Characteristics of fasciculations in amyotrophic lateral sclerosis and the benign fasciculation syndrome

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The aim of this study was to determine first, if benign fasciculations and those in amyotrophic lateral sclerosis can be distinguished on the basis of their waveforms or firing characteristics, and second to determine how fasciculation parameters evolved with progression of amyotrophic lateral sclerosis. Fasciculation potentials recorded from 63 muscles of 28 patients with definite amyotrophic lateral sclerosis were compared with those from 21 muscles of 11 patients with the benign fasciculation syndrome. In each muscle, at a single site, up to 15 identifiable fasciculation potentials could be recognized. Thus the characteristics of 430 fasciculations from patients with amyotrophic lateral sclerosis and 191 benign fasciculations were analysed. Fasciculation potential amplitude, area, turns, duration, firing interval, indices of waveform variability, evidence of axonal conduction block, evidence of axonal conduction variability and propensity to produce double fasciculations were measured. The waveforms of fasciculations in amyotrophic lateral sclerosis were on average of shorter duration and had a greater number of turns than benign fasciculations, but, although irregular in both conditions, the firing rate in amyotrophic lateral sclerosis was significantly higher. In both conditions, there was evidence of multifocal distal generation of fasciculations, axonal conduction block in the motor unit arborization and of variable axonal conduction. When severe weakness and marked chronic neurogenic change were present on electromyography, the firing rate of fasciculations in amyotrophic lateral sclerosis was higher but fasciculation potential amplitude, area and indices of waveform variability were little changed. Double fasciculations in which the waveforms of the two potentials were the same occurred in both conditions. The intervals were in two bands: an early band with 4–10 ms intervals showed identical waveforms of the two potentials, indicating the region of generation was the same. A second band of double fasciculation occurred in the tibialis anterior at an interval of 30–50 ms. Here, the first fasciculation waveform was variable in shape but the second fasciculation was the same on each occasion, suggesting reactivation of the fasciculation via the F-wave route. Double fasciculations in which the second discharge was different from the first had flat time-interval histograms, indicating no interaction between different fasciculations. In conclusion, benign and malignant fasciculations are not distinguishable on the basis of waveform; highly complex fasciculation potentials can be seen in both conditions. Fasciculation firing rate and the frequency of double fasciculations increases in amyotrophic lateral sclerosis when there is a marked lower motor neuron abnormality.

Keywords: fasciculation; amyotrophic lateral sclerosis; benign fasciculation syndrome

Abbreviations: DDFP = double different fasciculation potential; DSFP = double same fasciculation potential
Introduction

Fasciculations, random muscle twitches that can be observed clinically, are associated with electrical events recorded by a needle electrode as fasciculation potentials. A fasciculation potential represents the spontaneous discharge of a motor unit or part thereof. Although fasciculation potentials are seen in many conditions, e.g. peripheral neuropathy (Roth and Magistris, 1987), radiculopathy (Dumitru et al., 2001), peripheral nerve hyper-excitability syndromes (Newsom-Davis and Mills, 1993) and following the use of depolarizing blocking agents (Hartman et al., 1989); they are of particular importance in the diagnosis of motor neuron diseases. Lambert (1969), in providing the first guidelines for the diagnosis of amyotrophic lateral sclerosis, asserted that it would be difficult to entertain the diagnosis of amyotrophic lateral sclerosis without recording fasciculations. Fasciculation potentials also occur in otherwise healthy individuals (Falck and Alaranta, 1983; Mitskostas et al., 1998) in the condition termed benign fasciculation syndrome. This is a non-progressive chronic condition characterized by fasciculation, usually in the lower limbs, not associated with any other clinical abnormality. Long-term follow-up of these cases (Blexrud et al., 1993) has verified the benign nature of the condition. It is, however, a not uncommon problem, clinically, to distinguish between early amyotrophic lateral sclerosis and benign fasciculation syndrome.

In amyotrophic lateral sclerosis, most fasciculations are thought to be generated in the fine intramuscular axons innervating individual muscle fibres or at motor endplates (Desai and Swash, 1997). First, cutting the nerve supply to a fasciculating muscle (Forster and Alpers, 1944) or anaesthetic blockade of the nerve trunk supplying it (Denny-Brown, 1949) does not abolish fasciculation. Second, a study of the waveforms of fasciculation potentials shows that sometimes individual fibre components can be recognized that occur in a different order, implying different generator sites in the same motor unit arborization (Conradi et al., 1982). Third, it has been shown that fasciculation potentials evoke an F-response and, by using the fasciculation potential to trigger stimulation of the nerve trunk, blocking of the F-response only occurs when the stimulus is given such that collision occurs distally (Roth, 1982, 1984).

Some fasciculation potentials in amyotrophic lateral sclerosis however, may arise in more proximal sites (Denny-Brown and Pennybacker, 1938); it has been shown for instance, that some fasciculation potentials in amyotrophic lateral sclerosis can be driven by weak transcranial magnetic stimuli to the motor cortex (Mills, 1995; de Carvalho, 2000). Study of so-called combined fasciculations (Hirota et al., 2000), meaning fasciculation potentials with two or more components that could occur independently, suggested that these could arise at supraspinal sites although it was conceded that multiple distal generators could also explain the phenomenon. Furthermore, a recent multi-electrode surface EMG study (Drost et al., 2007; Kleine et al., 2008) has shown that inter-fasciculation intervals may follow either a Poisson distribution, as would be expected from a random rare event, or a more symmetrical distribution of shorter intervals (3–15 ms), generally referred to as double fasciculation potentials. On the basis of the known refractoriness of cell body membrane as opposed to axonal membrane, the former were postulated to be related to spinal motor neuron subexcitability from 50 to 80 ms post-discharge, whereas the latter were thought to occur during the phase of axonal superexcitability following the first discharge (Bostock et al., 1995; Kiernan et al., 2001).

