NÖRO PSİKİYATRİ ARŞİVİ
ARCHIVES OF NEUROPSYCHIATRY

RESEARCH ARTICLE

Arch Neuropsychiatry 2024;61:296–305
https://doi.org/10.29399/npa.28864

Selective Muscle Involvement in Amyotrophic Lateral Sclerosis: Evidence Inferred from the Point of Motor Unit Firing Rates

Sezin ALPAYDIN BASLO[1,2], Nermin Görkem ŞİRİN[3], Elif KOCASOY ORHAN[3], Mehmet Barış BASLO[3], Ali Emre ÖGE[3]

[1]Istanbul University, Graduate School of Health Sciences, Aziz Sancar Institute of Experimental Medicine, Deparment of Neuroscience, Electroneurophysiology Master Programme, Istanbul, Türkiye
[2]University of Health Sciences, Bakirkoy Prof. Dr. Mazhar Osman Training and Research Hospital for Psychiatric, Neurologic, and Neurosurgical Diseases, Department of Neurology, Istanbul, Türkiye
[3]Istanbul University, Istanbul Faculty of Medicine, Department of Neurology and Clinical Neurophysiology, Istanbul, Türkiye

ABSTRACT

Introduction: The aim of the study is to determine the role of upper motor neuron (UMN) or lower motor neuron (LMN) dysfunction as the primary initiator of distal-proximal and lateral-medial gradients of muscle involvement in amyotrophic lateral sclerosis (ALS).

Methods: Concentric needle electromyography recordings were performed in deltoid, abductor digiti minimi, and first dorsal interosseous (FDI) muscles in patients with ALS and controls during slight voluntary contraction needed to activate two motor units (MU). Five motor unit potential (MUP) pairs were recorded from each muscle. Motor unit potential analyses were performed offline using Multi-MUP analysis program. Quantitative MUP parameters, MU firing rate (FR), FR variability (FRV), and mean consecutive difference (MCD) were calculated. Motor-evoked potentials and the triple stimulation technique (TST) were performed to evaluate UMN involvement.

Results: Twenty patients with ALS along with 20 age and sex-matched healthy volunteers were enrolled. Quantitative MUP parameters compatible with denervation and reinnervation were found in patients with ALS, who also showed higher FR, FRV, and MCD values, most prominently in FDI. First dorsal interosseous FRV was lower in patients with abnormal central motor conduction time (CMCT). Firing rate and FRV were negatively correlated with CMCT, but not with TST.

Conclusion: Distal limb muscles, particularly FDI, revealed more prominent FR abnormalities in patients with ALS in parallel with the distal-proximal and lateral-medial gradients of the selective muscle involvement pattern which seems predominantly to be correlated with LMN dysfunction. Reduced FRV may be associated with the presence of UMN dysfunction in ALS.

Keywords: Amyotrophic lateral sclerosis; firing rate; motor unit potential; transcranial magnetic stimulation; triple stimulation technique

Cite this article as: Alpaydın Baslo S, Şirin NG, Kocasoy Orhan E, Baslo MB, Öge AE. Selective Muscle Involvement in Amyotrophic Lateral Sclerosis: Evidence Inferred from the Point of Motor Unit Firing Rates. Arch Neuropsychiatry 2024; 61:296–305.

INTRODUCTION

Clinical and electromyographic abnormalities are generally more prominent in distal muscles ('distal-proximal gradient')(1–3) and on the lateral side of the hand ('lateral-medial gradient')(4,5) in patients with amyotrophic lateral sclerosis (ALS). However, the role of upper motor neuron (UMN) or lower motor neuron (LMN) dysfunction as the promoter of these phenomena is still debated.

Motor unit firing and recruitment are essential for the graded tuning of the force exerted by voluntary muscle contraction (6). Motor units are recruited in an orderly fashion and to do so, spinal motor neurons need synaptic inputs from descending projections, spinal interneurons, and sensory afferents (7,8). Firing rate and recruitment properties of motor units (MU) could give indirect information about how these synaptic inputs and the spinal motor neurons themselves function both in health and disease (9,10).

Highlights

- **Firing rate, MCD and FRV were increased in ALS muscles.**
- **Firing rate, MCD and FRV increase was more prominent in distally located ALS muscles.**
- **Firing rate, MCD and FRV findings suggested LMN dysfunction keeping track of ALS gradients.**
- **Firing rate variability was lower in patients with abnormally long CMCT than those with normal results.**
- **Reduced FRV may be associated with UMN dysfunction in ALS.**

Correspondence Address: Sezin Alpaydın Baslo, University of Health Sciences, Bakirkoy Prof. Dr. Mazhar Osman Training and Research Hospital for Psychiatric, Neurologic, and Neurosurgical Diseases, Department of Neurology, Istanbul, Türkiye • **E-mail:** sznalpaydin@gmail.com
Received: 23.06.2024, **Accepted:** 08.08.2024, **Available Online Date:** 30.11.2024

In ALS, the most common motor neuron disease, a reduced number of recruited motor units with an increased firing rate is observed. However, the impact of UMN lesions on LMN firing rates is not well known in this disease. We aimed to focus on the origins of distal-proximal and lateral-medial muscle involvement gradients in ALS from the point of motor unit firing rate and recruitment. Upper motor neuron involvement was also evaluated using motor-evoked potentials (MEP) and the triple stimulation technique (TST).

METHODS

Subjects

Twenty patients with ALS, referred between December 2019 and September 2021, and 20 healthy volunteers were enrolled (11). Patients with mental and behavioral problems that could hinder cooperation with the procedure, and those with respiratory symptoms who were unable to lie supine comfortably were excluded. None of the subjects had any clinical symptoms or signs of additional neuromuscular disorders or any systemic disease that might affect the results. To be able to record motor unit potentials (MUPs) in a steady state voluntary contraction, extremities harboring the test muscles with relatively preserved strength (≥3/5 grade according to the Medical Research Council –MRC scale) were chosen. Routine nerve conduction studies were performed in the upper extremities of all subjects before the research protocol. To record MEPs clearly, only patients with ≥1 mV amplitude compound muscle action potentials (CMAPs) in abductor digiti minimi (ADM) were included.

The protocol was approved by İstanbul University İstanbul Faculty of Medicine Clinical Research Ethics Committee (22.11.2019/1366). Written informed consent was provided by all subjects.

A thorough clinic and neurologic examination was performed on each patient. Medical research council sum scores (0–60)(12), Amyotrophic Lateral Sclerosis Functional Rating Scale-revised (ALSFRS-r) scores (0–48) (13,14), and Turner UMN scores (0–15)(15) were calculated.

Electrophysiologic studies

Electrophysiologic studies were performed on the clinically more affected side in the ALS group and on the dominant side in controls. If the strength of any test muscle was not ≥3/5 MRC grade on the more affected side, contralateral side was chosen.

For nerve conduction and firing rate studies, a Natus Dantec Keypoint. Net version 2.40 device was used. Median and ulnar nerves were stimulated supramaximally at wrist, and CMAPs were recorded from the abductor pollicis brevis (APB), first dorsal interosseous (FDI), and ADM muscles by using a pair of self-adhesive round electrodes placed according to the belly-tendon montage. F waves were recorded from ADM in response to 20 consecutive supramaximal stimuli (16). Split hand index (SHI) was calculated according the formula: $SHI=CMAP_{APB}xCMAP_{FDI}/ CMAP_{ADM}$ (17). The cut-off value for SHI was accepted as 5.2(17). Neurophysiological index (NI) was calculated based on the CMAP recorded from ADM according to the formula: $NI=(CMAP_{ADM}/DML) \times F_{frequency}$ (18).

Motor unit potential analyses and firing parameters were studied in deltoid (Del), FDI, and ADM muscles in the same session. Concentric needle EMG recordings were performed using electrodes with a 0.07 mm^2 recording surface (Natus Medical Inc., Middleton, WI). The QEMG-Multi-MUP program residing in EMG equipment was used for analysis.

