Clinical Neurophysiology 130 (2019) 1954–1961

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

F Wave Analyzer, a system for repeater F-waves detection: Application in patients with amyotrophic lateral sclerosis

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ARTICLE INFO

Article history:
Accepted 12 August 2019
Available online 16 August 2019

Keywords:
Repeater F-waves
F-wave amplitude
F-wave persistence
Amyotrophic lateral sclerosis
Motor neurons
Automatic F Wave Analyzer

HIGHLIGHTS

- Repeater F-waves frequency is higher in ALS compared to healthy subjects.
- Latency and amplitude values of repeater vs non repeater F-waves are similar in the same recordings.
- F Wave Analyzer provides a fast, effortless and accurate estimation of repeater F-waves.

ABSTRACT

Objectives: We assessed the clinical usefulness of repeater F-waves (Freps) analysis in amyotrophic lateral sclerosis (ALS), using an automated computerized system (F Wave Analyzer).
Methods: Forty consecutive F-waves were recorded from the ulnar and peroneal nerve in 52 patients with ALS and 52 healthy control subjects. Data were imported into the F Wave Analyzer which identifies Freps and groups them. Parameters of Freps and non repeater F-waves (Fnonreps) were compared.
Results: Total number of repeating neurons, Freps persistence (100xFreps/40stimuli) and Index Total Freps (100xFreps/total number of F-waves) were significantly higher in the ALS compared to the control group ($P \leq 0.005$). There were no consistent differences of F-wave latency or amplitude measurements between Freps and Fnonreps for both studied groups, with the exception of prolonged Freps minimum latency in ALS.
Conclusion: In ALS, the high numbers of Freps, reduced overall F-wave persistence and increased F-wave amplitude measurements in a relatively unaffected nerve-muscle complex reflects excitability alterations of the corresponding motor neuron pool. Overall, automatic analysis facilitates accurate and fast detection of Freps and could be useful in other clinical settings.
Significance: Analysis of repeater F-waves is expected to provide new insight regarding ALS pathophysiology and utilized for monitoring in clinical drug trials.

1. Introduction

F-waves are late responses produced by backfiring of antidromically activated motor neurons, following peripheral electrical stimulation of motor fibers. F-wave latencies have been proven valuable as an index of conduction properties of motor neurons and are commonly assessed in routine electrophysiology studies (Panayiotopoulos and Chroni, 1996). Other F-wave parameters, such as persistence (percent of F-waves in a series of stimuli) or amplitude, are less frequently studied although they could provide additional information regarding the ability of individual motor neurons to generate F-waves (Mesrati and Vecchierini, 2004; Fisher, 2007). Notably, findings of decreased persistence and high

Abbreviations: ADM, abductor digiti minimi; ALS, amyotrophic lateral sclerosis; CMAP, compound muscle action potential; CMAP amp, negative amplitude of the CMAP; CMAP lat, distal motor latency of the CMAP; EDB, extensor digitorum brevis; FCD, F chronodispersion; Fnonreps, non repeater F-waves; F nonreps persistence, percent of Fnonrepeaters in a series of 40 stimuli; F persistence, persistence of F-waves, percent of traces with any F-waves in a series of 40 stimuli; Freps, repeater F waves; Freps persistence, percent of total Freps in a series of 40 stimuli; F-wave max amp, maximum peak-to-peak amplitude of the highest F-wave in a series of 40 stimuli; Famp max/M%, 100 × F-wave max amp/CMAP amp; F-wave mean lat, mean latency of F-waves in a series of 40 stimuli minus CMAP lat; Famp mean/M%, 100 × F-wave mean amp/CMAP amp; Index Total Freps, 100 × total number of F-wave repeaters/total number of traces with F-waves in the same nerve; MRC, Medical Research Council scores; RN, repeating neuron; Total Freps, total F-wave repeaters.

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https://doi.org/10.1016/j.clinph.2019.08.002
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amplitude F-waves, occasionally giant F-waves, have been reported in patients with amyotrophic lateral sclerosis (ALS) (Felice, 1998), probably reflecting alterations of lower motor neuron excitability and/or structural changes of the corresponding motor units. Another interesting parameter is F waveform similarities; normally, in a series of 20–40 consecutive stimuli, the elicited F-waves vary in latency and mainly in configuration. This inherent variability is explained by the estimated minimal possibility of each neuron within the population to backfire effectively after a single stimulus in order to generate an F response (Schiller and Stålberg, 1978). Repeater F-waves (Freps) i.e. F-waves identical in shape, latency and amplitude are considered to be produced by single neurons, so called repeated neurons (RNs), and rarely seen in healthy nerve recordings. In a series of consecutive traces, the loss of F waveform variability, denoted by repetition of identical F-waves, suggests that they are preferentially generated by some compared to other neurons. An increased frequency of Freps has been shown in patients with motor neuron or nerve dysfunction of various etiologies (Chroni et al., 2012). Previous studies have suggested the estimate of Freps as a sensitive electrodiagnositc finding of a neuropathic process (Macleod, 1987; Pastore-Olmedo et al., 2009).

To date, identification of Freps remains a time-consuming test applied mainly for research purposes. A specially designed computer program for automated identification of repeater F-waves has been developed in our laboratory aiming to facilitate and standardize the procedure in order to become suitable for clinical practice. We herein used this program, called F Wave Analyzer, to study Freps and their parameters in nerves of patients diagnosed with ALS compared to healthy subjects.