In the benign fasciculation syndrome, fasciculation potentials occur predominantly, but not exclusively, in the distal leg muscles. The origin of these fasciculation potentials is unknown. It has been suggested (Trontelj and Stalberg, 1977; Janko et al., 1989) that benign fasciculations may arise by ephaptic transmission from irregularly fibrillating fibres. Others suggest that benign fasciculations arise proximally (Sindermann et al., 1973). Study of benign fasciculations in upper limb muscles of three patients (de Carvalho and Swash, 1998) found fasciculation potentials had stable waveforms and a firing rate higher than those in amyotrophic lateral sclerosis. It has also been suggested (Guiloff, 1995) that benign fasciculation potentials are more likely to be able to be activated voluntarily than amyotrophic lateral sclerosis fasciculation potentials and have larger amplitudes than voluntary units in the same muscle. There has been no large-scale study, however, examining a wider group of parameters including an assessment of waveform stability, complexity and firing interval distribution in benign fasciculation syndrome.

The aim of the present study was to first, determine if benign and amyotrophic lateral sclerosis fasciculations can be distinguished on the basis of their waveform or firing characteristics. Second, the evolution of fasciculation potential parameters with disease progression was examined; the assumption being that muscles showing no weakness and no neurogenic EMG change were at an earlier stage of evolution than those showing severe weakness and marked neurogenic EMG change. The relationship of fasciculation potentials to the degree of lower motor neuron abnormality in amyotrophic lateral sclerosis remains controversial. For instance, it is not known whether or not fasciculation potentials arise solely in muscle that has undergone denervation and reinnervation (Guiloff and Modarres-Sadeghi, 1992); certainly fasciculation potentials can be detected clinically in muscles that are not weak (de Carvalho, 2000). Third, by studying the waveforms of double fasciculations, the site of generation of these double discharges could be examined.

Materials and methods

Patients with a potential diagnosis of amyotrophic lateral sclerosis or benign fasciculation syndrome attending a neurophysiology clinic were examined. The recordings made from patients were part of their standard EMG investigation for potential amyotrophic lateral sclerosis and the choice of muscle in each patient was dictated by the clinical situation. In order to maximize the clinically useful information, many muscles in patients with amyotrophic lateral sclerosis were not, for example, clinically weak. All patients underwent upper and lower limb motor and sensory nerve conduction studies to exclude other diagnoses. From an initial series of 57, there were 28 patients with amyotrophic lateral sclerosis who at their initial or at subsequent visits fulfilled the El Escorial criteria as modified by the Awaji consensus (Brooks, 1994; de Carvalho et al., 2008). There were 11 patients
with benign fasciculation syndrome who had fasciculation usually in lower limb muscles but no weakness or reflex change. These patients presented in a variety of ways: seven came to medical attention because they had themselves noticed leg muscle twitches and were concerned they might have amyotrophic lateral sclerosis, two had non-specific sensory disturbance in the legs or feet and were noted to have fasciculation by referring physicians and two had leg muscle stiffness thought by referring physicians to represent an upper motor neuron abnormality. There were six patients with probable amyotrophic lateral sclerosis who did not fulfil the criteria and 18 patients with a variety of other conditions (familial amyotrophic lateral sclerosis, primary lateral sclerosis, radiculopathy, neuropytotonia, multifocal motor neuropathy and old polio) whose data are not included here. The mean age (range) of the patients with amyotrophic lateral sclerosis was 63 (40–80) years and that of the patients with benign fasciculation syndrome was 52 (27–71) years. Onset of symptoms in the patients with amyotrophic lateral sclerosis was bulbar in 4 and limb in 24. The median (range) duration of history in amyotrophic lateral sclerosis patients was 18 (3–60) months and that of patients with the benign fasciculation syndrome was 65 (6–360) months. In each patient up to four muscles were examined depending on the clinical situation. In the patients with amyotrophic lateral sclerosis, a total of 63 muscles were examined (tibialis anterior = 26, biceps = 10, first dorsal interosseus = 23, trapezius = 2, vastus medialis = 1, extensor digitorum communis = 1) and in the patients with benign fasciculation syndrome a total of 21 muscles were examined (medial gastrocnemius = 9, tibialis anterior = 8, first dorsal interosseus = 3, biceps = 1). The firing characteristics of fasciculation potentials in amyotrophic lateral sclerosis in a subset of these data have already been reported (Mills, 2010).