The needle electrode was inserted into the muscle at rest and subjects were asked to initiate a mild but stable contraction to activate a single motor unit, and then to recruit a second one by increasing the force slightly. Minimal resistance was applied by the investigator and auditory feedback was provided by the EMG device to assist in fine-tuning the contraction force. Several seconds of rest were provided between the recording epochs in order to avoid the fatigue. After a steady state muscle activation was achieved, MUP pairs were recorded for at least 15-second duration epochs. In each muscle, five MUP pairs from five different sites, approximately 5 mm apart, were recorded by changing the needle position.

Multi-MUP analyses were performed offline with 5 Hz-10 kHz filter settings, 200 µV/div sensitivity, and 5 ms/div sweep speed (19).

Firing rate (FR) of MUs were analyzed using the epochs containing steadily firing MUP pairs. The MUP candidate to be analyzed was used for triggering the sweep and the entire recording was rastered for 20 traces on the screen. The sweep duration was re-arranged to view two identical signals representing the same MUP in the same line; the first one became the "triggering" and the following was the "jittering" potential. The time between those two potentials was the interpotential interval (IPI) and the mean FR of each MUP was calculated from the IPIs on 20 rastered lines (Figure. 1).

Firing rate variability (FRV) was calculated using the formula 'FRV=(a/b) ×100 [a=difference between the maximum and minimum IPIs, b=time frame between the triggering potential and the midpoint of 'a' (a/2)](20).

Mean consecutive difference (MCD) between the firing rates of MUPs in each pair was calculated using the formula '$MCD=|FR\_MUP_1\text{-}FR\_MUP_2| +|FR\_MUP_3\text{-}FR\_MUP_4|+ |FR\_MUP_{n-1}\text{-}FR\_MUP_n| / (n/2)$' (10).

A Nicolet Viking Select EMG system (version 11.1) and a Magstim 200 magnetic stimulator were used for TMS studies. A round coil with an outside diameter of 12 cm was located over the vertex to record MEPs from ADM during moderate voluntary contraction. Five to eight stimuli were applied with intensities obviously higher than the motor threshold (70% to 100% of the maximum stimulator output) in all participants except one patient in whom no motor response could be recorded with maximum stimulator output. The current direction in the coil was selected as the one that produced the highest amplitude motor responses (21). The shortest latency of the recorded MEPs was measured. Central motor conduction time (CMCT) was calculated using F-wave latencies according to the formula: 'CMCT=MEP-(F+M-1)/2' (22). The upper limit of normal for CMCT was calculated as 8.8 ms (mean +2SD of controls).

TST was performed by recording from ADM using the method described by Magistris et al. (21,23,24). TST% was calculated as the ratio of the TSTtest amplitude to that of the TSTcontrol, multiplied by 100 (Supplementary Figure.). The lower limit of normal for TST% was calculated as 84.6% (mean -2SD of controls).

Statistical analysis

IBM Statistical Package for Social Sciences (SPSS) program version 22.0 was used. Descriptive statistics were given as mean, standard deviation (SD), and frequency (%). Mixed analysis of variance was used to detect within (muscle) and between (group) subject factors for motor unit firing parameters. For parameters with significant difference levels, pairwise comparisons were performed using Mann-Whitney U test with Bonferroni correction. When comparing two groups, Student's t-test and Mann-Whitney U test were used for parametric and non-parametric variables, respectively. Chi-square test was used to compare categorical variables. Pearson's correlation analysis or Spearman's rho

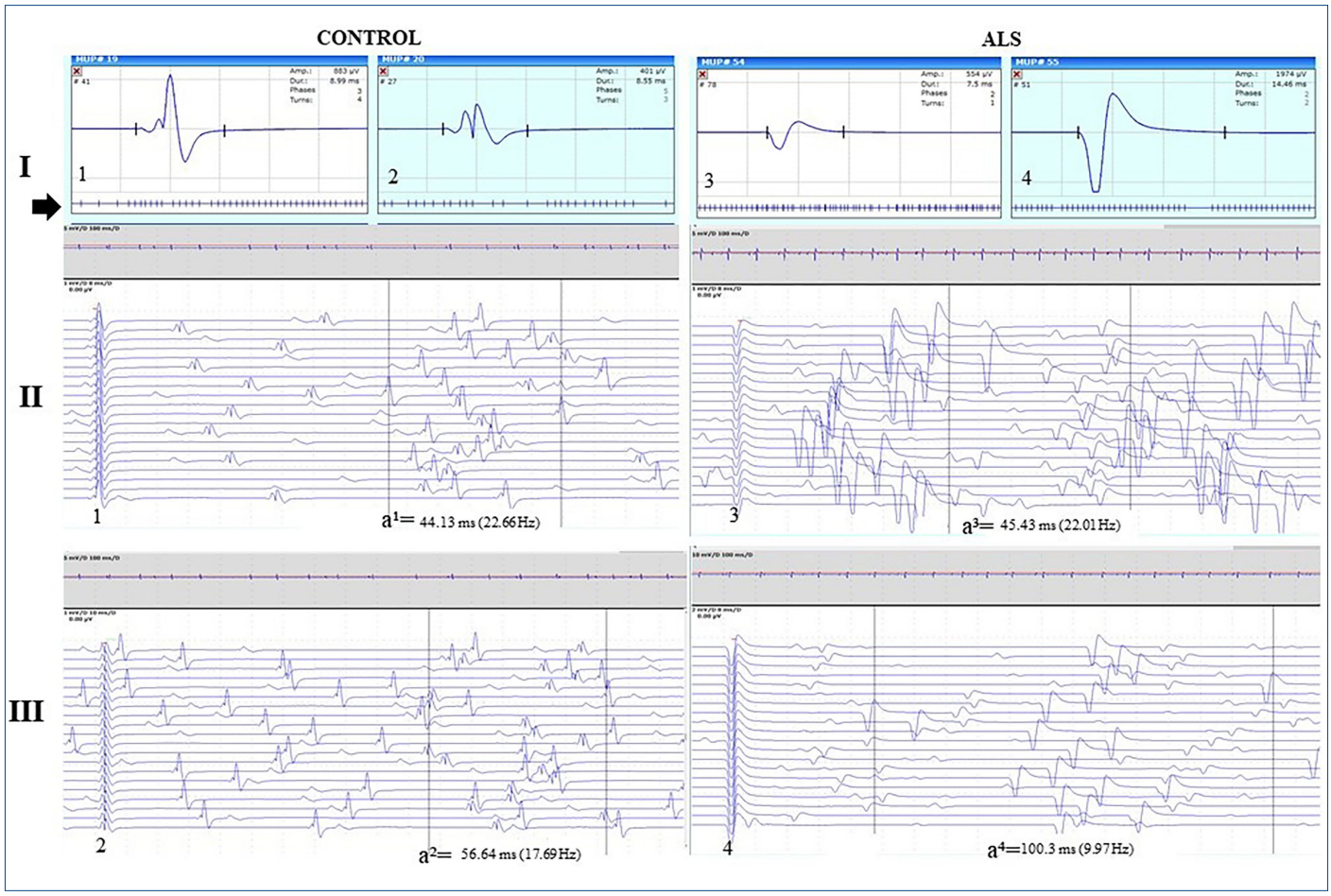

Figure 1. Two consecutive motor unit pairs recorded in a control (left) and a patient with ALS (right). First row (I): Multi-MUP software showing the two consecutive motor units with different amplitude, duration, phase and turn numbers in a control (1,2) and a patient with ALS (3,4). Note the relatively stable muscle contraction with only slight physiological variations in the inter-discharge intervals of each motor unit (arrow). Second (II) and third (III) rows: Each MUP was shown triggered and rastered in 20 lines in different windows. The sweep duration was re-arranged to view the triggering and jittering MUPs in the same line. Vertical lines indicate the maximum and minimum interpotential intervals and the difference between them depicted by 'a' ('a' values for each MUP; a1, a2, a3, a4). Note the higher 'a' values in ALS and the increased variability of motor unit firing rate.

were used according to the distribution characteristics of the data. A p-value <0.05 was accepted as statistically significant, except for Mann-Whitney U tests with Bonferroni correction where p<0.008 was accepted as significant.