2. Methods

2.1. Participants

Fifty-two patients with amyotrophic lateral sclerosis (ALS; 33 males, mean age 64.1 ± 10.2 years, mean height 172.3 ± 8.1 cm) and 52, gender-, age- and height-matched, healthy subjects (33 males, mean age 63.8 ± 11.9 years, mean height 172.5 ± 8.1 cm) who served as controls, participated in this study. Patients were clinically examined, Medical Research Council (MRC) scores and signs of upper and lower motor neuron involvement recorded. All patients had a diagnosis of clinically definite ALS based on clinical or electrophysiological criteria of the revised El Escorial (Brooks et al., 2000) and Awaji criteria (Costa et al., 2012). Controls were part of a pool of healthy subjects who had responded to advertising for volunteers, in order to collect normative data for nerve conduction studies, and none had symptoms of neuromuscular disease or a medical condition known to cause peripheral neuropathy, such as diabetes mellitus, alcohol or drug abuse. All participates gave written informed consent and the study was approved by the Research and Ethics Committee of Patras University Hospital (no. of approval 434, 11.12.2013).

2.2. Methods

For all studies, a two-channel Keypoint ver. 3.25 electromyographic device (Medtronic-Dantec Electronics, Skovlunde, Denmark) was used. Standard motor conduction and F wave studies of the ulnar and peroneal nerve were performed on the left side for all healthy subjects, and on the side of weaker muscle strength or on the left side if normal or similar strength for the patient group. The amplitudes of the CMAPs of the ulnar and peroneal nerve had to be $\geq$1 mV and $\geq$0.5 mV respectively. Patients with absent F-waves in either nerve were excluded. Firstly, the ulnar nerve was examined and subjects were instructed to remain relaxed placing the upper limb on an arm board. During the recording the skin temperature was maintained at >31 °C. A cathodal stimulation with supramaximal intensity was applied at the wrist and elbow level and the compound muscle action potential (CMAP) was recorded using a bipolar surface electrode, with a fixed 30 mm interelectrode distance, placed over the motor point of the abductor digiti minimi muscle (ADM). F-waves were elicited by a series of 40 consecutive stimuli (pulse frequency 1 Hz and duration 0.1 ms) delivered at the wrist. A similar procedure was followed for the study of the peroneal nerve. The examined muscle remained relaxed and skin temperature of the foot was maintained at >29 °C, throughout the procedure. The recording electrode was placed over the extensor digitorum brevis muscle (EDB) and CMAP responses were recorded following supramaximal stimulation at the ankle and knee. Forty consecutive stimuli were applied to the peroneal nerve at the ankle, at a frequency of 1 Hz and the evoked F-waves were recorded.

2.3. Data processing

The recorded myoelectric data was transferred and imported into the F Wave Analyzer for further processing. This program is installed in our electromyographic based personal computer and its detailed properties have been previously described (Chroni et al., 2017). Briefly, this software comprises multiple features and functionality for inspection of myoelectric recordings, identification of F-wave locations, grouping of repeaters and calculation of their parameters. It also gives the option to manually reset F-wave markers. The algorithm for wave detection consisted of the following stages. Frequency domain preprocessing is initially applied, in order to retain the signal frequencies that fall into the spectrum of an F-wave. High-frequency noise was eliminated from raw signal using a moving average filter with window size of 300 μs. The derivative was then refiltered and smoothed, to remove additional spikes probably caused by outliers. Afterwards, locations of F-waves are indentified based on the rate of signal change; when the derivate was below the preset threshold of 14.4 μV/ms the signal had a zero value, otherwise the value was 1. Any drop in the derivative below threshold for a less than 3 ms (arbitrary selected duration) was considered insignificant and ignored. Onset of the wave is the first non zero value and termination the first zero value obtained after that.

Repeater groups are now formed by pairwise comparison of all F-waves. The following quantities are computed for every pair of F-waves that collectively give a similarity index that is used to introduce the necessary tolerance. (a) The ratio of the absolute difference of the two potentials' area to that of the first potential's should be below 0.1. (b) Onset, termination and all intermediate peaks of the waveforms had to match in time with maximum tolerance of 0.05 ms, according to the estimated jitter of F-waves from a single motor axon (Schiller and Stålberg, 1978). (c) Difference in amplitudes should not exceed 20 μV, a value which we defined as the smallest recognizable F-wave. (d) A possible transient change of the potential direction should not exceed 2% of the total amplitude. After matching check of all forty traces, F-waves appearing more than once were categorized into different colored groups (Fig. 1). Finally, the correct recognition of all RN groups by the F Wave Analyzer was visually verified by the first author. Identical late responses in $\geq$16 of 40 traces with the same latency were considered A waves (Puksa et al., 2003) and therefore excluded from F-wave measurements.