In order to investigate whether fasciculation potential characteristics varied with the degree of weakness and denervation in a given muscle, a scheme, combining clinical and qualitative EMG findings in each muscle, was devised as follows: (i) Stage 1, no clinical weakness and no evidence of acute or chronic partial denervation on EMG; (ii) Stage 2, no clinical weakness but mild acute and/or chronic neurogenic signs on EMG; (iii) Stage 3, mild weakness (Medical Research Council 4) and signs of acute and/or chronic denervation on EMG; and (iv) Stage 4, moderate or severe weakness (Medical Research Council 3 and below) and marked acute and chronic neurogenic signs on EMG. The qualitative EMG features taken to indicate acute denervation were the presence of fibrillation potentials and/or positive sharp waves; these were judged as mild to marked depending on their density at five needle positions. Chronic partial denervation on EMG was assessed as mild to marked using the amplitude of early recruited motor unit potentials with values >10 mV taken as abnormal, a qualitative assessment of the stability of early recruited motor units, the completeness of the recruitment pattern and the firing rate of motor units during maximal activation.

A fine (30G) 25 mm concentric EMG needle was inserted into the relaxed muscle. No ‘tuning’ of needle position was undertaken apart from ensuring by observing a small voluntary activation that the needle was indeed in muscle. EMG signals were amplified and band pass filtered between 10 Hz and 10 kHz before digitization at a sampling rate of 20 kHz giving a time resolution of 50 μs. A threshold trigger was used to initiate data collection by a computer running Cambridge Electronic Design Signal software (Cambridge Electronic Design, Cambridge); 100 ms of data before and 100 ms after the triggering fasciculation potential were collected. The triggering level was set at ±25 μV. Recording continued until 50–200 fasciculation potentials had been recorded. The time of each fasciculation from the start of the recording was automatically logged allowing inter-fasciculation intervals to be computed. The mean duration of recordings ranged from 53.7 to 709.9 s in patients with amyotrophic lateral sclerosis and from 149.9 to 1009.4 s in patients with the benign fasciculation syndrome.

Data analysis

In all records, multiple fasciculation potential waveforms could be identified. The waveforms of all fasciculation potentials were examined by eye and grouped into sets with at least one constant component (Figs 1 and 2). The classification was verified by superimposing the waveforms. Waveforms that were clearly fibrillation potentials or positive sharp waves were eliminated from further analysis. Thus the shapes and firing characteristics of individual fasciculation potentials could be measured. Fasciculations occurring just once during the recording were also noted and their waveforms characterized, as was the percentage of these single fasciculations in the total recording. In all a total of 430 fasciculation potentials from patients with amyotrophic lateral sclerosis and 191 fasciculation potentials from patients with benign fasciculation syndrome were analysed.

The duration of each fasciculation potential was measured by estimating the mean baseline level and its standard deviation in the 50 ms at the start of the sweep. Levels at the mean ± 3 SD were used as cut-off points to determine fasciculation potential duration (i.e. the time in ms from the initial deviation from baseline to its final return to baseline). Between these two time points the rectified area and peak-to-peak amplitude of the fasciculation potential were measured. The number of turns in each fasciculation potential was determined, with a turn defined as a change in polarity of amplitude at least 3 SD of the baseline level. The waveforms of individual fasciculation potentials in both amyotrophic lateral sclerosis and benign fasciculation syndrome clearly showed that on some occasions an individual component of the fasciculation potential was absent (Figs 1, 2 and 4D). This was taken as evidence of intramuscular axonal conduction block. The fraction of fasciculation potentials showing evidence of axonal conduction block was measured in amyotrophic lateral sclerosis and benign fasciculation syndrome. In order to measure individual fasciculation potential stability, superimposed records were viewed and a horizontal cursor placed at the level of maximum time variability. The interval between two vertical cursors cutting the fasciculation potentials at this horizontal level measured the time variability (Fig. 4C). Two other measures of fasciculation potential stability were used: the coefficients of variation (SD/mean) of fasciculation potential area and fasciculation potential peak-to-peak amplitude.

In addition, the occurrence of double fasciculations was noted. A double fasciculation potential was, for this purpose, defined as any discharge occurring in the 100 ms following the triggering fasciculation potential. The inter-fasciculation interval was measured. These double discharges were further classified according to whether the second discharge was the same fasciculation potential (DSFP) (Fig. 9) as the first or different (DDFP).

The frequency distribution of each variable in the fasciculation potentials of amyotrophic lateral sclerosis and benign fasciculation syndrome was examined for normality using the Normal plot method. Essentially, the cumulative frequency distribution of the data was plotted against that of a normal distribution with the same mean and variance, linearity indicating the likelihood of the distribution being normal. All were found to have skewed distributions (Fig. 3A and C) indicated on Normal plots as curves. Log transformation, however, rendered the distributions normal, indicated by approximate linearity on the Normal plots (Fig. 3B and D). Thus testing the difference in these parameters between amyotrophic lateral...
**Figure 1** Fasciculations recorded from a single needle site in the first dorsal interosseous muscle in a patient with amyotrophic lateral sclerosis. In each case a number of waveforms have been superimposed to indicate waveform variability. Arrows indicate clear examples of components showing intermittent appearance suggestive of axonal conduction block. Bars indicate 0.1 mV and 5 ms.