RESULTS

Clinical characteristics

Twenty patients with ALS (11F: 9M) and 20 controls (10 F: 10M) were enrolled. There was no significant age or sex difference between the patients and controls. Clinical characteristics of controls and the patients are summarized in Table 1. Medical research council scores of the studied muscles were ≥4/5 in 19 out of 20 Del, 16 out of 20 FDI and 17 out of 20 ADM muscles. Comparison of muscle strengths of FDI and Del (as MRC scores) revealed that, FDI was weaker than Del in 14, Del was weaker than FDI in 1 while both muscles were equal in 5 patients. Comparison of muscle strengths of FDI and ADM revealed that both muscles were equal in 16, ADM was weaker than FDI in 3 and FDI was weaker than ADM in 1 patient. Taking into consideration of APB, split hand sign was present clinically in 11 (55%) patients.

Electrophysiologic studies

Compound muscle action potential amplitudes were lower, F-wave latencies, MEP latencies, and CMCT were longer, F-wave persistences, MEP amplitudes, and TST amplitude ratios were lower in the patients. Mean SHI and NI values were also significantly lower in them (Table 2).

Motor unit potential amplitudes were higher and MUP durations were longer in patients with ALS when compared with controls. The phase and turn numbers of Del MUPs were higher in the patients compared with those of the controls. Motor unit potential amplitudes of Del were lower than those of ADM and FDI in both patients and controls. Turn numbers of Del MUPs were higher than those of FDI in the patients (Table 3, Figure. 2).

The FR, MCD, and FRV values were higher in patients with ALS than those of the controls (Table 3, Figure. 2). Subgroup analyses revealed that MCD in all three muscles and FR and FRV in ADM and FDI were higher in the patients. Firing rate of FDI was higher than FR of ADM in the ALS group. Mean consecutive difference of FDI tended to be the highest among the three recording muscles in the patients. Firing rate variability of ADM was higher than that of Del and FDI in the controls. Firing rate variability of ADM and FDI were higher than that of Del but not significantly different from each other in patients with ALS.

The correlation analysis of electrophysiologic and clinical data is shown in Table 4. FR, FRV, and MCD showed positive correlations with each other for all three recording muscles of the patients (data not shown for ADM and Del). First dorsal interosseous FR was negatively correlated with

Table 1. Clinical characteristics of controls and patients with ALS

	Controls	Patients with ALS
Number (n)	20	20
Age [mean ± SD (min-max)] (years)	44.85±7.53 (34–64)	50.15±10.98 (33–71)
Sex (F: M)	10:10	11:9
Hand dominance (R: L)	16:4	17:3
Disease duration (months)		18.05±15.7 (3–72)
Onset region [n, (%)]		
Bulbar		2 (10)
Upper extremity		8 (40)
Lower extremity		10 (50)
MRC sum score [mean ± SD (min-max)]		53.6±7.88 (33–60)
Split-hand sign [n, (%)]		
Yes		11 (55)
No		9 (45)
Turner UMN score		9.45±4.03 (2–14)
ALSFRS-r score [mean ± SD (min-max)]		35.6±5.11 (26–44)
Conformity to diagnostic criteria rEl-Escorial criteria [n, (%)]		
Possible		3 (15)
Lab-supported probable		11 (55)
Probable		6 (30)
Definite		0 (0)
Awaji-Shima criteria [n, (%)]		
Clinically possible		5 (25)
Clinically probable		10 (50)
Clinically definite		5 (25)
Gold Coast criteria [n, (%)]		
ALS		20 (100)

ALSFRS-r: revised Amyotrophic Lateral Sclerosis Functional Rating Scale; F: female; L: left; M: male; MRC: Medical Research Council; R: right; UMN: upper motor neuron.

Table 2. Electrophysiologic data in controls and patients with ALS

	Controls (n=20)	ALS (n=20)	p*
APB_{CMAP} (mV)	9.7±2.2 (5.4–14.1)	5.7±3.9 (0.1–14)	**≤0.001**
ADM_{CMAP} (mV)	8.9±1.5 (6.5–11.6)	5.6±2.8 (1.8–12)	**≤0.001**
FDI_{CMAP} (mV)	13.1±2.4 (9.3–17.7)	5.2±3.5 (0.3–12.5)	**≤0.001**
$F_{latency}$ (ms)	23.8±2.02 (20.1–27.7)	25.5±2.3 (21–29.5)	**<0.05**
$F_{persistance}$ (%)	99±30 (90–100)	65±24 (10–100)	**≤0.001**
SHI	14.22±3.58	5.44±4.64	**≤0.001**
NI	3.37±0.72	1.35±1.02	**≤0.001**
	Control (n=19)	**ALS (n=19)§**	**p***
$Erb_{latency}$ (ms)	12.9±1.5 (10.6–16.2)	13.6±1.4 (11.7–17.1)	0.180
$Erb_{amplitude}$ (mV)	8.6±1.8 (5.2–12.2)	4.7±2.8 (1.3–11.2)	**≤0.001**
$MEP_{latency}$ (ms)	19.5±1.7 (16.5–22.7)	24.4±5.8 (18.1–36)	**0.002**
$MEP_{amplitude}$ (mV)	4.8±1.7 (1.9–8.1)	2.2±1.9 (0.1–6.3)	**≤0.001**
CMCT (ms)	6.8±0.9 (5–8.8)	11.5±7.8 (5–35.1)	**0.018**
MEP/M ratio	0.6±0.2 (0.2–0.9)	0.5±0.3 (0.05–1.1)	0.251
$TST_{amplitude\ ratio}$ (%)	100.9±8.2 (89.4–120.6)	70.1±29 (16–112.7)	**≤0.001**
$TST_{area\ ratio}$ (%)	97.1±10 (81.1–118.1)	64.9±28 (8.1–105.3)	**≤0.001**

*Mann-Whitney U test
§No motor response could be recorded in one patient with ALS, with stimulations up to 100% of the maximal output of the stimulator. The data of one control case could not be analyzed because of her refusal to complete the TMS studies.
ADM: abductor digiti minimi; APB: abductor pollicis brevis; CMAP: compound muscle action potential; CMCT: central motor conduction time; F: F-wave; FDI: first dorsal interosseus; MEP: motor evoked potential; MEP/M ratio: motor evoked potential amplitude/ motor response amplitude ratio; NI: neurophysiologic index; SHI: split hand index, TST: triple stimulation test. Data are given as mean ± SD (minimum-maximum).

