The site of origin of fasciculations in amyotrophic lateral sclerosis (ALS) and other disorders has proven difficult to determine. Denny-Brown and Pennybacker, who first addressed this question, considered that fasciculations probably arose from anterior horn cells but Forster et al found that fasciculations persisted after motor nerve block or section, suggesting a peripheral origin. F-wave and collision studies, and analysis of the fasciculation potential (FP) firing pattern, have also implied a more frequent distal than proximal origin. Additionally, FPs can sometimes be driven voluntarily or by cortical stimulation. Fasciculation potentials may be of complex or simple morphology, the latter possibly having an ephaptic origin from more than 1 axon, or even from the central nervous system. Norris recorded simultaneous FPs in 2 muscles on opposite sides of the body, lending credibility to the notion that a central excitatory influence could modulate fasciculation and implying a spinal origin. Time-locked firing of FPs originating from different motor units in a single muscle would suggest axonal ephaptic excitation of peripheral axons proximal to the distal end plate arborization, firing of 2 independent spinal motor neurons, or a cortical origin. We used a double-electrode recording technique in a single muscle to detect time-locked FPs to consider these issues.

Report of Cases

Methods

Patients With ALS
All patients gave written informed consent according to the requirements of the local research ethics committee in Lisbon, Portugal, which approved the study.

We studied 52 patients (29 men and 23 women) aged 36 to 75 years (mean [SD], 59.6 [10.1] years) clinically suspected as having sporadic ALS, referred for diagnostic testing (January 2010-March 2013). The mean (SD) disease duration from symptom onset was 11.1 (5.7) months (range, 2-24 months). Disease onset was bulbar in 16, axial in 5, upper limb in 20, and lower limb in 11.

In each, electromyogram (EMG) and nerve conduction studies confirmed the diagnosis of probable or definite El Escorial ALS, applying the Awaji recommendations for neurophysiological diagnosis. All progressed to definite ALS during subsequent follow-up. Patients with an associated frontotemporal dementia were excluded because of ethical and practical concerns regarding their ability to understand the study requirements. Patients with diabetes and those older than 74 years were also excluded. The investigation was made at the time of diagnostic evaluation. Each could stand unaided on the heel of the studied leg, indicating clinically normal strength in the tibialis anterior (TA). All had fasciculations in the TA muscle at the time of investigation.

Patients With Benign Fasciculations
We studied 11 patients with benign fasciculations, aged 38 to 70 years (mean [SD], 58.5 [11.7] years). These patients had normal strength and their EMG showed normal motor unit potential (MUP) analysis. There was no progression to other disorders in the following 2 years. Some had associated muscle cramps. No metabolic or medication-related cause was identified. The TA muscle was studied in each patient.

Neurophysiological Methods
We used a Natus Keypoint Net-G4 EMG machine in this investigation. The skin temperature was monitored and was always

IMPORTANCE Fascication potentials (FPs) may arise proximally or distally within the peripheral nervous system. We recorded FPs in the tibialis anterior using 2 concentric needle electrodes, ensuring by slight voluntary contraction and electrical nerve stimulation that each electrode recorded motor unit potentials innervated by different axons.

OBSERVATIONS Time-locked FPs recorded from both electrodes, suggesting a spinal origin, were most frequent in benign fasciculation syndrome (44%) (P < .001) and amyotrophic lateral sclerosis without reinnervation (27%). Fewer time-locked FPs were found (14%) in the reinnervated tibialis anterior in amyotrophic lateral sclerosis (P < .001).

CONCLUSIONS AND RELEVANCE We conclude that in chronic partial denervation FPs are more likely to arise distally and that FPs in benign fasciculation syndrome more frequently arise proximally.


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greater than 32°C. All recordings were made with a conventional concentric needle EMG electrode (recording area 0.07 mm²). We classified the TA muscles in the patients with ALS as with or without neurogenic change, according to our normative data for this muscle. This was defined by study of 20 different MUPs from at least 5 different sites in the TA during a slight voluntary contraction, using the “Multi-MUP” analysis program implemented on the EMG machine with a filter setting of 5 Hz to 10 kHz, gain of 200 mV/division (div), and sweep speed of 5 ms/division. Amplitude, duration, and area were analyzed for each MUP and mean values of these measurements were calculated for each muscle according to previously published standards.

FP Studies
We followed the definition of FPs as set out in the recommendations of the American Association of Electrodiagnostic Medicine, in brief, electrical activity associated with a fasciculation that has the configuration of a motor unit action potential but occurs spontaneously, although we accepted FPs only if their amplitude was greater than 100 μV.