**Figure 2** Fasciculation potentials recorded from a single needle site in the tibialis anterior in a patient with benign fasciculation syndrome. In each case a number of waveforms have been superimposed to indicate waveform variability. Arrows indicate clear examples of components showing intermittent appearance suggestive of axonal conduction block. Bars indicate 0.1 mV and 5 ms.
sclerosis and benign fasciculation syndrome and between groups of patients with amyotrophic lateral sclerosis at various stages of the disease was done using a $t$-test assuming unequal variances on the log-transformed data. In tables, data are presented for easier understanding as median and range.

**Results**

**General fasciculation potential characteristics**

At a single recording position up to 15 individual fasciculation potentials could be identified (Figs 1 and 2). The mean ($\pm$SD) number in amyotrophic lateral sclerosis was $7.2 \pm 2.6$ and $9.2 \pm 2.6$ in benign fasciculation syndrome. These means are significantly different [$t$-test: $t(34) = 3.06, P = 0.004$]. Fasciculations occurring just once during the recording amounted to an average of $19.7 \pm 13.0\%$ of the total number of fasciculation potentials in each record in amyotrophic lateral sclerosis and $29.5 \pm 13.0\%$ in benign fasciculation syndrome. These means are also significantly different [$t$-test: $t(34) = 2.99, P = 0.005$]. Double fasciculations of both types and evidence of axonal conduction block occurred in both amyotrophic lateral sclerosis and benign fasciculation syndrome. Amplitude, area, duration and turn count all showed skewed distributions in both amyotrophic lateral sclerosis and benign fasciculation syndrome, but none showed evidence of bimodality as might be expected if, as some authors (de Carvalho and Swash, 1998) have suggested, fasciculation potentials can be segregated into simple and complex or into stable and unstable. Turn count, for example, a simple measure of complexity, showed a continuous unimodal distribution in both amyotrophic lateral sclerosis and benign fasciculation syndrome fasciculation potentials (Supplementary Fig. 10).

**Comparison of amyotrophic lateral sclerosis fasciculations with benign fasciculations**

The measurements in amyotrophic lateral sclerosis fasciculation potentials and benign fasciculation syndrome fasciculation potentials are set out in Table 1. The peak-to-peak amplitude of amyotrophic lateral sclerosis fasciculation potentials did not differ significantly from that of benign fasciculation syndrome [$t$-test: $t(303) = 1.02, P = 0.31$], nor did fasciculation potential area [$t$-test: $t(325) = 0.49, P = 0.63$]. Fasciculation potential duration, however, was significantly longer in benign fasciculation syndrome compared with amyotrophic lateral sclerosis fasciculation potentials [$t$-test: $t(338) = 2.85, P = 0.005$]. The number of turns in amyotrophic lateral sclerosis fasciculation potentials was significantly greater than in benign fasciculation syndrome fasciculation potentials [$t$-test: $t(426) = 3.75, P = 0.0002$]. The time variability (Fig. 4C) was also significantly different between the two conditions.
greater in amyotrophic lateral sclerosis fasciculation potentials compared with benign fasciculation syndrome fasciculation potentials \([t\text{-test}: \ t(356) = 2.1, \ P = 0.04]\). However, the coefficients of variation in peak-to-peak amplitude were not significantly different \([t\text{-test}: \ t(339) = 1.55, \ P = 0.12]\), nor were the coefficients of variation in fasciculation potential area \([t\text{-test}: \ t(330) = 1.13, \ P = 0.26]\). The interval between fasciculation potentials (Fig. 3) was significantly shorter in amyotrophic lateral sclerosis compared with benign fasciculation syndrome \([t\text{-test}: \ t(346) = 6.99, \ P < 0.0001]\). Evidence of axonal conduction block was found in 55.5% of benign fasciculation syndrome fasciculation potentials and in 54.2% of amyotrophic lateral sclerosis fasciculation potentials; these percentages are not significantly different \([\chi^2(1) = 0.09, \ P > 0.2]\). DSFPs were seen in 15.4% of amyotrophic lateral sclerosis fasciculation potentials and 6.8% of benign fasciculation syndrome fasciculation potentials; this is a significant difference \([\chi^2(1) = 8.69, 0.01 < P > 0.001]\). Similarly, DDFPs occurred in 48.1% of amyotrophic lateral sclerosis fasciculation potentials and in 23.0% of benign fasciculation syndrome fasciculation potentials; again this is a highly significant difference \([\chi^2(1) = 34.6, P < 0.0001]\). Complex fasciculation potentials may be defined as those having either more than four phases, increased amplitude or increased duration compared to normal values for motor unit potentials in the specified muscle.

### Table 1 Fasciculation potential parameters in amyotrophic lateral sclerosis and benign fasciculation syndrome

<table>
<thead>
<tr>
<th>Measurement</th>
<th>ALS Median (Range)</th>
<th>BFS Median (Range)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>430</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>0.41 (0.03–10.0)</td>
<td>0.31 (0.04–7.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Area (mV ms)</td>
<td>16.8 (6.9–400.0)</td>
<td>15.4 (1.4–350.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>14.6 (4.5–69.3)</td>
<td>16.9 (4.8–67.7)</td>
<td>0.005</td>
</tr>
<tr>
<td>Turns</td>
<td>4.7 (1.8–24.5)</td>
<td>4.3 (2.1–13.8)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Time variability (ms)</td>
<td>1.06 (0.07–12.61)</td>
<td>1.05 (0.05–7.30)</td>
<td>NS</td>
</tr>
<tr>
<td>Amplitude coefficient of variation</td>
<td>7.3 (0.01–60.9)</td>
<td>7.1 (0.02–85.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Area coefficient of variation</td>
<td>12.0 (0.03–70.3)</td>
<td>11.1 (0.05–63.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Discharge interval (s)</td>
<td>24.7 (0.41–631.6)</td>
<td>57.4 (0.47–761.9)</td>
<td>&gt;0.00001</td>
</tr>
</tbody>
</table>

*A\text{-test} on log-transformed data.
ALS = amyotrophic lateral sclerosis; BFS = benign fasciculation syndrome; NS = not significant.

