

Physiology of the fasciculation potentials in amyotrophic lateral sclerosis: which motor units fasciculate?

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Abstract We set out to study whether in amyotrophic lateral sclerosis (ALS) fasciculation potentials (FPs) arise from the most excitable motor units (MUs). We studied 70 patients with ALS and 18 subjects with benign fasciculation syndrome (BFS). Of the 56 eligible ALS patients, 31 had signs of reinnervation in the right first dorsal interosseous muscle selected for study, and 25 did not. Two needle electrodes were placed in different MUs in each studied muscle. We defined the most excitable MU as that first activated by minimal voluntary contraction. In muscles without reinnervation, the recording site with most frequent FPs had a higher probability of showing the first recruited MU ($p < 0.001$). No significant difference was found in other patients or in BFS subjects. In very early affected muscles, fasciculating MUs are the most likely to be recruited volitionally. This probably represents hyperexcitability at lower motor neuronal level.

Keywords Amyotrophic lateral sclerosis · Benign fasciculations · Fasciculation potentials · Hyperexcitability · Motor units · Origin of fasciculation potentials

Abbreviations

1st DI	First dorsal interosseous
ALS	Amyotrophic lateral sclerosis
BFS	Benign fasciculation syndrome
EMG	Electromyography
FP	Fasciculation potential
IQR	Interquartile range
MU	Motor unit

Introduction

The first detectable abnormality in muscles in amyotrophic lateral sclerosis (ALS), by clinical observation, electromyographic (EMG) studies [1] and by ultrasound imaging of muscle [2], is fasciculation. It is likely that in these fasciculating motor units the site of origin of the fasciculation discharge is at the lower motor neuron cell body, since there is no evidence of regenerative sprouting of the peripheral axonal arborisation at this early stage [2], although a supraspinal origin for some fasciculations cannot be entirely excluded [3]. Later in the course of the disease, fasciculations originate more frequently from peripheral sites, associated with nodal excitability in unstable regenerating axons [4, 5]. In ALS, there is evidence for increased cortical excitability very early in the disease [6] and even, in patients with SOD1 mutations [7], before the clinical onset of the disease. We suggest that early in ALS fasciculations are indicative of increased anterior horn cell excitability, possibly related to membrane instability. We have addressed this hypothesis by assessing whether the fasciculating motor unit in this very early stage of the disease is more likely to be activated by volitional effort.

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Methods

Amyotrophic lateral sclerosis (ALS)

We studied 70 patients (41 men and 29 women) with definite (12), probable (51) or possible (7) ALS as defined by the revised El-Escorial [8] and Awaji criteria [9]. None had coincidental polyneuropathy, diabetes or ulnar neuropathy. Those older than 75 years, with disease duration greater than 24 months, with weak 1st dorsal interosseous muscle (1st DI) of the right hand on clinical examination, with respiratory distress lying down or with cognitive change, were excluded. The median age was 62 years [range 30–75 years, 1st–3rd interquartile range (IQR) 52–69 years]. The disease was of upper limb onset in 11 patients, lower limb onset in 30 patients, bulbar onset in 28 patients and axial onset (drop-neck) in 1. Disease duration before study entry ranged from 2 to 24 months (median 14 months, 1st–3rd IQR 8–18 months). All the patients showed clinical progression in subsequent follow-up. Of these 70 patients, 14 were excluded from the protocol because they could not cooperate with the experimental protocol, which required minimal muscle contraction (10 subjects) or because no fasciculation potential (FP) was detected by needle EMG (4 subjects). The 1st DI muscle of the right hand was studied in each patient. Patients were evaluated within the diagnostic workup period, before riluzole treatment was started.

Benign fasciculation syndrome (BFS)

Eighteen subjects (15 men and 3 women) were studied (median age 53.5 years; range 29–68; 1st–3rd IQR 50 to 60 years). These subjects had all been symptomatic for several years, and all had been followed for more than 5 years by one of the authors (MdC). In all of them, motor conduction studies, sensory action potentials and regularly repeated EMG were normal, including motor unit potential analysis in the right 1st DI muscle that was selected for investigation. In addition, the FPs were of normal morphology [1].

