as a function of the predictors is illustrated. Significant predictors are indicated by asterisks; positive and negative coefficients are illustrated with β^+ and β^- , respectively; significance p -values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. In (B and D), scatter plots of the comparison between actual and predicted values are reported. The figure was made using Matlab 2019a. ALS = myotrophic lateral sclerosis; ECAS = Edinburgh Cognitive and Behavioural ALS Screen

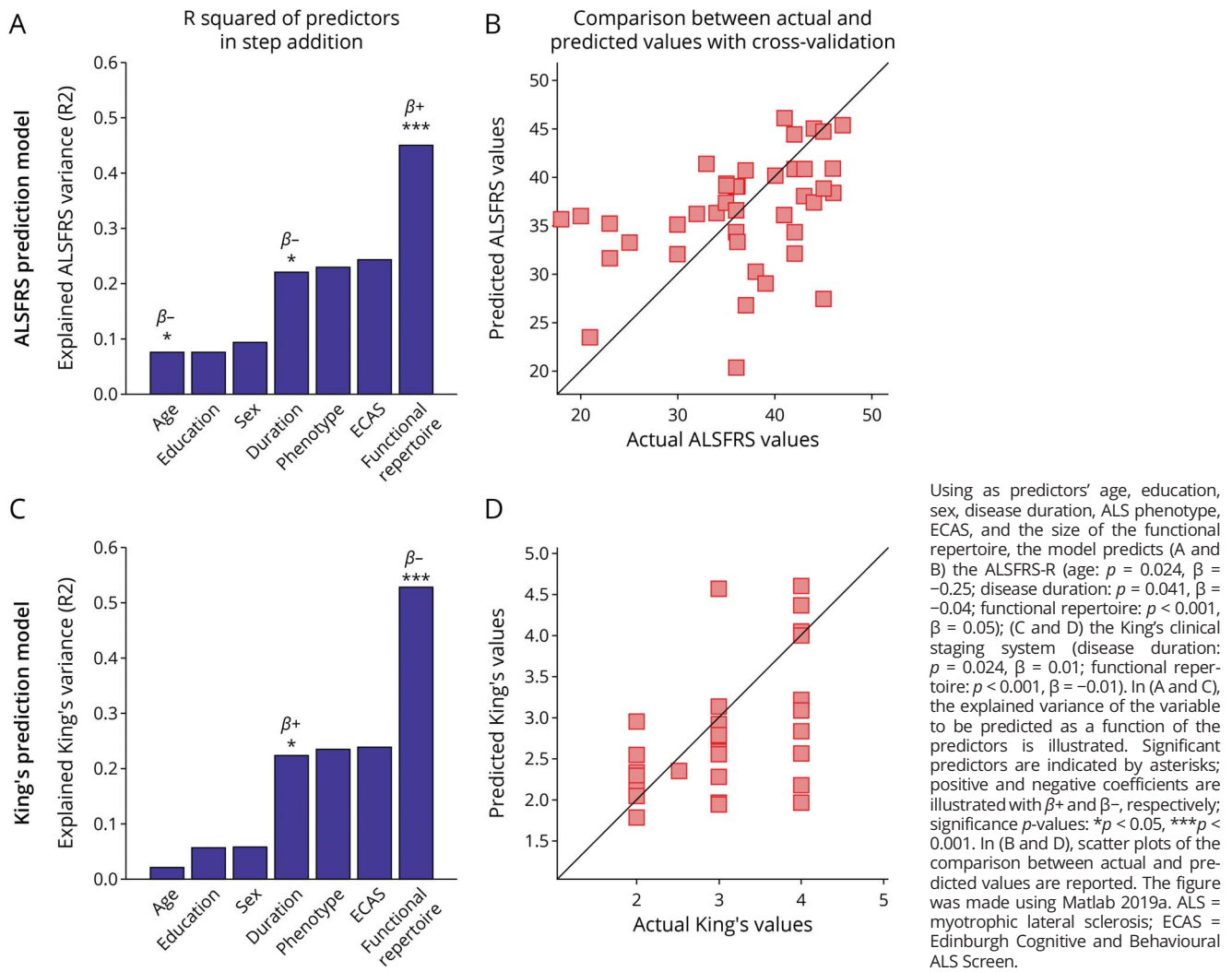
and the theta frequency bands. Particularly, in the delta band, the larger the functional repertoire, the lower the clinical impairment, as measured by the ALSFRS-R ($R = 0.37$, $p = 0.019$; Figure 4A), and the better the clinical staging, as measured by both the King's ($R = -0.51$, $p = 0.002$; Figure 4B) and the MiToS ($R = -0.41$, $p = 0.015$; eFigure 1A, links.lww.com/WNL/C297) disease staging systems. Similar results were observed in the theta frequency band, where the number of avalanche patterns was larger in the least-compromised patients (correlation with ALSFRS-R: $R = 0.40$, $p = 0.015$; Figure 4C), and smaller in more advanced patients, again as measured by the King's ($R = -0.53$, $p = 0.002$; Figure 4D) and the MiToS ($R = -0.47$, $p = 0.005$; see eFigure 1B) staging systems. These results could also be observed in the theta frequency band, where the number of avalanche patterns correlated positively with the ALSFRS-R ($R = 0.40$, $p = 0.015$; Figure 4C) and negatively with the King's ($R = -0.53$, $p = 0.002$; Figure 4D) and the MiToS ($R = -0.47$, $p = 0.005$; see eFigure 1B) staging systems. Conversely, there were no significant correlations between the number of avalanche patterns and the ECAS total score, neither in the delta