2.4. Definition of variables

(1) CMAP distal latency and amplitude from baseline-to-negative peak. (2) F-wave minimum (min) and maximum (max)

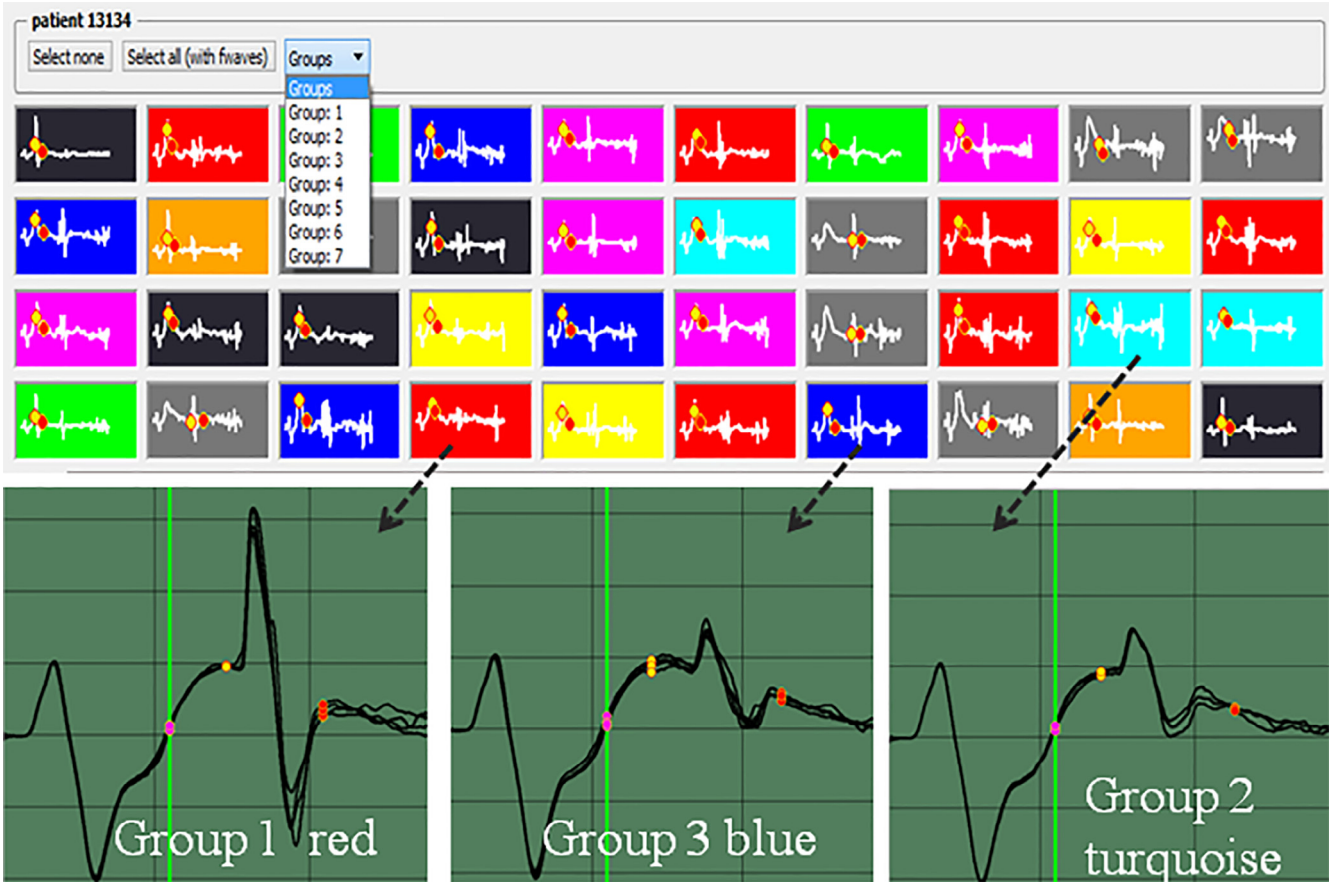


Fig. 1. F wave recordings of the ulnar nerve in a 66 year-old female patient with lower limb onset ALS. CMAP parameters are within normal limits and MRC score of the examined left ADM muscle is 5. Every one of the 40 traces is displayed in the upper screen in different colored boxes; grey boxes indicate traces with absent F-waves, black include Fnonreps and each of the other colored boxed refers to a group of Freps. Identical F-waves are depicted in the same color. In the lower screen three groups of repeater F-waves are magnified. A total of 23 Freps, grouped into 7 RNs, are recognized (Freps persistence: 57.5%, Index total Freps: 74%).

values of latency, F chronodispersion (FCD), peak-to-peak maximum (max) and mean amplitude; these parameters were estimated for each nerve in a series of 40 traces separately for RNs and F-waves that appeared just once (Fnonreps). F-wave amplitude estimates were also expressed as a percentage of CMAP amplitude (Famp max/M % and Famp mean/M %). (3) Persistence of F-wave (F persistence), percent of traces with a least 1 F-wave in a series of 40 stimuli. 4. Repeater F-wave frequency variables as described previously (Chroni et al., 2012), were the following: (1) RNs, F waveforms that appear identical at least twice in a series of 40 stimuli; (2) Total Freps, all RNs and their repetitions; (3) Persistence of Freps (Freps persistence), percent of total Freps in a series of 40 stimuli. (4) Index total Freps, 100 × total number of Freps/total number of traces with F-waves. If more than one F-wave were elicited in a trace, they all counted for Freps persistence estimations.

2.5. Statistics

Analysis was performed using IBM SPSS version 24 for Windows (IBM SPSS Inc., Chicago, Illonois, USA). Descriptive variables were generated for all variables. For the analysis of F-wave parameters, each RN was included once, irrespective of the number of repetitions, in order to avoid bias in favor of repeater F-waves. Data for most variables, despite transformation, did not satisfy normality criteria according the Kolmogorov-Smirnov test and therefore, the nonparametric Mann-Whitney test for independent samples and Wilcoxon test for dependent samples were used. Correlation was assessed using the Pearsons correlation coefficient. The significance level was set at $P < 0.05$.