Table 3. Quantitative MUP and MU firing rate parameters in controls and patients with ALS

	Controls (n=20)				ALS (n=20)			
	Del	**ADM**	**FDI**	**All muscles***	**Del**	**ADM**	**FDI**	**All muscles***
Analyzed MUP numbers	200	200	200	600	198	199	152	549
Amplitude (μV)	525.9±324.9 (88–2086)	703.2±401.87 (132–2006)	760.3±487 (106–2400)	663.1±420.9 (88–2400)	887.4±586.5 (77–4682)	1127.8±829.5 (135–3536)	1237±790.9 (97–3592)	1072.1±752.6 (77–4682)
Duration (ms)	8.6±2.3 (2.1–13.6)	8.5±2.02 (3.9–13.4)	8.4±2.1 (2.4–13.2)	8.6±2.1 (2.1–13.6)	11.9±3.1 (4.3–24.9)	11.5±3.6 (5.1–33.4)	11.5±2.9 (4.3–18.2)	11.6±3.2 (4.3–33.4)
Phase #	2.9±0.8 (1–6)	3.04±0.8 (2–6)	3.07±0.9 (1–6)	3.0±0.8 (1–6)	3.3±0.3 (2.8–3.8)	3.1±0.9 (1–8)	3.04±0.9 (1–8)	3.2±0.9 (1–8)
Turn #	2.8±1.2 (1–7)	3.1±1.5 (1–10)	2.9±1.1 (1–7)	2.9±1.3 (1–10)	3.6±1.6 (1–11)	3.3±1.5 (1–9)	3.16±1.4 (1–10)	3.4±1.5 (1–11)
FR (Hz)	10.8±1.9 (6.6–17.1)	10.2±2.4 (4.8–19.5)	10.05±2.02 (6.3–17.7)	10.4±2.1 (4.8–19.5)	11.8±3.4 (4.3–29.9)	11.7±4.04 (4.5–27.9)	13.4±5.8 (5.3–34.9)	12.2±4.5 (4.3–34.9)
MCD (Hz)	1.3±1.01 (0.01–4.1)	1.5±1.2 (0.03–5.4)	1.6±1.4 (0.03–6.3)	1.5±1.2 (0.01–6.25)	2.4±2.6 (0.005–18.8)	3.1±3.4 (0.04–17.6)	3.7±4.5 (0.006–23.3)	3.03±3.5 (0.005–23.3)
FRV (%)	54.8±28.9 (20.8–410.2)	63.5±17.2 (30.5–125.8)	58.1±17.3 (22.4–126.6)	58.8±22.1 (20.8–410.2)	57.9±23.2 (15.7–198.7)	72.3±41.7 (29.5–589.5)	69.7±30.6 (16.2–164.8)	66.4±33.5 (15.7–589.5)

ADM: abductor digiti minimi; ALS: amyotrophic lateral sclerosis; Del: deltoid; FDI: first dorsal interosseus; FR: firing rate; FRV: firing rate variability; MCD: mean consecutive difference; MU: motor unit; MUP: motor unit potential; #: number. Data are given as mean±SD (minimum-maximum). * Quantitative MUP (for amplitude, duration, turn number p ≤0.001; for phase number p<0.008) and MU firing rate parameters (p ≤0.001) of all studied muscles were significantly different between controls and the patients.

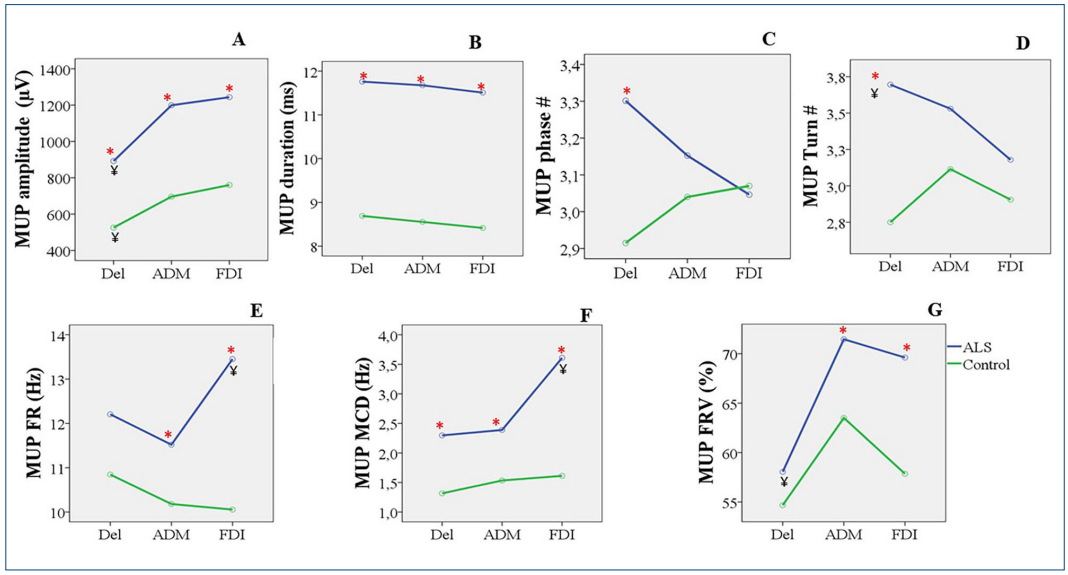

Figure 2. Quantitative MUP, firing rate and recruitment properties in patients with ALS (blue) and controls (green). Motor unit potential amplitudes were higher and MUP durations were longer in patients with ALS when compared with controls (p≤0.001). Note the increased phase and turn number of Del MUPs in patients with ALS compared with the controls (p≤0.001) and the increased turn number of Del MUPs compared with those of FDI in patients with ALS (p=0.006). The FR, MCD, and FRV of MUPs were higher in patients with ALS than those of the controls (p≤0.001). Also note the higher FR and MCD of FDI, and the lower FRV of Del in patients with ALS. * indicates to significant differences between (group) subject factors, ¥ indicates to significant differences within (muscle) subject factors (A-Del vs ADM and FDI, D-Del vs FDI, E-FDI vs ADM, F-FDI vs ADM and Del, G–Del vs ADM and FDI).

MRC sum score, CMAP amplitude, SHI, and CMCT, but not correlated with TST. Central motor conduction time was negatively correlated with turn number, CMAP amplitude, TST_{area} and FRV of FDI, and positively correlated with Turner UMN score. None of the FR parameters were correlated with other electrophysiologic and clinic data, except FR and MRC sum score in ADM and Del with a negative correlation and FRV and NI with a positive correlation.

There was no statistically significant difference between patients with abnormal and normal TST results regarding any of the clinic or electrophysiologic parameters. However, in patients with abnormally long CMCT, CMAP amplitudes were lower and ADM MUP durations were longer than those elicited in patients with normal CMCT. Turner UMN scores as well as MRC sum scores were higher and FRV in FDI was lower in patients with abnormally long CMCT than in those with normal results (Table 5).

In nine patients whose SHI was lower than the cut-off value of 5.2, mean FR of FDI was higher than in those with normal SHI (19.35±8.2 vs. 11.28±3.6, p=0.004). However, there was no statistically significant difference concerning MEP and TST parameters between those groups (Supplementary table).

DISCUSSION

One of the main results of the present study, conforming to both distal-proximal and lateral-medial gradients of ALS, was the higher MU FR in the patients, especially in FDI. We interpreted the increased FR as an indicator of LMN involvement because of the negative correlation of FR with CMAP amplitudes and MRC scores, along with its known property of being a hallmark of LMN loss (11). Lower motor neuron loss and motor neuronal hyperexcitable state together can explain the increased MU FRs in ALS; either with earlier recruitment of the fast firing MUs or more rapidly

Table 4. Correlation analyses of MUP parameters, electrophysiologic and clinic scores for FDI muscle