Experimental Protocol
Recordings were made from 2 adjacent sites in the TA muscle using 2 concentric needle electrodes placed at least 1 cm apart perpendicular to the vertical axis of the muscle (Figure). The peroneal nerve was stimulated at low intensity at the fibular head to confirm that the 2 electrodes were recording from motor units innervated by different axons (Figure, D). In addition, it was often possible to confirm this separation by noting that during slight voluntary contraction voluntarily activated motor units could be recorded from only 1 of the 2 electrodes (Figure, C). In each pair of recording sites, recordings were made during periods of 10 seconds using a sweep speed of 1 s/div to detect FPs at 1 or both electrodes (Figure, A). One to 5 consecutive periods were analyzed in the same pair of recording sites, depending on the number of FPs observed. Fasciculation potentials apparently firing synchronously at the 2 sites were evaluated by changing the sweep speed to 200 ms/div (Figure, B). Between 6 and 8 pairs of recording sites were used in each participant. We quantified the relative number of FPs recorded at a single site and the FPs time-locked at both sites in each recording. To be certain the recording was not contaminated by incidental voluntary activity or electrical artifact, time-locked potentials smaller than 20% of the amplitude of the larger FP were not considered as simultaneous.

Results

Patients With ALS: TA Muscles With Neurogenic Change
Thirty-two of the 52 patients with ALS showed neurogenic change in the TA muscle. In these patients, 5 to 146 FPs per patient (mean [SD], 34.3 [22.5]) were recorded. A total of 1096 FPs were recorded; 941 (85.7%) of these were recorded from only 1 of the 2 simultaneous recording sites and the remaining 155 (14.3%) were recorded time-locked from both sites.

Patients With ALS: TA Muscles Without Neurogenic Change
In the 20 TA muscles without neurogenic change, 5 to 97 FPs per patient (mean [SD], 28.1 [25.4]) were recorded. A total of 544 FPs were recorded: 394 (72.7%) were recorded from only 1 of the 2 electrode sites and 150 (27.3%) were recorded time-locked from both recording sites.

Patients With Benign FP
In these 11 patients, 5 to 43 FPs per patient (mean [SD], 21.3 [13.0]) were recorded. A total of 234 FPs were recorded: 129 (55.1%) were recorded from only 1 electrode and 105 (44.9%) were recorded time-locked from the 2 sites.

Data Analysis
The mean number of FPs noted in each of the 3 groups was similar (P = 0.62, Kruskal-Wallis test). The probability of recording time-locked FPs was greater in the benign fasciculations group than in the 2 groups of patients with ALS (P < .001, χ² test). The probability of time-locked FPs from the 2 recording sites was significantly greater in the ALS group without neurogenic changes than in the ALS group with neurogenic changes (P < .001, χ² test). The results were similar comparing patients with or without prominent upper motor signs in the lower limbs.

Discussion
Our results show that time-locked FPs recorded from 2 motor units innervated by different axons in a muscle are more frequently encountered in benign fasciculation than in ALS. In addition, in ALS, FPs were more common in TA muscles without denervation and reinnervation than in muscles in which there was chronic partial denervation. We conclude that in the absence of neurogenic change, as in benign fasciculation and in ALS before the onset of chronic partial denervation, FPs are more likely to arise proximally, perhaps in the motor neuron pool in the cord innervating the studied muscle, whereas in ALS with neurogenic change on EMG, FPs more frequently arise distally in the peripheral nervous system. Ephaptic “cross-talk” between adjacent motor axons, another possible explanation for time-locked FPs, is unlikely in benign fasciculation syndrome. The reduced frequency of time-locked FPs as ALS advances is best explained by loss of contiguity and therefore of synaptic contact between remaining neurons as they become more isolated from one another.

Roth16,5 studied 100 FPs in 31 patients with neurogenic disorders but did not specify their diagnoses. He studied the resulting single-unit F waves and also used a collision technique in some of the recordings, concluding that a distal axonal origin was likely in about 80%, regardless of the underlying disorder. In the remaining 20%, a more proximal origin in the peripheral nervous system was postulated. Kleine et al16 suggested from interspike interval measurements in surface recordings of FPs that 75% had a distal origin. In our dual-electrode recordings of FPs in the TA muscles of 32 patients with neurogenic change due to ALS, we found that 14% of FPs were time-locked in the 2 recording sites, suggesting a proximal origin for these FPs, whereas in ALS without neurogenic change twice as many FPs were recorded time-locked in the 2 recording sites. This accords with the more frequent volun-
The possible role of central spinal excitation and inhibition is untested experimentally, although commensurate with reduced fasciculation frequency during sleep.\textsuperscript{14}

A. Recordings from 2 adjacent sites in the tibialis anterior muscle using 2 concentric needle electrodes placed at least 1 cm apart and perpendicular to the vertical axis of the muscle. Time-locked (double arrowhead) and non–time-locked (single arrowhead) FPs are shown. B, Changing the sweep speed to 200 ms/division confirms that the FPs are time locked (double arrowhead). Repeated FPs are observed at sites 1 (*) and 2 (†). C, Slight contraction activates motor unit firing at site 1 but not at site 2, implying that the electrodes are recording electrical activity from 2 different motor units. In addition, time-locked (double arrowhead) and non–time-locked (single arrowhead) FPs are observed during muscle activation. D, Low-intensity electrical stimulation at the fibula stimulates the lower-threshold axon innervating the motor unit recorded at site 1 but not the one innervating the motor unit recorded at site 2.
Author Contributions: Drs de Carvalho and Swash had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: All authors.

Acquisition of data: de Carvalho.

Analysis and interpretation of data: All authors.

Drafting of the manuscript: All authors.

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REFERENCES