3. Results

3.1. Neurological examination findings of the patients

Disease duration at the time of examination ranged between 6–15 months (mean 10 ± 5.3 months). The presenting symptom was muscle weakness of the upper limb in 21 subjects, the lower limb in 19 and bulbar region in 12. Signs of upper motor neuron involvement in the examined limb were present in all patients, confirmed by presence of brisk deep tendon reflexes, Modified Ashworth Scale 0–1+, and/or a Babinski sign, although all had a predominant lower motor neuron syndrome. MRC score in the ADM muscle was 5 in 23 patients, 4 in 17, 3 in 8 and 2 in 4. EDB muscle strength had a MRC score of 5 in 26 patients, 4 in 17, 3 in 6 and 2 in 3.

3.2. CMAP parameters

There were statistical significant differences in CMAP measurements between the two study groups in both examined nerves: in patients' vs healthy group, mean ± SD CMAP amplitude for ulnar was 4.4 ± 2.3 vs 5.5 ± 1.1 mV and for peroneal 2.1 ± 1.2 vs 3.0 ± 1.0 mV. CMAP latency for the ulnar was 3.1 ± 0.5 vs 2.7 ± 0.4 ms and for peroneal 4.7 ± 0.8 vs 4.0 ± 0.6 ms respectively

(all P values ≤ 0.001). It should be noted, however, that the vast majority of the patients had values within the normal range of our laboratory.

3.3. F-wave findings

According to our exclusion criteria, a total of 17 nerves were excluded from further analysis due to absence of F-waves. In 3 patients no F-wave recordings were obtained from either both ulnar or both peroneal nerves and they did not participate in this study. Whereas, 11 of the ALS participants had one nerve without F-waves and studies were thereby performed on the less affected side. Mean amplitude of the CMAP in the excluded nerves were 0.7 mV for the ulnar and 0.5 mV for the peroneal nerve. All recordings were satisfactory, free of artifacts and forwarded for automated analysis. Measurements of the F Wave Analyzer were extracted into an excel file. An example of an F-waves typical analysis is illustrated in Fig. 1.

3.3.1. Persistence of F waves

The frequency-related statistical findings for all F waves and Freps are presented in Table 1. Freps occurred more frequently in the peroneal nerve than the ulnar in both groups. RNs with more than 5 repetitions at the ulnar nerve was detected in 32 patients (vs 0 in healthy) and at the peroneal in 39 (vs 8 in healthy). In a series of 40 stimuli, 4 or more different RNs were detected in 28 ulnar and 30 peroneal nerves from ALS group as opposed to 2 ulnar and 13 peroneal from the healthy group.

3.3.2. F-waves parameters

Latency min and max measurements in all F-waves, repeaters and non repeaters, for both nerves were significantly prolonged in the patient compared to the healthy group ($P < 0.001$); FCD of the ulnar was significantly higher in the patients ($P < 0.001$). Famp mean/M % and Famp max/M %, in both nerves were significantly higher in the ALS group (data not shown, all P-values ≤ 0.01).

3.3.3. Comparisons between Freps and Fnonreps parameters

Intra-group: Table 2 shows the results of Freps vs Fnonreps comparisons within each group separately. Statistical inconsistencies of amplitude values between Freps and Fnonreps were demonstrated for both nerves and groups. Min latency values of Freps were significantly longer in both studied nerves and groups, while the max latency values of Freps were shorter in the healthy but tended to be similar to Fnonreps in the patient group.

Inter-group: Famp max/M and F amp mean/M % values of Freps were significantly higher in patients compared to controls for the ulnar nerve ($P < 0.001$), but not for the peroneal nerve ($P \geq 0.135$). Freps latency min was significantly prolonged in the patient's ulnar and peroneal nerve as opposed to the healthy ($P < 0.03$ and $P < 0.07$ respectively).

Table 1
Repeater F-wave frequency in 52 healthy subjects and 52 ALS patients.

	Patients	Healthy	*P* value
Ulnar nerve			
F-wave persistence† (%)	78.13 ± 25.76	98.65 ± 2.00	<0.001
Freps persistence† (%)	37.07 ± 15.95	6.73 ± 6.67	<0.001
Index total Freps† (%)	53.15 ± 25.82	6.81 ± 6.73	<0.001
Nerves with Frep*	52 (100%)	34 (65.4%)	N/A#
RN's†	4.44 ± 2.12	1.27 ± 1.22	<0.001
Total Freps†	15.19 ± 6.77	2.73 ± 2.64	<0.001
RNs ≥ 5 repetitions†	0.94 ± 0.93	0	N/A#
Peroneal nerve			
F-wave persistence† (%)	55.53 ± 25.51	80.87 ± 15.95	<0.001
Freps persistence† (%)	39.38 ± 18.12	17.84 ± 12.74	<0.001
Index total Freps† (%)	75.02 ± 20.54	23.58 ± 18.37	<0.001
Nerves with Frep*	52 (100%)	46 (88.5%)	N/A#
RN's†	4.10 ± 2.50	2.77 ± 1.77	0.005
Total Freps†	16.56 ± 8.79	7.23 ± 5.19	<0.001
RNs ≥ 5 repetitions†	1.30 ± 1.29	0.20 ± 0.40	<0.001

Freps persistence: percent of total Freps in a series of 40 stimuli; Index total Freps: 100 × total number of Freps/total number of traces with F-waves; RNs: F waveforms that appear identical at least twice in a series of 40 stimuli; Total Freps: all RNs and their repetitions.
* Variables expressed as number.
† Variables expressed as mean values ± SD.
# Not applicable.