FDI	Amp	Dur	Phase #	Turn #	FR	MCD	FRV	APB CMAP	ADM CMAP	FDI CMAP	NI	SHI	MRC SUM	MRC FDI	MRC ADM	MRC APB	Turner	ALSFRS-r	MEP LAT	MEP AMP	CMCT	MEP/M	TST AMP	TST AREA
Amp																								
Dur	**0,574‡**																							
Phase #	0,106	**0,190***																						
Turn #	0,133	**0,272‡**	**0,536‡**																					
FR	0,129	-0,013	-0,116	-0,155																				
MCD	0,082	-0,075	-0,065	**-0,268***	**0,648‡**																			
FRV	**0,174***	0,054	0,022	-0,073	**0,501‡**	**0,481‡**																		
APB_{CMAP}	0,235	0,435	-0,147	0,132	**-0,591***	**-0,584***	-0,343																	
ADM_{CMAP}	0,179	0,128	0,118	0,327	-0,232	-0,418	0,168	**0,689‡**																
FDI_{CMAP}	0,211	0,099	-0,246	0,045	**-0,490***	**-0,625***	-0,252	**0,703‡**	**0,742‡**															
NI	-0,034	-0,283	0,53	0,145	-0,319	-0,211	0,005	0,269	**0,551***	**0,829‡**														
SHI	0,119	0,181	-0,298	-0,004	**-0,581***	-0,279	-0,439	**0,589†**	0,264	**0,844‡**	**0,586†**													
MRC_{SUM}	-0,124	0,051	0,261	0,102	-0,230	0,041	-0,167	0,025	-0,238	0,349	0,219	0,278												
MRC_{FDI}	0,325	0,468	0,182	0,133	**-0,515***	-0,288	0,088	**0,611†**	**0,672‡**	0,435	0,338	0,159	0,193											
MRC_{ADM}	0,228	0,378	0,391	0,258	-0,362	-0,174	0,165	0,333	0,428	0,205	**0,479***	0,063	0,354	**0,776‡**										
MRC_{APB}	0,158	0,383	0,016	0,149	**-0,582***	-0,428	0,066	**0,733‡**	**0,831‡**	**0,567***	0,442	0,278	-0,038	**0,830‡**	**0,676‡**									
Turner	-0,056	-0,045	-0,224	-0,290	-0,436	-0,169	**-0,475***	0,048	-0,252	-0,091	0,208	0,082	0,211	0,078	0,324	-0,002								
ALSFRSr	**-0,544***	-0,072	0,115	0,237	-0,216	-0,234	-0,237	0,112	-0,077	0,112	0,065	0,247	**0,586***	0,087	0,080	0,049	-0,035							
MEP_{LAT}	-0,230	-0,142	-0,337	**-0,511***	-0,276	0,079	**-0,509***	-0,203	**-0,605†**	-0,333	-0,234	-0,030	0,295	-0,164	-0,120	-374	**0,587†**	-0,017						
MEP_{AMP}	0,061	0,026	0,104	0,308	-0,132	-0,455	0,182	0,439	**0,677‡**	**0,570***	0,383	0,271	0,025	**0,545***	0,231	**0,536***	-0,337	0,039	-0,359					
CMCT	-0,174	-0,021	-0,383	**-0,482***	**-0,490***	-0,044	**-0,645†**	-0,049	**-0,498***	-0,156	-0,026	0,154	0,260	-0,125	-0,034	-0,252	**0,748‡**	-0,031	**0,909‡**	-0,329				
MEP/M	-0,185	-0,231	-0,043	-0,012	0,216	-0,124	0,195	-0,110	0,035	0,082	0,024	0,053	0,189	0,087	-0,137	-0,020	-0,361	0,240	-0,125	**0,692‡**	0,219			
TST_{AMP}	-0,222	-0,202	-0,126	0,009	0,257	-0,282	0,240	0,153	0,325	0,207	0,057	0,061	-0,116	0,132	-0,085	0,204	-0,351	0,108	-0,365	**0,691‡**	-0,388	**0,807‡**		
TST_{AREA}	-0,092	-0,094	-0,058	0,128	0,168	-0,374	0,263	0,360	**0,521***	0,309	0,075	0,030	-0,107	0,350	0,068	0,402	-0,295	0,085	-0,446	**0,723‡**	**-0,486***	**0,677‡**	**0,918‡**	

Spearman's correlation, * :p<0.05, † : p<0.01, ‡ : p≤0.001. ADM: abductor digiti minimi; ALSFRS-r: revised Amyotrophic Lateral Sclerosis Functional Rating Scale; Amp: amplitude; APB: abductor pollicis brevis; CMAP: compound muscle action potential; CMCT: central motor conduction time; Dur: duration; FDI: first dorsal interosseus; FR: firing rate; FRV: firing rate variability; MCD: mean consecutive difference; MEP_{AMP}: amplitude of motor evoked potential; MEP_{LAT}: latency of motor evoked potential; MEP/M: ratio of motor evoked potential amplitude to motor response amplitude; MRC: Medical Research Council; NI: neurophysiologic index; SHI: split hand index; TST_{AMP}: amplitude of triple stimulation technique response; TST_{AREA}: area of triple stimulation technique response.

Table 5. Electrophysiologic and clinical scores of patients with ALS with normal and abnormal TST and CMCT results