Experimental protocol

In all the subjects, in both experimental groups, preliminary conventional concentric needle EMG was performed in the 1st DI muscle. In the group of patients with a diagnosis of ALS, the 1st DI muscles were subgrouped into those with neurogenic motor unit potentials (MUPs) on quantitative analysis and/or presence of fibrillations and positive sharp waves (fibs-sw) (ALS-1) and those in whom quantitative analysis revealed MUPs of normal morphology and no fibs-sw (ALS-2). Four needle positions were

used for the conventional study of MUPs and fibs-sw. For detection of the presence of FPs, a 2-min recording was made in the four different sites [10].

Neurophysiological methods

A Keypoint-Net device was used (Dantec-Natus, Denmark) for all investigations. Motor (both ulnar nerves at wrist, below and above elbow, and both peroneal nerves, including F-waves) and sensory nerve fibers (both ulnar and both sural nerves) were assessed to exclude peripheral neuropathy and ulnar nerve lesion. For motor studies, standard amplifier filter settings of 20 Hz and 10 kHz were used. The latency measurements were performed with a gain of 200 $\mu\text{V}/\text{division}$. Sensory responses were recorded by bar electrodes using filter settings of 20 Hz–2 kHz, and a gain of 10 $\mu\text{V}/\text{division}$. A ground electrode was placed at the wrist. Following routine evaluation of the 1st DI muscle, we introduced two disposable concentric facial needle electrodes (recording area 0.017 mm^2 ; Dantec-Natus) connected to two different channels of the Keypoint device (double-EMG recordings). Filter settings were 500 Hz–10 kHz. Data was saved on the hard-disk of the device for offline analysis.

One concentric facial needle electrode (needle A) was placed in the most lateral part of the muscle (closer to the thumb) and the other (needle B) was placed more medially. The two needle electrodes were placed ≥ 25 mm apart, perpendicular to the long axis of the limb, 2–3 mm deep in the muscle, and in the territories of different motor units [11]. The position of the needle electrodes was maintained using a tape to hold the cables on the patient's arm. To ensure that the needles were recording different motor units, we performed two tasks. We required that one or both of these tests confirmed that the needle electrodes were recording from different motor units. We first asked the patient to make a very slight voluntary contraction of the muscle and noted whether the voluntarily activated motor units (≥ 50 μV) could be recorded from only one of the two electrodes (Fig. 1). Secondly, the ulnar nerve was stimulated at threshold intensity at the wrist and elbow to confirm that the response from a single motor unit was recorded from only one needle electrode (Fig. 2). A single motor unit response was supported by the observation of an all-or-none response at near-threshold axon stimulation (Fig. 2). A similar surface stimulation technique was used by others in stimulation SFEMG studies of single motor units [12]. We have previously validated the method [11, 13]. Following confirmation that the needles were recording from the territories of different motor units at two different loci, a 2-min recording period was used to detect FPs in both motor unit territories [10]. Typically, each