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**Figure 4** Fasciculation potentials recorded from patients with amyotrophic lateral sclerosis and benign fasciculation syndrome. (A and B) Fasciculation potential from a patient with amyotrophic lateral sclerosis showing multifocal distal triggering; the arrowed component fires at different times within the fasciculation potential. (C) Fasciculation potential from a patient with benign fasciculation syndrome showing time variability of a single component; the time variability is measured as shown. (D) Fasciculation potential from a patient with benign fasciculation syndrome showing intermittent conduction block of one component.
(de Carvalho et al., 2008). Using this definition, 39.7% of amyotrophic lateral sclerosis fasciculation potentials and 32.1% of benign fasciculation syndrome fasciculation potentials were complex, i.e. the incidence of complex fasciculation potentials in benign fasciculation syndrome and amyotrophic lateral sclerosis does not differ significantly \( \left( \chi^2(1) = 3.3, P = 0.1 \right) \).

### Differences between weak and normal muscles in amyotrophic lateral sclerosis

Measurements on fasciculation potentials in weak and normal strength muscles are set out in Table 2. The peak-to-peak amplitude of amyotrophic lateral sclerosis fasciculation potentials in weak muscles did not differ significantly from that of fasciculation potentials in normal strength muscles \( \left( t\text{-test: } t(350) = 0.16, P = 0.87 \right) \), nor did the fasciculation potential area \( \left( t\text{-test: } t(327) = 1.9, P = 0.06 \right) \), but the duration of fasciculation potentials was significantly shorter in weak muscles \( \left( t\text{-test, } t(329) = 2.93, P = 0.004 \right) \). The number of turns in amyotrophic lateral sclerosis fasciculation potentials in weak muscles was not significantly different from those in normal strength muscles \( \left( t\text{-test: } t(352) = 2.26, P = 0.024 \right) \) nor was the time variability \( \left( t\text{-test: } t(327) = 1.32, P = 0.19 \right) \). However, the coefficient of variation of area \( \left( t\text{-test: } t(420) = 2.83, P = 0.005 \right) \) was smaller in the fasciculation potentials in weak muscles compared to those in normal strength muscles. Amyotrophic lateral sclerosis fasciculation potentials in weak muscles occurred at significantly shorter intervals than in muscles of normal strength \( \left( t\text{-test: } t(357) = 4.49, P < 0.0001 \right) \). Evidence of axonal conduction block was found in 52.4% of fasciculation potentials in weak muscles and in 55.3% of fasciculation potentials in non-weak muscles; these percentages are not significantly different \( \left( \chi^2(1) = 0.34, P < 0.2 \right) \). DSFPs were found in 21.7% of fasciculation potentials in weak muscles and in 11.7% of non-weak muscles; this is a significant difference \( \left( \chi^2(1) = 7.66, 0.01 < P < 0.001 \right) \). DDFPs occurred in 48.2% of fasciculation potentials from weak muscles and in 48.1% of fasciculation potentials from normal strength muscles; this is not a significant difference \( \left( \chi^2(1) = 0.1, P < 0.9 \right) \).

### Fasciculation potential parameters in relation to degree of neurogenic abnormality

Fasciculation potential parameters at the various stages of muscle weakness and denervation are set out in Table 3. Amplitude, area, turns and time variability of fasciculation potentials showed no significant changes across the stages. Fasciculation potential duration was significantly shorter in Stage 3 than in Stage 1 \( \left( t\text{-test: } t(142) = 5.1, P < 0.001 \right) \). Fasciculation potential variability, as assessed by the coefficient of variation of amplitude, was significantly greater in Stage 4 when compared to Stage 1 \( \left( t\text{-test: } t(111) = 1.9, P = 0.06 \right) \) and the coefficient of variation of fasciculation potential area was significantly greater in Stage 3 compared with Stage 1 \( \left( t\text{-test: } t(162) = 2.1, P = 0.04 \right) \) and in Stage 4 compared with Stage 1 \( \left( t\text{-test: } t(194) = 2.3, P = 0.03 \right) \). Discharge interval (Fig. 5) was significantly shorter in Stage 4 compared with Stage 1 \( \left( t\text{-test: } t(210) = 4.43, P < 0.0001 \right) \) and shorter in Stage 4 compared with Stage 2 \( \left( t\text{-test: } t(208) = 6.5, P < 0.0001 \right) \). There were no significant differences, however, in the percentage of fasciculation potentials showing evidence of axonal conduction block across the four stages. The incidence of DSFPs increased significantly in Stage 4 (Fig. 6); comparing Stages 1 and 4, the percentage of DSFPs increased by a factor of three \( \left[ \text{Chi square test: } \chi^2(1) = 12.2, P < 0.001 \right] \). DDFPs, however, showed no significant changes in incidence over the four stages.