	TST normal (n=7)	TST abnormal (n=12)	p*	CMCT normal (n=7)	CMCT abnormal (n=12)	p*
$CMAP_{APB}$ (mV)	7.3±4.7 (0.5–14)	4.9±3.35 (0.13–9.9)	0.227	6.32±4.33 (0.13–14)	4.90±3.31 (0.50–9.90)	0.482
$CMAP_{ADM}$ (mV)	6.6±3.5 (1.8–12)	5.2±2.4 (2.1–8.9)	0.384	**6.70±2.68 (3.20–12.00)**	**4.06±2.37 (1.84–7.70)**	**0.028**
$CMAP_{FDI}$ (mV)	6.1±4.5 (1–12.5)	5.2±2.9 (1.2–10.9)	0.820	6.14±3.91 (1.18–12.50)	4.42±2.27 (1.00–7.20)	0.375
$F_{latency}$ (ms)	26.71±3.18 (21–29.50)	24.91±1.60 (22–27.14)	0.067	26.02±2.48 (21.00–29.50)	24.48±1.70 (22.00–26.00)	0.180
$F_{persistance}$ (%)	0.64±0.24 (0.40–1.00)	0.70±0.20 (0.40–1.00)	0.646	0.67±0.20 (0.40–1.00)	0.71±0.23 (0.40–1.00)	0.660
NI	1.3±1.2 (0–36)	1.5±0.9 (0.3–3.4)	0.682	1.51±1.09 (0.00–3.61)	1.24±0.85 (0.29–2.54)	0.682
SHI	6.03±5.8 (0–14.5)	5.5±4.04 (0.04–12.5)	0.837	5.83±5.32 (0.00–14.56)	5.52±3.40 (0.27–9.66)	0.999
MRC sum score	52.29±8.88 (36–60)	54.50±7.88 (33–60)	0.592	**51.00±9.08 (33–60)**	**58.29±2.43 (54–60)**	**0.036**
Turner UMN score	7.71±4.1 (3–14)	10.3±3.9 (2–14)	0.261	**7.92±3.87 (2–12)**	**11.71±3.49 (4–14)**	**0.013**
ALSFRS-r score	36.4±4.3 (33–44)	35.7±5.5 (26–44)	0.837	35.42±5.23 (26–44)	36.86±4.85 (30–44)	0.483
Del FR (Hz)	14.4±5.3 (10.1–25.1)	12.4±6.1 (7.6–29.9)	0.261	14.7±6.65 (7.75–29.94)	10.53±2.31 (7.61–13.33)	0.120
ADM FR (Hz)	11.8±5 (7.3–20.5)	11.8±4.1 (7.1–21.1)	0.773	3.43±5.10 (0.14–18.77)	1.62±0.97 (0.69–3.29)	0.837
FDI FR (Hz)	15.7±5.2 (9.4–24.05)	13.6±7.9 (6.9–34.7)	0.285	68.13±35.05 (32.88–151.85)	56.95±14.43 (33.83–74.06)	0.999
Del MCD (Hz)	1.95±1.9 (0.34–5.7)	3.2±5.01 (0.14–18.8)	0.432	11.39±4.20 (7.10–21.12)	12.68±4.73 (8.65–20.48)	0.592
ADM MCD (Hz)	3.44±3.1 (0.74–9.3)	2.2±2.4 (0.04–7.04)	0.340	2.63±2.12 (0.11–7.04)	2.73±3.60 (0.04–9.33)	0.592
FDI MCD (Hz)	1.8±1.4 (0.006–3.5)	3.69±3.6 (0.5–12.4)	0.313	64.79±15.16 (39.04–92.65)	64.04±22.38 (45.49–108.31)	0.592
Del FRV (%)	64.6±30.9 (32.9–126.2)	63.6±29.5 (33.8–151.9)	0.999	15.96±7.89 (6.92–34.74)	11.34±2.74 (8.78–16.38)	0.213
ADM FRV (%)	67.4±23.4 (39.03–108.3)	62.8±14.04 (45.5–92.6)	0.837	3.24±3.75 (0.01–12.35)	2.57±1.54 (0.54–4.13)	0.875
FDI FRV (%)	78.4±31.4 (55.04–147.1)	83.2±44.5 (32.2–155.3)	0.724	**95.88±39.53 (51.65–155.27)**	**52.17±14.08 (32.17–70.56)**	**0.013**
Del amplitude (μV)	1035±309.5 (569–1582)	1142.9±1162 (358–4682)	0.432	1245.42±1130.33 (506–4682)	861.00±361.79 (358–1423)	0.482
Del duration (ms)	11.4±3.18 (6.29–14.95)	12.9±2.72 (5.7–16.6)	0.482	11.77±3.39 (5.67–16.36)	13.35±1.58 (11.51–16.60)	0.650
Del phase #	2.9±0.69 (2–4)	2.9±0.51 (2–4)	0.837	3.00±0.60 (2–4)	2.71±0.49 (2–3)	0.432
Del turn #	3.0±1.29 (1–5)	3.8±2.48 (2–11)	0.650	3.83±2.52 (1–11)	3.00±1.15 (2–5)	0.482
ADM amplitude (μV)	1232.7±1203.4 (245–3422)	1603.4±814.47 (381–2677)	0.384	1168.92±954.03 (245–3422)	1977.57±784.27 (381–2677)	0.100
ADM duration (ms)	14.2±7.78 (7.68–29.98)	11.7±3.21 (5.08–16.06)	0.902	**10.71±3.41 (5.08–17.80)**	**15.99±6.48 (10.43–29.98)**	**0.022***
ADM phase #	3.1±0.90 (2–4)	2.8±0.84 (2–5)	0.432	3.08±0.9 (2–5)	2.71±0.76 (2–4)	0.432
ADM turn #	3.7±1.62 (1–6)	3.5±1.17 (2–6)	0.711	3.50±1.51 (1–6)	3.57±0.98 (2–5)	0.837
FDI amplitude (μV)	1622±1089 (491–3580)	1711.3±600.38 (549–2407)	0.659	1766.83±835.11 (491–3580)	1496.00±747.23 (549–2407)	0.616
FDI duration (ms)	11.7±4.17 (7–17)	12.77±3.30 (7.66–18.21)	0.596	12.25±3.53 (7.04–17.04)	12.55±4.04 (7.66–18.21)	0.999
FDI phase #	2.9±0.69 (2–4)	3.09±1.04 (2–5)	0.724	3.00±1.04 (2–5)	3.00±0.63 (2–4)	0.892
FDI turn #	2.9±1.35 (1–5)	2.64±0.81 (1–4)	0.860	2.83±1.11 (1–5)	2.5±0.84 (1–3)	0.682

*Mann-Whitney U test. ADM: abductor digiti minimi; ALSFRS-r; revised Amyotrophic Lateral Sclerosis Functional Rating Scale; CMAP: compound muscle action potential; CMCT: central motor conduction time; Del: deltoid; F: F-wave; FDI: first dorsal interosseous; MRC: Medical Research Council; NI: neurophysiological index; SHI: split hand index; TST: triple stimulation technique; #: number.

firing of the remaining slow MUs as a sign of compensatory reinnervation (25,26). The increased motor neuronal excitability in the lateral side of hand in ALS has been discussed at both central and peripheral levels (27). The findings of an electrophysiologic study comparing ADM and FDI, were interpreted as an indication of more favorable reinnervation capacity of ADM due to the lower excitability and prolonged survival of its motor neurons (28). Similarly, the increased FR of FDI found in the present study may be explained by a lesser reinnervation capacity of FDI due to increased motor neuronal excitability either at the central or peripheral levels.

The other striking finding of this study is that reduced FRV in FDI was associated with longer CMCT, which was suggested as a good biomarker of UMN dysfunction in ALS (29). This finding is also supported by the negative correlation between FR of FDI and CMCT. The reverse correlation of CMCT with FR and FRV could be explained by the stabilization of membrane potential, less reactive to small inputs and more stable FR as CMCT prolongs due to UMN involvement (9,30–32). In an earlier study, de Carvalho et al. used Multi-MUP analysis to evaluate FR of MUs in six subject groups and showed a lower coefficient of variance of FR in patients with PLS, ALS with spasticity, and UMN involvement due to other causes (9). They similarly concluded that MU firing variability might be useful in assessing UMN versus LMN involvement and it tended to be greater in diseases affecting LMN (9). Later, they described the MCD parameter to identify motor unit firing rate variability and found less MCD in subjects with spinal cord lesions and PLS when compared to ALS and controls. They interpreted the reduced MCD in patients with marked pyramidal signs as being a result of lower threshold for LMN firing with lesser FR variability and proposed the method as a tool to detect UMN involvement (10). However, in our study, MCD values failed to show a significant correlation with the parameters of UMN dysfunction in ALS, probably because our patient group had similar UMN and LMN dysfunction. The lack of a patient control group with pure UMN involvement stands as a limitation of the present study.

The irregularity of MU firing revealed by increased MCD in our group, in parallel with LMN involvement could be explained by the increased spontaneous oscillations of the membrane potential due to synaptic noise and inconstant presynaptic flow (9,33). Similar to our results, Yalinay Dikmen et al. reported higher FRV in patients with motor neuron disorders and discussed this finding as a consequence of both upper and lower motor neuronal excitability changes (34). MUPs of distally located muscles, FDI and ADM, were firing more unsteadily in our study, according to FRV, conforming to the distal-proximal gradient. These findings could be speculated to be caused by increased vulnerability of motor neurons innervating fast twitch muscle fibers (35), because studies on SOD1 mutant mice suggested that distal leg muscles contained higher percentages of fast-twitch muscle fibers and the motor units innervating them degenerated preferentially (2,36). However, fiber composition of different human muscles has not been fully elucidated yet.

Higher MUP amplitudes and longer MUP durations in the patient group were compatible with the chronic denervation and reinnervation process. The higher phase and turn number of Del MUPs in patients with ALS compared to controls and the higher turn number of Del MUPs compared to FDI MUPs in the patients might point to the relatively early reinnervation stage in Del muscle rather than the physiologic properties of deltoid MUPs known to have a polyphasic rate of 25% (37). This probably more immature reinnervation in Del muscle compared to FDI suggested us the temporal profile of denervation which might led the collateral reinnervation to reach at a more mature stage in distally located FDI than the proximal Del.

Central motor conduction time prolongation in ALS is explained by the degeneration of fastest conducting corticospinal fibers (38). On the other hand, TST uses a collision technique and provides a quantitative and reproducible measure of functional motor neurons. Both CMCT and TST were abnormal in nearly two-thirds of our patients with ALS. Unlike the TST amplitude ratio, there were statistically significant correlations between CMCT and CMAP amplitude of the recording muscle, UMN score and FR and FRV of FDI in the patients. This could be related to the methodologic differences between these two TMS techniques, which probably made one of them more efficient to show the effects of UMN dysfunction on FR variability and might be considered from the perspective of recent restoration of the reputation of CMCT in showing the UMN dysfunction, particularly when it is masked by LMN involvement (29).