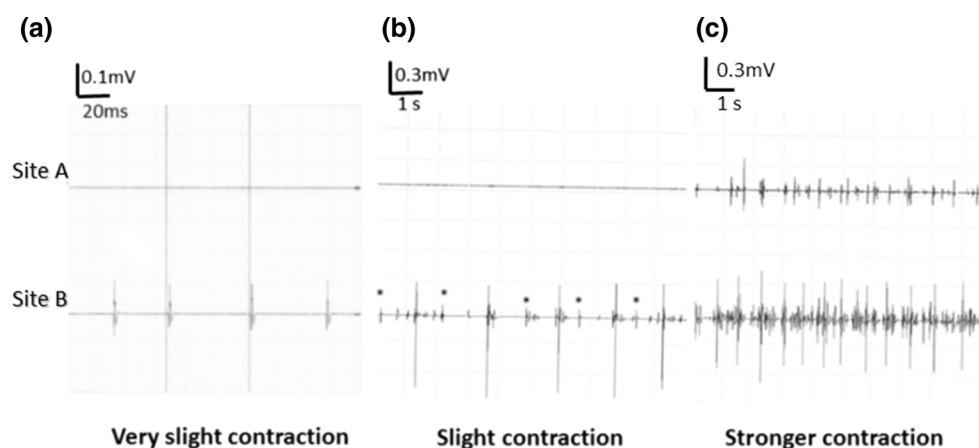
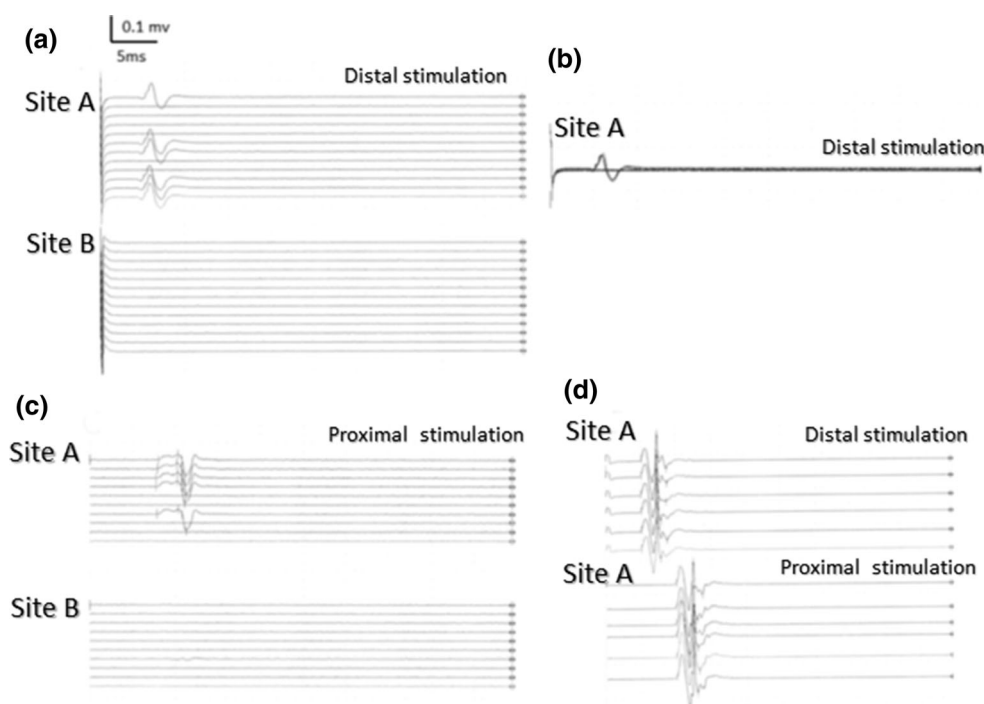


Fig. 1 Early activated motor unit using a double-recording. Recordings of motor unit potentials from two sites (*Site A* and *Site B*) within a 1st DI muscle. Initially, one MU was recorded only from site B (**a**), showing that the two electrodes were recording from different motor units. With increasing force of contraction different MUs were

recorded from the two electrode sites (**b**, **c**). During stronger contraction (gain of the recording was changed because larger motor units were recruited) the first recruited motor unit is still identified (*asterisk*)

Fig. 2 Nerve stimulation and single motor unit recording.

(**a**) Near-threshold stimulation of the right ulnar nerve at wrist excited one axon at *Site A*, but not at *Site B*, showing that the two electrodes were recording from different motor units. To confirm this conclusion, a typical all-or-none response of the same motor unit was required. (**b**) The excited motor unit shows a stable morphology. (**c**) Near-threshold stimulation of the right ulnar nerve at elbow in this subject recruited another single motor unit (all-or-none response). (**d**) In another subject, the same motor unit was recruited at wrist and at elbow (traces with absent responses were deleted) to show that the same excitable axon can be stimulated at different sites in some patients



facial needle electrode recorded from 2 to 3 easily recognisable and separable motor units. The total number of FPs was calculated in the recordings from each of the two sites in each muscle studied in the ALS and BFS groups of subjects. We followed the definition of the American Academy of Electrodiagnostic Medicine [14] and accepted as FP the electric activity with the configuration of a motor unit activation potential, but occurring spontaneously with the muscle at rest. For this study, we accepted FPs only if their amplitude was greater than

100 μ V. The motor point, located proximally in this muscle [15], was avoided.