3.3.4. Correlations of F-wave findings

The possible relations of Freps parameters with those of Fnonreps and CMAP parameters were examined and the meaningful results were as follows:

1. The values of Famp mean/M % for Freps and Fnonreps had a positive correlation in both studied nerves in the control group, but not in the patient group (Fig. 2).
2. Index total Freps had a strong negative correlation with CMAP amplitude, used as a measurement of the weakness of the examined muscle, for both nerves in the patient group (Fig. 3).
3. A negative correlation was found between F persistence and Index total Freps in the ulnar and peroneal nerve of the patients, but not in the healthy (the latter is not depicted). (Fig. 4).

4. Discussion

Several experimental studies have found that the occurrence of repeater F-waves increased in neurogenic conditions (Peioglou-Harmoussi et al.,1987; Chroni et al., 2012; Fang et al., 2015). The usefulness of this information has not been fully appreciated in routine studies due to the impractical methods used so far for the repeaters' detection. This report presented a software program, named F Wave Analyzer, for identification of repeater F-waves and measurement of their parameters. Its main advantage is the fast processing of data, which requires approximately 5 minutes, making it suitable for application in a clinical setting. It also provides objective measurements, which are based on predefined criteria, common for all recordings, instead of determining identicality on an individual basis. Finally, the particular lay-out of the F-wave data divided into separate bins, and the optional corrections by the examiner are additional convenient features. For all these reasons, the analyzer resulted in prevention of: a. underestimation of repeaters' occurrence when some comparisons have been neglected, even with the superimposition of F-waves which is available in modern EMG apparatus and b. overestimation when minor differences in F-waves shape have been ignored. Other research groups have also expressed the need for computer aided detection of Freps (Pastore-Olmedo et al., 2009; Hachisuka et al., 2015). Very recently, another research group presented their own automatic analysis program for repeater F-waves recognition, and employed it to calculate motor unit number estimation values in 10 ALS and healthy participants. Although their results were not comparable to ours, these authors also commented on the significance of the software application for this purpose (Artug et al., 2019).

Our findings have clearly shown that all repeater F-wave indices were increased in ALS patients as opposed to healthy subjects. Table 3 summarizes the findings of previous studies, all of which employed manual methods for repeater F-waves frequency estimations in the ulnar nerve. We acknowledge that direct comparison is not applicable between the different populations in these studies, however the following methodological factors could account for deviations of their results: (a) The lowest amplitude limit of a potential to be identified as F-wave; this is usually set

Table 2
Parameters of repeater (Freps) and non repeater (Fnonreps) F-waves in 52 healthy subjects and 52 ALS patients.

	Patients			Healthy		
	Freps	Fnonreps	*P* value	Freps	Fnonreps	*P* value
A. Ulnar nerve						
Flat min (ms)	29.2 ± 3.1	28.9 ± 4.1	0.005	27.8 ± 2.7	25.4 ± 2.2	<0.001
Flat max (ms)	33.5 ± 5.9	33.5 ± 4.4	0.235	28.7 ± 2.6	29.3 ± 2.4	<0.001
FCD (ms)	4.3 ± 4.6	5.0 ± 2.5	0.005	1.5 ± 1.2	3.9 ± 0.1	<0.001
Famp max (μV)	684 ± 407	761 ± 624	0.539	467 ± 272	634 ± 275	<0.001
Famp mean(μV)	404 ± 280	275 ± 248	<0.001	384 ± 230	228 ± 96	<0.001
Famp max /M (%)	18.5 ± 13.8	18.7 ± 16.6	0.834	9.0 ± 5.6	11.6 ± 4.9	<0.001
Famp mean/M (%)	10.7 ± 7.8	7.8 ± 11.2	<0.001	7.4 ± 5.0	4.2 ± 1.9	<0.001
B. Peroneal nerve						
Flat min (ms)	52.4 ± 7.0	50.3 ± 7.0	0.01	47.7 ± 5.1	45.4 ± 5.1	<0.001
Flat max (ms)	58.4 ± 8.9	57.5 ± 8.1	0.472	50.4 ± 5.9	52.6 ± 5.8	<0.001
FCD (ms)	6.9 ± 6.2	7.9 ± 4.7	0.415	3.1 ± 2.5	7.2 ± 2.0	<0.001
Famp max (μV)	548 ± 510	440 ± 300	0.990	344 ± 365	497 ± 325	<0.001
Famp mean(μV)	301 ± 227	214 ± 157	0.171	208 ± 147	179 ± 125	0.100
Famp max /M (%)	30.1 ± 27.3	22.8 ± 16.4	0.885	12.4 ± 12.3	17.4 ± 11.3	<0.001
Famp mean/M (%)	17.9 ± 18.6	12.2 ± 12.1	0.272	7.5 ± 5.2	6.3 ± 4.7	0.107

All variables are expressed as mean values ± SD.