The dissociated muscle weakness and wasting in ALS have been documented as different split muscle phenotypes since 'the split hand' phenomenon was originally reported (4,17,39–43). The split hand phenomenon is accepted as a good clinical biomarker with the aid of neurophysiologic verification studies (5,17,44,45). Four main hypotheses have been proposed to explain its pathophysiological origins (46); 1: cortical dysfunction/hyperexcitability (47–52), 2: abnormal spinal circuitry/LMN dysfunction (5,53), 3: LMN hyperexcitability/axonal membrane channel dysfunction (27), and 4: motor endplate dysfunction (46,54). In the present study, SHI was abnormal in less than half of the patients, which could be explained by the MRC and CMAP amplitude criteria used for inclusion. First dorsal interosseous was the representative muscle of lateral side of the hand in the present study and is expected to be affected preferentially in this context. From the standpoint of MU FR and recruitment, the split hand phenomenon was reflected electrophysiologically by the higher FDI FR and MCD values in the whole group of patients, and by the decreased FDI FRV in 12 patients with prolonged CMCT. Moreover, the FR of FDI was higher in patients with abnormally low SHI, pointing out the predominating LMN involvement in this muscle. No such difference could be shown between the MEP and TST findings of the patients with abnormally low and normal SHI.

There are several limitations of the present study. First, the more clinically affected side was chosen to observe the distal-proximal gradients better, taking the possible risk of masking UMN involvement with LMN predominance. Second, it would be more conclusive to study TST and CMCT in each recording muscle in the study. However, to apply these methods to proximal muscles is technically very difficult. Third, there is no follow up recordings to investigate the evolution of FR parameters.

In conclusion, distal-proximal and lateral-medial gradients of muscle involvement reflected by the firing rate variability parameters suggested that the LMN dysfunction keeps track of preferential muscle involvement in ALS. In relation to those gradients, some indication for UMN dysfunction like reduced FRV was also revealed by CMCT. Although the majority of the results pointed to the predominant LMN involvement as the primary pathology underneath them, the question 'does LMN dysfunction hinder the clues of UMN involvement, because of recording from the already diseased end organs, remains unanswered.

Ethics Committee Approval: The protocol was approved by İstanbul University İstanbul Faculty of Medicine Clinical Research Ethics Committee (22.11.2019/1366).

Informed Consent: Written informed consent was provided by all subjects.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept- SAB, NGS, EKO, MBB, AEÖ; Design- SSAB, NGS, EKO, MBB, AEÖ; Supervision- SAB, NGS, EKO, MBB, AEÖ; Resource- SAB, NGS, EKO, MBB, AEÖ; Materials- SAB, NGS, EKO, MBB, AEÖ; Data Collection and/or Processing- SAB, NGS, EKO, MBB, AEÖ; Analysis and/or Interpretation- SAB, NGS, EKO, MBB, AEÖ; Literature Search- SAB, NGS, EKO, MBB, AEÖ; Writing- SAB, NGS, EKO, MBB, AEÖ; Critical Reviews- SAB, NGS, EKO, MBB, AEÖ.

Conflict of Interest: The authors declared that there is no conflict of interest.