band ($R = 0.16$, $p = 0.30$) nor in the theta band ($R = 0.14$, $p = 0.39$) (data not shown).

Then, we used a multilinear model analysis with *k*-fold cross-validation to evaluate if demographics, clinical, and brain dynamics features could predict the ALSFRS-R and the disease stage. We found that the size of the functional repertoire significantly improves the predictive capacity of the model in both the delta and the theta frequency bands (Figures 5 and 6).

In the delta band, the model provided significant predictions of the ALSFRS-R (Figure 5, A and B, $R^2 = 0.46$), and both the King's (Figure 5, C and D, $R^2 = 0.56$) and the MiToS (eFigure 2 A, B, links.lww.com/WNL/C297, $R^2 = 0.52$) clinical staging systems. Similarly, in the theta band, the ALSFRS-R (Figure 6 A, B, $R^2 = 0.45$), and both the King's (Figure 6, C and D, $R^2 = 0.53$) and the MiToS (eFigure 2 C, D, $R^2 = 0.50$) staging systems were predicted. The comparison between actual and predicted values obtained through the *k*-fold validation method³⁰ is shown in the

Figure 6 Multilinear Model With *k*-Fold Cross-validation in the Theta Frequency Band



panels B and D of both Figures 5 and 6, while the residual distribution is shown in eFigure 3 (delta band) and eFigure 4 (theta band), in the Supplement. No significant contribution of education level, sex, ALS phenotype, and ECAS was observed.

We also validated our model using the LOOCV approach. The results were confirmed in both the delta (eFigures 5, 6, 7 in the Supplement, links.lww.com/WNL/C297) and the theta (eFigures 6, 8, 9 in the Supplement) frequency bands.

Discussion

In this work, we set out to predict clinical impairment in ALS from the flexibility of brain dynamics (i.e., reduced functional repertoire). Our results showed that patients with ALS have a restricted functional repertoire as compared with healthy controls. This was demonstrated by the lower number of distinct (i.e., unique) avalanche patterns. The size of the functional repertoire correlated directly with the ALSFRS-R and negatively with both the King's and the MiToS clinical staging systems.