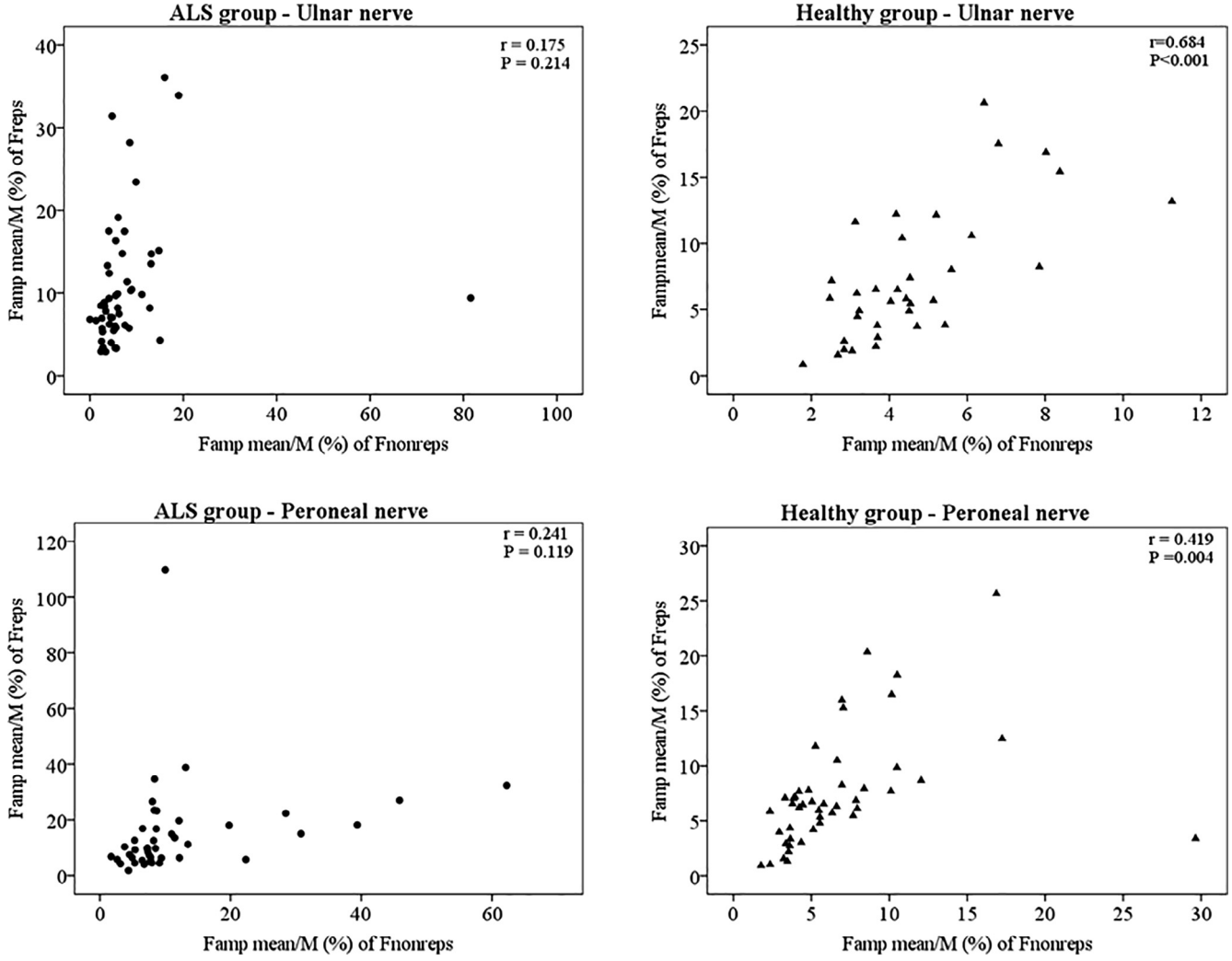
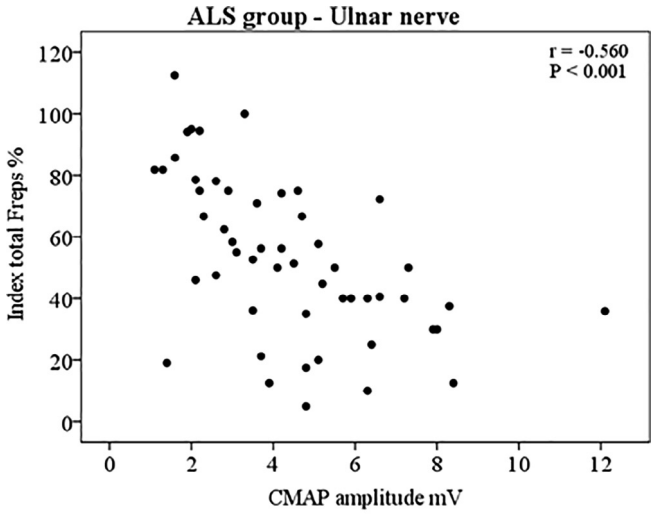
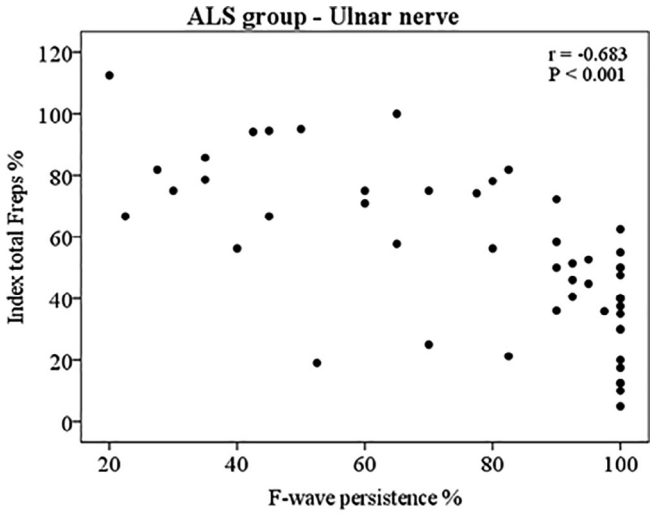
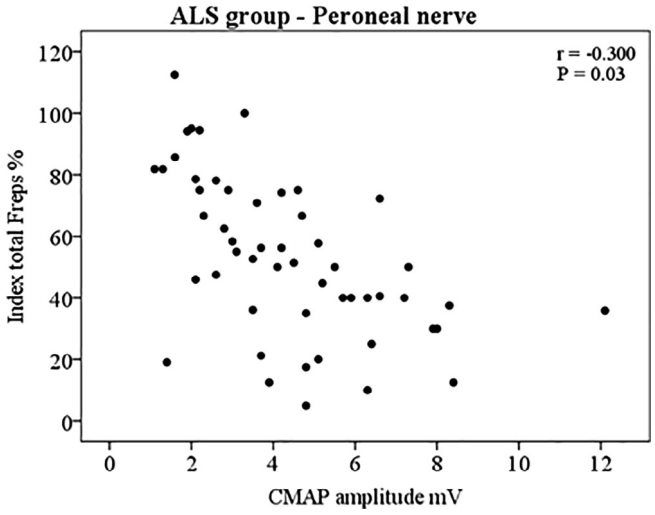