### Double fasciculation potentials

Pooled time interval histograms of DDFPs and DSFPs were constructed to compare amyotrophic lateral sclerosis with benign fasciculation syndrome (Fig. 7) and to compare different muscles in amyotrophic lateral sclerosis (Fig. 8). In all muscles and in both amyotrophic lateral sclerosis and benign fasciculation syndrome, there was an early phase of double discharge with a 4–10 ms interval. Comparing DSFPs from tibialis anterior in amyotrophic lateral sclerosis and benign fasciculation syndrome, the mean inter-fasciculation interval in this early band was 6.95 (±1.9) ms in benign fasciculation syndrome and 7.02 (±2.8) ms in amyotrophic lateral sclerosis. These are not significantly different.

#### Table 2 Fasciculation potential parameters in weak and non-weak muscles in amyotrophic lateral sclerosis

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Muscle weakness Median (range)</th>
<th>No muscle weakness Median (range)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>166</td>
<td>264</td>
<td></td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>0.38 (0.04–2.21)</td>
<td>0.41 (0.034–10.01)</td>
<td>NS</td>
</tr>
<tr>
<td>Area (mV/ms)</td>
<td>14.84 (1.02–135.00)</td>
<td>18.76 (0.92–399.10)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>12.92 (4.46–69.30)</td>
<td>15.94 (4.54–60.69)</td>
<td>0.004</td>
</tr>
<tr>
<td>Turns</td>
<td>4.5 (2–19)</td>
<td>5 (1.8–24.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Time variability (ms)</td>
<td>1.03 (0.13–12.61)</td>
<td>1.07 (0.07–11.59)</td>
<td>NS</td>
</tr>
<tr>
<td>Amplitude coefficient of variation</td>
<td>8.30 (0.04–44.59)</td>
<td>6.48 (0.10–60.85)</td>
<td>NS</td>
</tr>
<tr>
<td>Area coefficient of variation</td>
<td>12.82 (0.51–790.27)</td>
<td>11.04 (0.03–61.32)</td>
<td>0.005</td>
</tr>
<tr>
<td>Discharge interval (s)</td>
<td>18.01 (0.41–231.48)</td>
<td>30.40 (0.53–631.56)</td>
<td>&gt;0.0001</td>
</tr>
</tbody>
</table>

*t-test on log-transformed data. NS = not significant.
Comparing DSFPs from weak and strong muscles in amyotrophic lateral sclerosis, the mean inter-fasciculation interval in weak muscles was 6.39 ± 2.32 ms and in strong muscles was 8.33 ± 3.43 ms. The interval was significantly longer in non-weak muscles \( t \)-test: \( t(22) = 2.03, P = 0.05 \). DSFPs were too infrequent to allow a comparison across the different stages of disease. Of the 48 DSFPs in amyotrophic lateral sclerosis, the waveforms of the two fasciculation potentials in this early peak were identical in 38 (79.1%) (Fig. 8A).

In tibialis anterior and medial gastrocnemius, DSFPs in both amyotrophic lateral sclerosis and benign fasciculation syndrome occurred also at an interval of 30–50 ms (Fig. 8B); this later peak of double firing was not detected in other muscles. In five amyotrophic lateral sclerosis muscles, DSFPs were frequent enough to permit a more detailed analysis (Fig. 9). It was noted that whereas the first discharge could have a variable waveform suggesting multiple distal axonal triggering, the second discharge was identical on all occasions. In benign fasciculation syndrome, DSFPs at ~40 ms were identical on 13/14 (93%) occasions.

Interval histograms of DDFPs were flat, indicating a uniform probability of a different fasciculation potential occurring after the triggering fasciculation potential; this held true in both amyotrophic lateral sclerosis and benign fasciculation syndrome, across muscles in amyotrophic lateral sclerosis and at the various stages of neurogenic change in amyotrophic lateral sclerosis.

**Discussion**

The question as to whether benign fasciculation syndrome and amyotrophic lateral sclerosis fasciculation potentials can be

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**Table 3 Fasciculation parameters in relation to degree of neurogenic change**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Stage 1 Median (range)</th>
<th>Stage 2 Median (range)</th>
<th>Stage 3 Median (range)</th>
<th>Stage 4 Median (range)</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>111</td>
<td>161</td>
<td>62</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>0.41 (0.06–4.6)</td>
<td>0.44 (0.03–10.0)</td>
<td>0.31 (0.04–1.7)</td>
<td>0.41 (0.07–2.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Area (mV ms)</td>
<td>17.7 (1.4–112.2)</td>
<td>20.4 (0.92–400)</td>
<td>8.9 (1.0–135)</td>
<td>15.7 (1.0–129.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>15.9 (6.8–60.7)</td>
<td>16.4 (4.5–69.3)</td>
<td>11.3 (4.5–41.9)</td>
<td>14.2 (4.5–62.9)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Turns</td>
<td>5.0 (2–20)</td>
<td>5.0 (1.8–24.5)</td>
<td>3.5 (2–11)</td>
<td>5.0 (2–19)</td>
<td>NS</td>
</tr>
<tr>
<td>Time variability (ms)</td>
<td>0.97 (0.07–4.7)</td>
<td>1.2 (0.08–11.6)</td>
<td>1.03 (0.16–7.24)</td>
<td>0.97 (0.13–12.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Amplitude coefficient of variation</td>
<td>6.5 (0.04–60.9)</td>
<td>6.4 (0.01–39.3)</td>
<td>7.6 (0.84–36.3)</td>
<td>9.4 (0.04–44.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Area coefficient of variation</td>
<td>10.8 (0.03–61.3)</td>
<td>11.2 (0.04–52.3)</td>
<td>12.7 (1.0–70.3)</td>
<td>13.3 (0.5–57.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Discharge interval (s)</td>
<td>25.9 (1.0–261.3)</td>
<td>33.4 (0.52–631.5)</td>
<td>26.3 (0.4–182.3)</td>
<td>11.9 (0.6–231.5)</td>
<td>&gt;0.001</td>
</tr>
</tbody>
</table>