Financial Disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

SUPPLEMENTARY

https://www.noropsikiyatriarsivi.com/uploads/NPA_28864_EN_SUPPLEMENTARY.pdf

REFERENCES

1. Babu S, Pioro EP, Li J, Li Y. Optimizing muscle selection for electromyography in amyotrophic lateral sclerosis. Muscle Nerve. 2017;56(1):36–44. [Crossref]
2. Shayya L, Babu S, Pioro EP, Li J, Li Y. Distal predominance of electrodiagnostic abnormalities in early-stage amyotrophic lateral sclerosis. Muscle Nerve. 2018;58(3):389–395. [Crossref]
3. de Carvalho MA, Pinto S, Swash M. Paraspinal and limb motor neuron involvement within homologous spinal segments in ALS. Clin Neurophysiol. 2008;119(7):1607–1613. [Crossref]
4. Wilbourn AJ. Dissociation of muscle wasting of ulnar innervated and muscles withmotor neuron disease. Third International Symposium on ALS/MND, November 1992, Solihull, UK
5. Kuwabara S, Mizobuchi K, Ogawara K, Hattori T. Dissociated small hand muscle involvement in amyotrophic lateral sclerosis detected by motor unit number estimates. Muscle Nerve. 1999;22(7):870–873. [Crossref]
6. Adrian ED, Bronk DW. The discharge of impulses in motor nerve fibres: Part II. The frequency of discharge in reflex and voluntary contractions. J Physiol. 1929;67(2):i3–151. [Crossref]
7. Henneman E, Somjen G, Carpenter DO. Functional significance of cell size in spinal motoneurons. J Neurophysiol. 1965;28(3):560–580. [Crossref]
8. Grimby L, Hannerz J. Recruitment order of motor units on voluntary contraction: changes induced by proprioceptive afferent activity. J Neurol Neurosurg Psychiatry. 1968;31(6):565. [Crossref]
9. de Carvalho M, Turkman A, Swash M. Motor unit firing in amyotrophic lateral sclerosis and other upper and lower motor neurone disorders. Clin Neurophysiol. 2012;123(11):2312–2318. [Crossref]
10. de Carvalho M, Poliakov A, Tavares C, Swash M. Interplay of upper and lower motor neuron degeneration in amyotrophic lateral sclerosis. Clin Neurophysiol. 2017;128(11):2200–2204. [Crossref]
11. de Carvalho M, Dengler R, Eisen A, England JD, Kaji R, Kimura J, et al. Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol. 2008;119(3):497–503. [Crossref]
12. Kleyweg RP, van der Meche FG, Schmitz PI. Interobserver agreement in the assessment of muscle strength and functional abilities in Guillain-Barre syndrome. Muscle Nerve. 1991;14(11):1103–1109. [Crossref]
13. Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, et al. The ALSFRS-R. a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci. 1999;169(1-2):13–21. [Crossref]
14. Koç F, Balal M, Demir T, Alparslan ZN, Sarica Y. Adaptation to Turkish and reliability study of the revised amyotrophic lateral sclerosis functional rating scale (ALSFRS-R). Noro Psikiyatr Ars. 2016;53(3):229–233. [Crossref]
15. Turner M, Cagnin A, Turkheimer F, Miller C, Shaw C, Brooks D, et al. Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [11C](R)-PK11195 positron emission tomography study. Neurobiol Dis. 2004;15(3):601–609. [Crossref]
16. Drory VE, Kovach I, Groozman GB. Electrophysiologic evaluation of upper motor neuron involvement in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2001;2(3):147–152. [Crossref]
17. Menon P, Kiernan MC, Yiannikas C, Stroud J, Vucic S. Split-hand index for the diagnosis of amyotrophic lateral sclerosis. Clin Neurophysiol. 2013;124(2):410–416. [Crossref]
18. Swash M, de Carvalho M. The neurophysiological index in ALS. Amyotroph Lateral Scler Other Motor Neuron Disord. 2004;5 Suppl 1:108–110. [Crossref]
19. Stalberg E, Falck B, Sonoo M, Stalberg S, Astrom M. Multi-MUP EMG analysis -a two year experience in daily clinical work. Electroencephalogr Clin Neurophysiol. 1995;97(3):145–154. [Crossref]
20. Stalberg EV, Nogues MA. Automatic analysis of heart rate variation: I. Method and reference values in healthy controls. Muscle Nerve. 1989;12(12):993–1000. [Crossref]
21. Oguz Akarsu E, Sirin NG, Kocasoy Orhan E, Erbas B, Dede HO, Baslo MB, et al. Repeater F-waves in amyotrophic lateral sclerosis: electrophysiologic indicators of upper or lower motor neuron involvement? Clin Neurophysiol. 2020;131(1):96–105. [Crossref]
22. Rossini PM, Berardelli A, Deuschl G, Hallett M, Maertens de Noordhout AM, Paulus W, et al. Applications of magnetic cortical stimulation. The International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol Suppl. 1999;52:171–185. PMID: 10590986.
23. Magistris MR, Rosler KM, Truffert A, Myers JP. Transcranial stimulation excites virtually all motor neurons supplying the target muscle. A demonstration and a method improving the study of motor evoked potentials. Brain. 1998;121(Pt 3):437–450. [Crossref]
24. Magistris MR, Rosler KM, Truffert A, Landis T, Hess CW. A clinical study of motor evoked potentials using a triple stimulation technique. Brain. 1999;122(Pt 2):265–279. [Crossref]
25. Noto YI, Kitaoji T, Watanabe K, Mizuno T. Assessment of motor unit firing by high-density surface electromyography detects motor neuronal hyperexcitable state in amyotrophic lateral sclerosis. Muscle Nerve. 2023;68(2):149–156. [Crossref]
26. Kiernan MC, Park SB. Hyperexcitability, neurodegeneration, and disease progression in amyotrophic lateral sclerosis. Muscle Nerve. 2023;68(2):103–105. [Crossref]
27. Shibuya K, Misawa S, Nasu S, Sekiguchi Y, Mitsuma S, Beppu M, et al. Split hand syndrome in amyotrophic lateral sclerosis: different excitability changes in the thenar and hypothenar motor axons. J Neurol Neurosurg Psychiatry. 2013;84(9):969–972. [Crossref]
28. Cengiz B, Mercan M, Kuruoglu R. Spinal excitability changes do not influence the mechanisms of split-hand syndrome in amyotrophic lateral sclerosis. Muscle Nerve. 2018;58(4):503–508. 3 [Crossref]
29. Tokimura R, Murakami T, Ugawa Y. Central motor conduction time reveals upper motor neuron involvement masked by lower motor neuron impairment in a significant portion of patients with amyotrophic lateral sclerosis. Clin Neurophysiol. 2020;131(8):1896–1901. [Crossref]
30. Lee RH, Heckman CJ. Bistability in spinal motoneurons in vivo: systematic variations in rhythmic firing patterns. J Neurophysiol. 1998;80(2):572–582. [Crossref]
31. de Carvalho M, Eisen A, Krieger C, Swash M. Motoneuron firing in amyotrophic lateral sclerosis (ALS). Front Hum Neurosci. 2014;8:719. [Crossref]
32. Rosenfalck A, Andreassen S. Impaired regulation of force and firing pattern of single motor units in patients with spasticity. J Neurol Neurosurg Psychiatry. 1980;43(10):907–916. [Crossref]
33. Stalberg E, Thiele B, Hilton-Brown P. Effect of succinylcholine on single motor end-plates in man. Acta Anaesthesiol Scand. 1973;17(2):108–118. [Crossref]
34. Yalinay Dikmen P, Kocasoy Orhan E, Baslo MB. Analysis of motor unit firing characteristics in patients with motor neuron diseases. Somatosens Mot Res. 2016;33(1):16–19. [Crossref]
35. Piotrkiewicz M, Hausmanowa-Petrusewicz I. Motoneuron afterhyperpolarisation duration in amyotrophic lateral sclerosis. J Physiol. 2011;589(Pt 11):2745–2754. [Crossref]
36. Hegedus J, Putman CT, Tyreman N, Gordon T. Preferential motor unit loss in the SOD1 G93A transgenic mouse model of amyotrophic lateral sclerosis. J Physiol. 2008;586(14):3337–3351. [Crossref]
37. Preston DC, Logigian EL. Positive paraneoplastic panels: probabilities, perils, and pearls. Muscle Nerve. 2022;65(5):489–491. [Crossref]
38. Lemon RN. The cortical "upper motoneuron" in health and disease. Brain Sci. 2021;11(5):619. [Crossref]
39. Simon NG, Lee M, Bae JS, Mioshi E, Lin CS, Pfluger CM, et al. Dissociated lower limb muscle involvement in amyotrophic lateral sclerosis. J Neurol. 2015;262(6):1424–1432. [Crossref]
40. Wang ZL, Cui L, Liu M, Zhang K, Liu S, Ding Q, et al. Reassessment of split-leg signs in amyotrophic lateral sclerosis: differential involvement of the extensor digitorum brevis and abductor hallucis muscles. Front Neurol. 2019;10:565. [Crossref]
41. Khalaf R, Martin S, Ellis C, Burman R, Sreedharan J, Shaw C, et al. Relative preservation of triceps over biceps strength in upper limb-onset ALS. the 'split elbow'. J Neurol Neurosurg Psychiatry. 2019;90(7):730–733. [Crossref]

42. Sonoo M, Takahashi K, Hamada Y, Hokkoku K, Kobayashi S. Split-finger syndrome in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2020;91(11):1235–1236. [Crossref]
43. Hamada Y, Kanbayashi T, Takahashi K, Kamiya H, Kobayashi S, Sonoo M. Weak shoulder and arm sparing signs in amyotrophic lateral sclerosis. Muscle Nerve. 2022;65(3):311–316. [Crossref]
44. Kuwabara S, Sonoo M, Komori T, Shimizu T, Hirashima F, Inaba A, et al. Dissociated small hand muscle atrophy in amyotrophic lateral sclerosis: frequency, extent, and specificity. Muscle Nerve. 2008;37(4):426–430. [Crossref]
45. Henderson RD, Eisen A. ALS split phenotypes -to what extent do they exist? Clin Neurophysiol. 2020;131(4):847–849. [Crossref]
46. Corcia P, Bede P, Pradat PF, Couratier P, Vucic S, de Carvalho M. Split-hand and split-limb phenomena in amyotrophic lateral sclerosis: pathophysiology, electrophysiology and clinical manifestations. J Neurol Neurosurg Psychiatry. 2021;92(10):1126–1130. [Crossref]
47. Eisen A, Bede P. The strength of corticomotoneuronal drive underlies ALS split phenotypes and reflects early upper motor neuron dysfunction. Brain Behav. 2021;11(12):e2403. [Crossref]
48. Eisen A, Braak H, Del Tredici K, Lemon R, Ludolph AC, Kiernan MC. Cortical influences drive amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2017;88(11):917–924. [Crossref]
49. Ludolph AC, Emilian S, Dreyhaupt J, Rosenbohm A, Kraskov A, Lemon RN, et al. Pattern of paresis in ALS is consistent with the physiology of the corticomotoneuronal projections to different muscle groups. J Neurol Neurosurg Psychiatry. 2020;91(9):991–998. [Crossref]
50. Weber M, Eisen A, Stewart H, Hirota N. The split hand in ALS has a cortical basis. J Neurol Sci. 2000;180(1-2):66–70. [Crossref]
51. Menon P, Kiernan MC, Vucic S. Cortical dysfunction underlies the development of the split-hand in amyotrophic lateral sclerosis. PLoS One. 2014;9(1):e87124. [Crossref]
52. Bae JS, Menon P, Mioshi E, Kiernan MC, Vucic S. Cortical hyperexcitability and the split-hand plus phenomenon: pathophysiological insights in ALS. Amyotroph Lateral Scler Frontotemporal Degener. 2014;15(3-4):250–256. [Crossref]
53. Sirin NG, Oguz Akarsu E, Kocasoy Orhan E, Erbas B, Artug T, Dede HO, et al. Parameters derived from compound muscle action potential scan for discriminating amyotrophic lateral sclerosis-related denervation. Muscle Nerve. 2019;60(4):400–408. [Crossref]
54. de Carvalho M, Swash M. The split hand in amyotrophic lateral sclerosis: a possible role for the neuromuscular junction. Amyotroph Lateral Scler Frontotemporal Degener. 2019;20(5-6):368–375. [Crossref]