Fig. 2. Correlation of amplitude between Freps and Fnonreps reveals a parallel increment in healthy subjects only.

at 40 μV compared to the 20 μV used herein. (b) The number of traces studied. In the distant past, experimental studies with a small number of participants used a large number of stimuli, ranging from 200 to 300, in order to fully indentify individual F-waves (Peioglou-Harmoussi et al., 1987; Doherty et al., 1994). Nonetheless, we herein generally accepted a series of 40 stimuli adequate for routine F-wave studies to demonstrate differences between healthy and diseased nerves, as the best compromise between







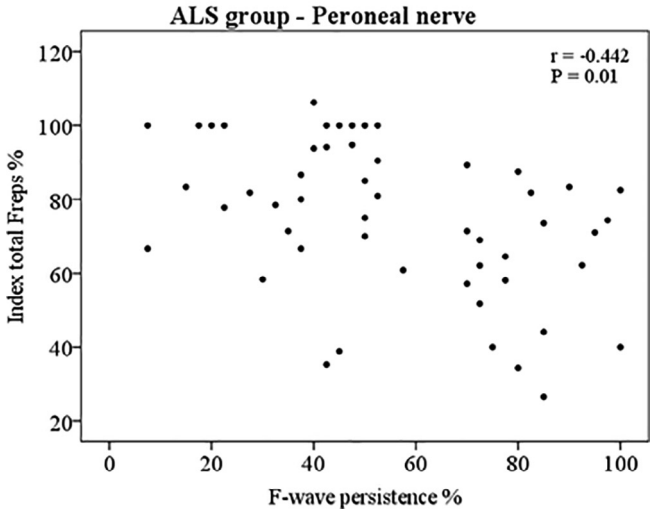


Fig. 3. The CMAP amplitude reduction in ALS patients has a negative effect on the frequency of index total Freps in a nerve.

Fig. 4. Relation of persistence between indices of repeater F-waves and overall F-waves in ALS subjects. It shows that the lower the percentage of F-waves in a recording, the higher the repeaters' frequency. The observed ceiling effect for values of 100% frequency reduced the power of statistics.

accuracy, patients' comfort and practicality (Panayiotopoulos and Chroni, 1996). (c) The severity of clinical involvement of the examined limb in ALS subjects (Fang et al., 2016). (d) Method of analysis; our measurements for the healthy subjects were intermediate compared to those of visual – manual superimposition methods, whereas the results for the ALS patients were towards the higher published values. This trend was repeated in our findings in the peroneal nerve compared to our previous study, which was based on visual inspection (Chroni et al., 2012).

The enhanced repeater occurrence in ALS patients could theoretically be due to a combine effect of upper and lower motor neuron process. Reduction in the number of motor neurons capable to produce F-waves is a simple explanation. However, the appearance of several repetitions of RNs implied that the surviving motor neurons generate F-waves more frequently that that expected according to single-fiber study (Schiller and Stålberg, 1978). In our previous study we showed that submaximal stimulation of the ulnar nerve in healthy individuals resulted in enhanced Freps persistence, though in no cases RNs had 4 or more repetitions in a trial (Chroni et al., 2017). This could be attributed to an abnormally increased ability of the remaining intact neurons to generate recurrent impulses, either as a compensation mechanism or due to release from central or local inhibitory control (Petajan 1985; Mesrati and Vecchierini, 2004). The role of glutamate excitotoxicity causing corticomotoneuronal hyperexcitability (Bae et al., 2013; de Carvalho et al., 2014) which results in overproduction of F-waves by some neurons, as a transient phenomenon prior to their degeneration, could also be considered. It should be noted, however, that repeater F-waves is not a pathognomonic finding for ALS. In conditions such as radiculopathy, carpal tunnel syndrome, and ulnar mononeuropathy repeaters were also increased (Macleod, 1987; Pastore-Olmedo et al., 2009; Chroni et al., 2012). In general, it is reasonable to assume that repeaters accumulate in situations where, for any reason, some neurons become incapable to generate F-waves. This concept was confirmed in the current setting, where a reversed relation between reduced F-wave persistence and increased Freps was found. Comparison of Freps indices and parameters across groups with different neurogenic processes was beyond the scope of the present study, which focused on evaluation of the F Wave Analyzer program.