In each of these double-EMG recordings, a sweep speed of one second/division (Fig. 3a) was used to detect and quantify FPs (number of FPs during the 2-min recording in each channel) [11, 13]. The morphology of the recorded FPs was evaluated using a sweep speed of 20 ms/division (Fig. 3b). After the 2-min recording of the muscle at rest, without allowing movement of the needle electrodes, each patient was asked to perform a very slight contraction of

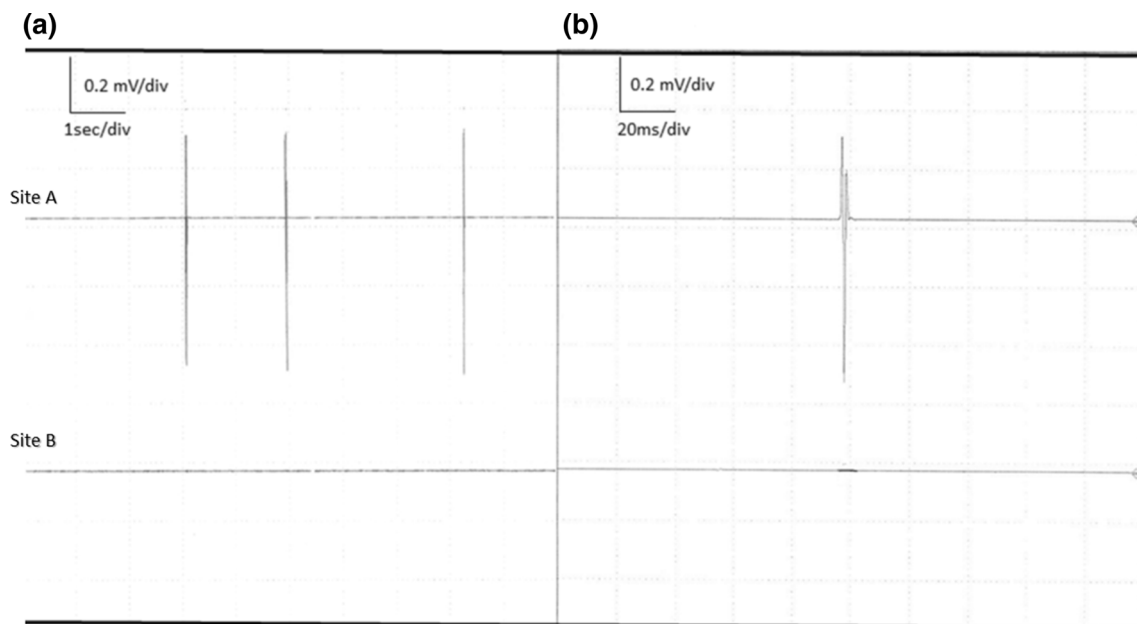
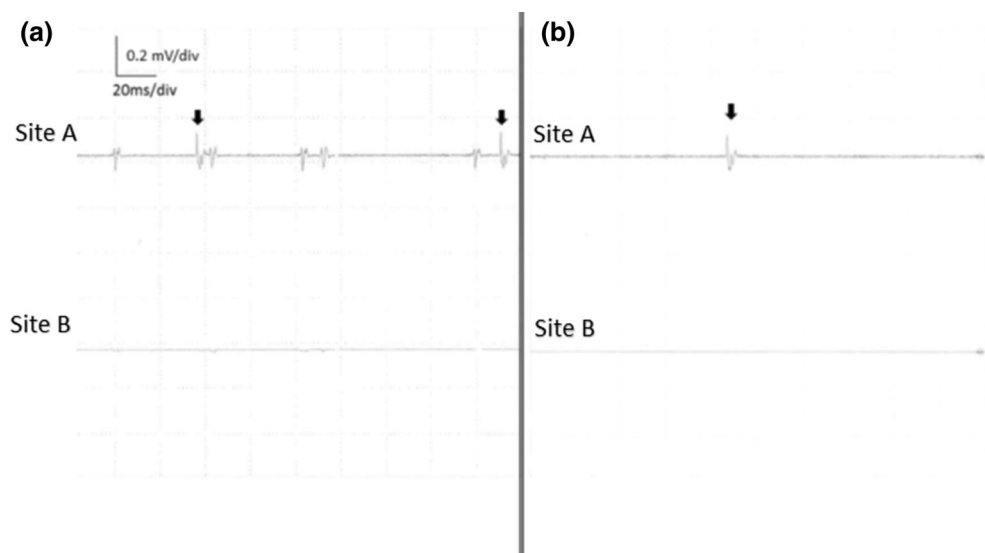