It has been proposed that the healthy brain operates in a regime that maximizes flexibility, thereby facilitating adaptive behavior. The brain in ALS might be operating in a suboptimal dynamical regime, resulting in more stereotyped activity. We borrow from statistical mechanics the concept of *neuronal avalanches*: fast, aperiodic bursts of activations that spread across the whole brain.¹¹ If the brain is operating in a regime that allows flexible activity, neuronal avalanches efficiently reconfigure themselves over time.²⁶ The restriction of the functional repertoire observed in patients with ALS might reflect the effect of pathophysiologic processes on the large-scale brain dynamics, as previously observed in other neurodegenerative diseases.^{12,36}

Our results were specific to the delta and the theta frequency bands. However, alterations in regional power or static functional connectivity have been described in all frequency bands.^{37,38} Ours is the first M/EEG study directly addressing the dynamic, aperiodic, scale-free activity in ALS. Hence, on the one hand, comparing our results with previous results, that were based on power spectra or static connectivity, is not trivial. On the other

hand, preliminary evidence from fMRI shows altered low-frequency brain dynamics in ALS,³⁹ which corroborates our results (given the comparable time scales). However, comparing the results should be performed cautiously.

To test whether some brain regions were specifically important in determining pathologic patterns of activity, we analyzed shared and group-specific avalanche patterns. We classified avalanche patterns either as shared, if they occurred in both groups, or as group-specific, if they occurred in either group. Our results highlighted several brain regions being recruited significantly more often in ALS-specific neuronal patterns. In particular, basal ganglia were more often involved in group-specific patterns, in both the delta and the theta frequency bands. This finding may suggest the key role of subcortical regions in the coherent recruitment of cortical areas⁴⁰ and the association between the altered functioning of these regions and the cognitive and behavioral deficits in ALS,^{41,42} Finally, it corroborates the wide-spread involvement on the brain in ALS.⁴³

Subsequently, we reasoned that if the *functional repertoire* is capturing a pathophysiologic process, then it should be related to the stage of the disease and, in turn, allow its prediction. We found, as said, a positive correlation with the ALSFRS-R and a negative correlation with both the King's and the MiToS clinical staging systems. Our findings might be framed within the hypothesis that the neuropathologic mechanisms in ALS shift the operational regime of the brain to a (presumably) suboptimal state that no longer allows sufficient flexibility to support correct behavior, thereby relating to disability and staging (as measured by the ALSFRS-R). Consistent with our work, time-resolved approaches showed that reduced temporal variability in ALS relates to disease severity.⁴⁴

Using a multilinear model analysis, we showed that the size of the functional repertoire is a significant predictor of both clinical staging and impairment, even after accounting for age, education, gender, disease duration, phenotype, and ECAS. This supports the hypothesis that pathophysiologic mechanisms impair brain flexibility, which could then be used as a noninvasive readout.

Our work straightforwardly characterizes qualitative alterations of the whole-brain dynamics. This framework is mathematically grounded linking microscopic mechanisms to changes observed in the data. Hence, in the framework of personalized medicine, it might be possible to use our approach to tailor large-scale mechanistic models to the individual patients. However, to this end, longitudinal studies are warranted. Furthermore, despite the lower spatial resolution, neuronal avalanches can be observed using EEG, which might allow the application of our methodology on a wider scale.

In conclusion, our work shows that (1) pathophysiologic changes in ALS are reflected in reduced flexibility and, possibly, less effective large-scale communication; (2) subcortical regions contribute to brain dynamics and are affected by the pathophysiologic processes of ALS (however, we found these

regions from a post hoc analysis that was not corrected for multiple comparison, and this finding should be regarded as merely explorative); and (3) the reduction of flexibility in ALS predicts disease stage and clinical impairment.

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Disclosure

The authors report no relevant disclosures. Go to Neurology.org/N for full disclosures.

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Emmanuel Troisi Lopez, PhD	University of Naples “Parthenope”, Italy	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Roberta Minino, PhD	University of Naples “Parthenope”, Italy	Drafting/revision of the manuscript for content, including medical writing for content
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Antonella Romano, PhD	University of Naples “Parthenope”, Italy	Drafting/revision of the manuscript for content, including medical writing for content
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Appendix (continued)

Name	Location	Contribution
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Giuseppe Sorrentino, MD, PhD	Institute of Applied Sciences and Intelligent Systems of National Research Council	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
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