\( t \)-test on log-transformed data. NS = not significant.
distinguished based on their waveform of firing characteristics is first addressed. The amplitude, area, time variability of components and complexity as measured by the coefficients of variation of amplitude or area are not different. Evidence of axonal conduction block is also present in both conditions; so are DSFPs, but they are far more frequent in amyotrophic lateral sclerosis. Firing interval is random in both conditions but is significantly longer in benign fasciculation syndrome. Clearly then, in the individual case, there are no features distinguishing the two conditions.

Instability and complexity have generally been held to be characteristic of the fasciculation potentials seen in amyotrophic lateral sclerosis, whereas those in benign fasciculation syndrome are thought to have relatively simple waveforms. The current results refute this and show that in both conditions fasciculation potentials can be highly complex.

It is worth considering the sources of this complexity, and three main factors should be considered. Assuming that most fasciculation potentials arise from distal generator sites, one source of such complexity is intermittent axonal conduction block evidenced by some components of the fasciculation potential making an intermittent appearance (Figs 1, 2 and 5). Evidence of axonal conduction block has been found in both amyotrophic lateral sclerosis and benign fasciculation syndrome. This would argue that membrane abnormalities making axons intermittently subject to block are present in both amyotrophic lateral sclerosis and benign fasciculation syndrome, although the mechanisms of block may not necessarily be the same in each condition.

A second factor contributing to complexity is variability in axonal conduction time, manifesting as variability in the discharge of an identifiable component of the fasciculation potential. Again, evidence of such variability has been found in both amyotrophic lateral sclerosis and benign fasciculation syndrome (Fig. 4). The time variability parameter, measuring the maximal time variation of a fasciculation potential component in relation to the triggering component, was not significantly different between the two conditions. Thus factors causing variation in axonal conduction, which may be the same as the mechanisms causing axonal block, are also present in both conditions. Factors such as axonal diameter, internodal distance, state of myelination, temperature and so on, clearly play no role in these short-term variations in axonal conduction. It is more probable that there are changes in membrane potential due to imbalance of ionic conductances.

A third factor that may contribute to complexity of fasciculation potentials is multifocal and possibly intermittent distal axonal triggering of fasciculation potentials. The effect of this will be to produce a different order of firing of the components of a given fasciculating motor unit (Fig. 4A and B). Because all components of the fasciculation potential may be subject to additional time variability and/or conduction block, the effect of having multiple distal generator sites may be masked. Distinguishing multifocal distal triggering from extreme timing variability due to insecurity of axonal conduction is difficult. However, the fact that up to 15 identifiable waveforms can be recognized at a single recording site sheds some light on this. A simple consideration of the numbers of fibres within range of the needle tip shows that a needle of the dimensions used here will record from ~200 fibres. Assuming a random distribution of fibres within the motor unit, this means that fibre representation from about 40 motor units will be seen by the needle (~5 fibres per motor unit). In muscles undergoing denervation and reinnervation, grouping of fibres will further reduce the number of motor units within the pick-up volume. If we see 15 identifiable waveforms, then ~40% of motor units within the pickup volume of the needle would be fasciculating, which seems counterintuitive. However, if each fasciculating motor unit has 2 or more generator sites, then the percentage of fasciculating units is reduced commensurately. It is easy to show by modelling that summation of, for example, five fibre components to produce a fasciculation potential can produce quite different waveforms if each fibre’s contribution varies by a few milliseconds on each occasion. This latter argument also has an effect upon what has hitherto been referred to as fasciculation discharge interval; in effect what is probably being reported is the discharge interval of individual generator sites. Furthermore, the fact that many identifiable waveforms at a single needle site are seen in both amyotrophic lateral sclerosis and benign fasciculation syndrome suggests that distal multifocal triggering also occurs in both conditions.