Fig. 3 Fasciculation potentials recording. **(a)** Recordings from the two sites in the 1st DI muscle (*Sites A and B*), with the muscle at rest (1 s/division). We observed 3 FPs from site A and none from site B

during the recording time (10 s). **(b)** Same recording with a sweep-speed 20 ms/division, in order to observe the morphology of the FPs

Fig. 4 Voluntary recruitment of the fasciculation potential. **(a)** Recruitment of 2 motor units of simple morphology on slight volitional contraction (from *Site A*). **(b)** A single FP in the resting muscle (from *Site A*), its morphology identical to that of one of the recruited motor units (arrow)



the 1st DI muscle in order to voluntarily activate a single motor unit. Online analysis with offline review was used to compare the morphology of each FP with the morphology of the voluntarily activated motor unit (Fig. 4). The comparison was done by careful visual inspection, taking into account that during slight contraction the relationship between the electrode's recording surface and nearby muscles fibres changes a little, implying a very minor difference in amplitude of the potential (<20 %), which was considered in the analytical process; however, we required a similar potential duration and number of phases.

All subjects gave written informed consent as required by the local Research Ethics Committee.

Statistical analysis

In each of the three groups of patients (ALS-1, ALS-2 and BFS), we calculated the number of FPs recorded from each of the two needle sites, and the difference between the pairs of recordings in each subject. The percentage differences in these paired groups of data were not normally distributed. These percent differences were therefore ranked and the

20 % of pairs of recordings with the least differences were discarded as not being sufficient to warrant further analysis. This 20 % cut-off derived from a preliminary study of ten ALS patients, in whom 20 % was the largest variation observed in the number of FPs in a single site in two consecutive 2-min recording periods. The remaining 80 % of paired recordings (with more marked asymmetry in the number of FPs between sites) were analysed to determine the relation of the electrode site with more frequent FPs and the site of the first recorded motor unit potential (electrode A or B) following voluntary activity. These categorical relationships were evaluated using the Fisher exact test for significant differences. A p value <0.05 was considered significant. The median number of FPs in each group was compared using the Kruskal–Wallis test.

Results

In total, we observed 2775 FPs, 1356 in the 31 patients in the ALS-1 group, 917 in the 25 ALS-2 patients, and 482 in 18 patients in the BFS group. The median number of FPs was 35 (IQR 25–55) in the ALS-1 group, 27 (IQR 15.5–47.5) in the ALS-2 group, and 16.5 (IQR 11.3–39) in the BFS group. The median number of FPs in patients in the ALS-1 group was significantly greater than in the BFS group ($p = 0.001$). There was no significant difference between the median number of FPs recorded in patients in the ALS-2 and BFS groups. In total, there was no significant asymmetry between needle sites A and B in the different groups ($p > 0.3$ for each comparison).