F-wave amplitude measurements were significantly higher in the ALS compared to the healthy group, confirming older studies (Panayiotopoulos and Chroni, 1996; Drory et al., 2001; Mesrati and Vecchierini, 2004), but these large F waves were either repea-

Table 3
Repeater F-waves occurrence in the ulnar nerve in various studies.

Study	Method	N of stim	LL of F amp	Healthy			ALS patients		
				N subj	Freps persist	Index total Freps	N subj	Freps persist	Index total Freps
Peioglou-Harmoussi et al. 1987	Copy on transparent paper	200	40	21		3.4 3.3*	17		37 31*
Guiloff and Modarres-Sadeghi 1991	Copy on transparent paper & superimposed	20 100	40 40	11 11		5.7 11.2			
Fisher et al. 1994	Visual inspection	100	20	11	19	22			
Chroni et al. 2012	Visual inspection	20	40	50	0*	0*	50	10*	16*
Fang et al. 2015	Visual inspection	20 100	40 40	25 25		0* 3*	50 50		#p34/ nonp10* #p75/ nonp15*
Fang et al. 2016	Manually superimposed	100	40	20		1.5	40		51 in severe 5 in mild
Present study	F Wave Analyzer	40	20	52	6.7 5*	6.8 5*	52	37 40*	53 50*

N of stim: number of stimuli per nerve; LL of F amp: the lower limit of a potential amplitude accepted as F-wave (in μV); N subj: number of subjects per group; Freps persist: percent of total repeater F-waves in a series of 40 stimuli (mean group value); Index total Freps: 100 × total number of Freps/total number of traces with F-waves (mean group value).
* Median value is given.
# p: pyramidal, nonp: without pyramidal sings.

ters or non repeaters. More so, in the healthy subject group, where the number of repeaters was small, the highest amplitude F-waves belonged to the Fnonreps category. Amplitude increase in ALS may be the result of F-waves generation by enlarged, reinnervated motor units and/or a higher number of motor units per F-wave, which were synchronized due to the pyramidal lesion (Felice, 1998; de Carvalho et al., 2002). Latency measurements of Freps in both nerves in ALS group, showed a shift towards the longer values, implying that they are more likely to be generated by small type I, slow conducting neurons. It is known from experiments studies in human and animal models that the subpopulation of fast conducting, type II neurons are particularly vulnerable to degeneration in ALS (Kanning et al., 2010). Thus, one can speculate that type II motor neurons become less capable of responding due to their early involvement in the pathology, while the surviving small, type I neurons continue to elicit F-waves, possibly more so under the influence of increased central excitability (Mesrati and Vecchierini, 2004). In the healthy subjects, however, where the absolute numbers of Freps were small, the latency range of Freps was narrow and fit in the middle of the Fnonreps range; this is an expected finding from a statistical point of view and did not suggest a preferential generation of Freps by a certain motor neuron subgroup.

Although statistical difference of repeater frequency between ALS and healthy groups was evident in both ulnar and peroneal nerves, this was more obvious in the ulnar nerve, which under normal conditions is characterized by high persistence and variety of F waveforms. Indeed, none of the healthy subjects showed 5 or more repetitions of an individual F-wave, or 5 or more different RNs in a 40 stimuli trial of the ulnar nerve and we therefore, consider this nerve as more suitable for repeater F-waves estimation. In our practice, when a patient is referred with a clinical suspicion of ALS, we supplement standard nerve conduction studies with the F Wave analyzer, which makes possible the incorporation of repeater recognition for clinical purposes. Prior to the necessary electromyographic assessment, we study F-waves in well preserved muscles with normal or borderline conduction measurements of the corresponding nerves as a preliminary method. Findings of increased Freps indices together with reduced F-wave persistence and increased F-wave amplitudes in such nerve-muscle complex, constitute a red flag, urging us to carry on with a thorough needle electromyography in several body domains. Such F-wave patterns, even in cases with equivocal electromyographic findings strongly suggest a repetition of the neurophysiological examination.

In conclusion, the F-wave study in ALS showed infrequent and high amplitude F-waves, many of which were Freps. A lower motor neuron in order to generate an F response at any given time does not act in isolation but in conjunction with other neurons within the pool, intermediate neurons and under the influence of central drive. Therefore, it is assumed that changes in the appearance of F-waves in a nerve reflect the status of the motor neuron pool as a whole. In a chronic degenerating process, like ALS, signs of motor neuron pool dysfunction are expected to be present long before the obvious clinical manifestations in the same domain. Once repeater F-waves evaluation becomes part of the routine, the usefulness of the above described ALS profile for monitoring over time could be assessed along with the existing markers (de Carvalho and Swash, 2016) in clinical trials concerning new drugs efficacy. Overall, a quick and reliable automatic method to identify F-repeaters could be proven useful in more clinical settings than just ALS. With this intention, the next step will be to refine the F Wave Analyzer software and standardize the procedure, which will be available free of charge to use by every interested scientist for off-line analysis of F-wave data.

Acknowledgments

We would like to thank Sonia Malefaki, Assistant Professor of Department of Mechanical Engineering & Aeronautics, School of Engineering, University of Patras, for her assistance with statistical analysis.

Funding

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

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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