Of the 31 patients in the ALS-1 group, 25 showed a significantly different number of FPs between recording sites A and B as defined above. In these patients in the ALS-1 group, the first volitionally recruited motor unit was observed at the site with more FPs in 9 patients and in the site with less FPs in 1, while in the remainder no preferential site was detected ($p = 0.52$). Of the 25 patients in the ALS-2 group, 20 showed a different number of FPs between recording sites A and B. In these patients in the

ALS-2 group, the first volitionally recruited motor unit was observed at the site with more FPs in 17 patients and at the site with fewer FPs in 3, while in the remaining subjects, no preferential site was noted ($p < 0.001$). Of the 18 subjects in the BFS group, 15 showed a significantly different number of FPs at the two recording sites A and B. In this BFS group, the first recruited motor unit was observed at the site with more FPs in 9 patients and at the site with fewer FPs in 3 ($p = 0.08$). Table 1 summarizes these results.

With axonal stimulation at wrist or at elbow, in each of the investigated groups of subjects, the preferential axon stimulated was not associated with the site with greater number of FPs ($p > 0.05$).

In 8 of the 31 patients (23 %) in the ALS-1 group, we found that the first recruited motor unit had a similar morphology to that of the observed FP. In most patients, the FPs were complex and of variable morphology (Fig. 5), or represented part of the recruited MU shape (Fig. 6). In 18 patients (72 %) in the ALS-2 group, we found FPs with a morphology identical to the first recruited MU (Fig. 4). In subjects with BFS, an identical MU compared to a FP was observed in general (15 of 18; 83 %). This percentage was significantly lower for the ALS-1 group as compared with the remaining groups ($p = 0.04$).

Discussion

It is relatively well established in ALS that FPs and neurogenic spontaneous activity are distributed multifocally within an affected muscle, indicating that some motor units are affected to a greater or lesser extent than others [16–19]. The two needle electrodes we used to record FPs at two different sites in the 1st DI muscle were sufficiently separated to ensure they did not record from the same motor unit, even in reinnervated muscle. Motor unit territory has been carefully investigated in the past with the use of a 12- or 14-lead electrode [16–19]. It has been shown that the motor unit territory extends over about 7 mm in

Table 1 Summary of the results

Groups	Number of patients	Number of FPs	Hands with no significant difference in the number FPs between sites	Hands with more FPs in the site of the earliest recruited MU	Hands with more FPs in the site of the late recruited MU	p value
ALS-1	31	1356	6 (19.4 %)	9 (29 %)	16 (51.6 %)	0.52
ALS-2	25	917	5 (20 %)	17 (68 %)	3 (12 %)	<0.001
BFS	18	482	3 (16.7 %)	9 (50 %)	3 (16.7 %)	0.08

Significant p value shown in bold

ALS-1 patients with ALS in whom the first dorsal interosseous muscle showed neurogenic changes on electromyography, *ALS-2* patients with ALS in whom the first dorsal interosseous muscle showed no neurogenic changes on electromyography, *BFS* subjects with benign fasciculations syndrome, *FPs* fasciculation potentials

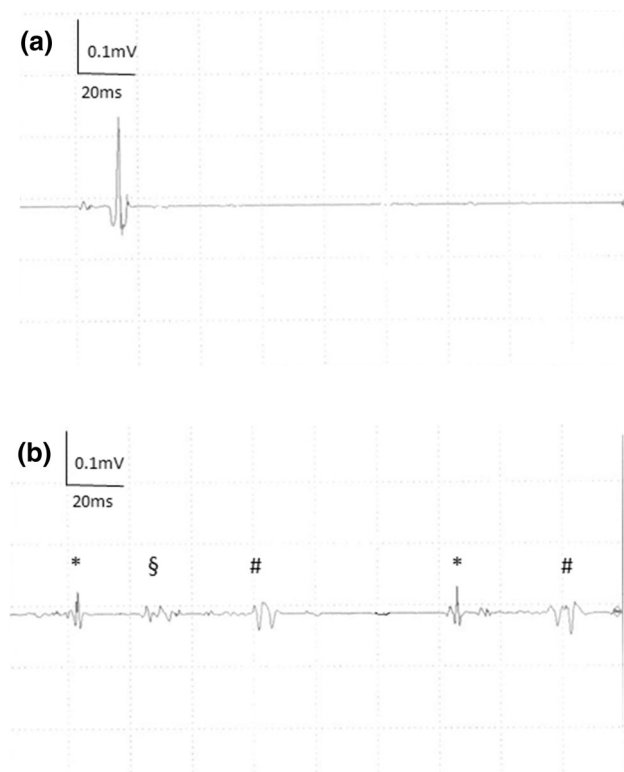
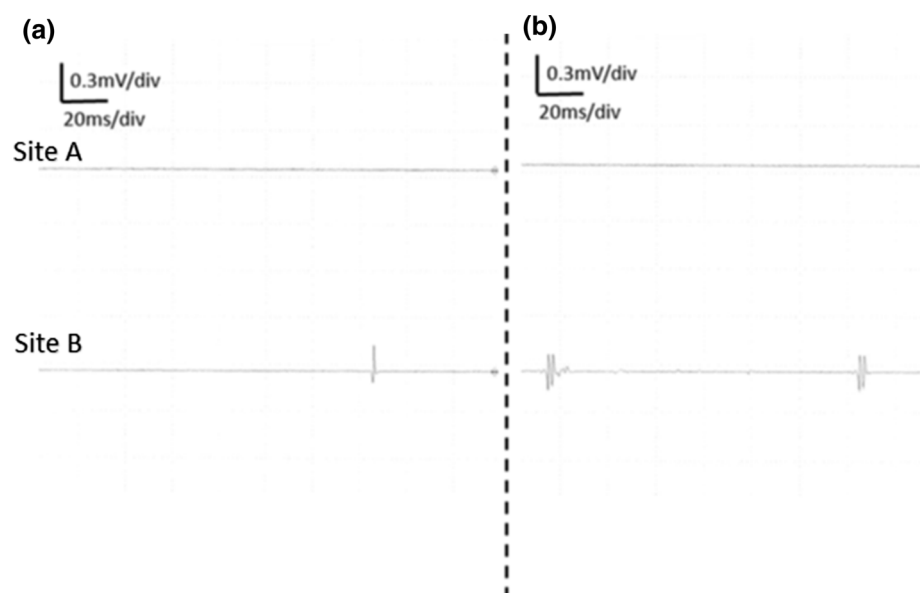


Fig. 5 No voluntary recruitment of the fasciculation potential. (a) A FP with a morphology that differs from the first 3 recruited motor units (b). (b) Early recruitment of 3 unstable motor units are seen in a 1st DI muscle (marked as *asterisk*, *section sign* and *hash*) with neurogenic changes (motor unit *hash* at a firing rate of approximately 8.3 Hz, motor unit *asterisk* at a firing rate of approximately 7 Hz and motor unit *section sign* at a firing rate lower than 7 Hz)

Fig. 6 Recruitment of a fasciculation potential representing a component of the motor unit. The FP recorded in (a) resembles the first component of the voluntarily activated motor unit in (b), suggesting that the FP sometimes represents spontaneous activity of only a part of a motor unit



healthy subjects [19] and, in patients with chronic neurogenic disorders, that motor unit territory does not enlarge more than 40 % [20]. Indeed, these observations are in agreement with the histological description that axonal sprouting is confined to a distance shorter than 2 mm [21]. Moreover, to ensure that the needle electrodes were recording from different motor units, we required volitional recruitment of a single motor unit from only one of the two recording sites, in very slight muscle contraction, and studied single axon stimulation by near-threshold nerve stimulation at wrist and elbow. These techniques, which depend on the ‘all or nothing principle’, have been used previously to confirm recording from different motor units in the same muscle [11, 13].

Basmajian [22] studied volitional recruitment of single motor units in the 1st DI muscle using concentric needle electrodes and others have studied recruitment in other muscles [23]. Voluntary contraction recruits motor units in order of increasing size [24, 25] as predicted by Henneman’s size principle [26]. The first recruited motor units on volitional contraction are the same as those following low-intensity cortical stimulation, suggesting that they represent the most excitable motor neurons of the motor pathway [27]. We utilised this method to investigate if fasciculating motor units arose from the first volitionally recruited motor unit in a muscle in ALS or BFS. For this identification, we relied on the constant position of the electrodes in the muscle and the morphology of the MUP activated voluntarily, compared with the spontaneously firing FP. In many recordings, one such volitional motor unit potential could be distinguished as resembling one of two motor unit

potentials firing spontaneously in the 2-min recording of the muscle at rest.

In order to avoid false-positive results derived from spurious asymmetry in the number of FPs originating from different motor units, we tested pairs of motor units associated with very dissimilar numbers of FPs in each muscle. We found a highly significant probability that the fasciculating units in a focal area of a muscle in non-re-innervated 1st DI muscles (in ALS-2) were those most likely to be recruited by volitional activity. This relationship was not found in ALS-1 in which there were established neurogenic changes, or in BFS. Taking into account the high significance of this finding ($p < 0.001$), it is unlikely to be due to chance. An analysis made of the whole dataset, not utilising a cut-off for a significant asymmetry in the number of FPs between sites, confirmed that the probability of FPs originating from the more excitable MUs in ALS-2 remained significant ($p = 0.005$). These results suggest that the most excitable motor units, in which the resting membrane potential is close to threshold, are those most likely to fire spontaneously as FPs at a stage in the disease when there are no other abnormal clinical or electrophysiological findings. When there is neurogenic change, a phase in ALS at which FPs probably arise more frequently from distal nodal activation and from discharges associated with peripheral axonal sprouting than from spontaneous motoneuronal firing [4], this relationship is lost. In BFS, there was no associated preferential recruitment of motor units in muscle regions in which we recorded FPs. It is perhaps most likely that FPs and cramps in BFS arise from axons in similar fashion to neuromyotonia [28]. Applying low-threshold stimuli to the ulnar nerve, we could not confirm that the most excitable axons in the nerve were related to the motor units from which FPs arose. However, we should be cautious about this observation, since we know that stimulation of a nerve with low-intensity current will only excite superficial fibres and these will not be constantly located in the anatomical cross-sectional area of the nerve. In addition, peripheral nerves show a subtle spiral structure, to allow for lengthening during joint movement—the spiral bands of Fontana described in 1779 [29]. Our study has another related limitation. Our method does not allow us to test all the axons in a nerve. We studied two random motor units, representing two different motor axons, in each tested muscle in a large group of patients. We compared the frequency of spontaneous FPs in motor units activated early or later during very mild volitional contraction. This method non-selectively assessed type 2 motor units, the first to be activated on mild contraction.

In ALS, there is evidence of abnormal glutamate metabolism, with reduced glutamate re-uptake in spinal glia as a result of impaired glutamate transport [30]. CSF glutamate levels are increased in ALS [31]. There is also

neurophysiological evidence for increased excitability in the spinal cord in ALS. Raynor and Shefner [32] reported reduced recurrent inhibition in patients with ALS, as defined by a conditioned H reflex technique, although this Renshaw cell dysfunction is considered unlikely to be a primary feature of the disease [33]. Kiernan and his colleagues have reported reduced inhibition in the cerebral cortex in human ALS [6]. Increased lower motor neuron excitability has been observed in the second post-natal week of the SOD1 animal model, with more depolarized membrane potential associated with changes in the threshold and intensities of Na(+) and Ca(2+) persistent inward currents [34], which could facilitate spontaneous lower motor neuron discharge causing FPs. Moreover, other authors using the same mouse model have confirmed that motoneurons innervating slow-contracting muscle fibres, which are more resistant to degeneration in ALS, displayed increased excitability, as judged by lower rheobase and hyperpolarised spiking thresholds [35], while the more susceptible fast-type motoneurons did not show hyperexcitability.

In conclusion, our findings are consistent with the association of FPs and hyperexcitability of the first recruited lower motor neurons in the early phase of ALS, before neurogenic changes are detectable by EMG.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Ethical statement All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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