**Double fasciculation potentials**

In both amyotrophic lateral sclerosis and benign fasciculation syndrome, double fasciculation potentials with the two discharges
from the same motor unit were seen in all muscles investigated. The incidence of these DSFPs was significantly higher, however, in amyotrophic lateral sclerosis. The interval between fasciculation potentials was in the range 4–10 ms; an interval that did not differ significantly between muscles or between amyotrophic lateral sclerosis and benign fasciculation syndrome. It is well known that after the discharge of an action potential the axonal membrane first becomes refractory and then some 3–20 ms afterwards a phase of superexcitability is seen (Bostock et al., 1995; Mogyoros et al., 1998; Kiernan et al., 2001). It is tempting therefore to attribute the early range DSFPs to axonal superexcitability. If the axon containing the fasciculation potential generator is situated proximal to the terminal arborization within the muscle and before branch points, then the fibres of the fasciculating unit will fire in the same order on each occasion and therefore the fasciculation potential waveform will be invariant (ignoring axonal conduction block and variation in fibre conduction time). Similarly, if there is just one fasciculation potential generator situated in one of the branches of the terminal arborization, then again the fasciculation potential waveform will be the same on each occasion. If however, there are more than one fasciculation potential generators in the terminal arborization, then the fasciculation potential waveform will vary because of a different order of fibre activation, dependent on which fasciculation potential generator is active. Thus the fact that short interval DSFPs were almost always of identical waveform suggests that the double discharge arose from the same point in the motor unit arborization.

The findings in the tibialis anterior are also of interest with a peak of interfasciculation intervals at about 40 ms. Purely on timing grounds, this could represent either an F-response or a monosynaptic reflex transmitted over fast conducting afferent fibres. A fasciculating motor unit could activate stretch or tension receptors...
in the muscle. The lack of interaction between different fasciculation potentials, however, argues against a reflex cause for double fasciculation potentials. From Fig. 9 it can be seen that the second of the two discharges has exactly the same waveform on each occasion, whereas the first may vary. If we postulate that after generation in the terminal arborization, impulses propagate antidromically to the motor neuron and then back to the muscle, then the order of fibre activation would be identical for the latter but may vary for the first fasciculation potential.

The question of how fasciculation potentials in amyotrophic lateral sclerosis evolve as the disease progresses is next addressed. Comparing weak and strong muscles in amyotrophic lateral sclerosis, it has been found that waveform parameters such as amplitude, area, time variability and turns in fasciculation potentials did not differ, but fasciculation potential duration was longer in strong muscles and area variability was greater in weak muscles. The most marked difference was in fasciculation potential discharge rate being faster in weak muscles. Comparing fasciculation potentials in muscles showing no weakness and no EMG neurogenic change (Stage 1) with those showing marked weakness and clear neurogenic change (Stage 4) also shows no great differences in fasciculation potential parameters, apart from area variability and discharge rate. It is then argued that the major change, as amyotrophic lateral sclerosis progresses, is the rate of generation of fasciculation potentials. The abnormal membrane properties in amyotrophic lateral sclerosis distal axons must therefore be such that they produce greater and greater instability as the disease evolves.

Axonal excitability studies (Krishnan et al., 2009) have been used to make inferences about axonal conductances in amyotrophic lateral sclerosis axons. Dysfunction of voltage-dependent K⁺ channels was first postulated (Bostock et al., 1995) and subsequently an abnormality of persistent Na⁺ channels (Mogyoros et al., 1998) was implicated as causes of the axonal superexcitability responsible for fasciculation. More recently, knowing that the predominant generator of fasciculation potentials was distally in the terminal arborization, studies of axonal excitability at the motor point showed more pronounced abnormalities than over the nerve trunk (Nakata et al., 2006). Results supported both persistent Na⁺ channel and voltage-gated K⁺ channel abnormalities as factors in the generation of fasciculation potentials. Therefore it is established that a distal membrane abnormality, probably causing a reduction of resting membrane potential, is present in amyotrophic lateral sclerosis. No such abnormalities were found, however, in six patients with benign fasciculation syndrome (Bostock et al., 1995). The DSFPs reported here occur at intervals of 4–10 ms, the time of axonal superexcitability in the post-action potential phase (Kiernan et al., 2000). Thus the membrane abnormality in amyotrophic lateral sclerosis could also manifest as double discharge of the fasciculation potential generated from the same area of membrane, as has been shown in these studies.

It is worth considering to what extent these findings affect the criteria for diagnosis of amyotrophic lateral sclerosis. Fasciculations should never, of course, be considered in isolation but should always be viewed in the context of other clinical and EMG findings. The retrospectively validated Awaji-shima criteria for the diagnosis of amyotrophic lateral sclerosis give equal emphasis to clinical and EMG findings (Boekestein et al., 2010; Douglass et al., 2010). For the evaluation of abnormalities in a given body region without clinical signs of a lower motor neuron abnormality (e.g. wasting), EMG features of chronic neurogenic change are now used in the diagnosis. Specifically, in the presence of chronic neurogenic change, fasciculation potentials carry the same weight as fibrillations and positive waves. The criteria, however, state that fasciculation potentials should preferably have a complex morphology; given the findings in the current study of a spectrum of fasciculation potential complexity from simple to highly complex in amyotrophic lateral sclerosis, it may render the criteria even more sensitive if all fasciculations in amyotrophic lateral sclerosis were taken as evidence of denervation (Kleine et al., 2010). It should also be noted that whilst fasciculation potentials have considerable diagnostic importance, they hold no prognostic information (Mateen et al., 2008).

In conclusion, fasciculation potentials in amyotrophic lateral sclerosis and benign fasciculation syndrome are indistinguishable on grounds of fasciculation potential waveform parameters, but those in amyotrophic lateral sclerosis have a higher discharge rate.
In both amyotrophic lateral sclerosis and benign fasciculation syndrome, multifocal distal triggering, axonal conduction variability and axonal conduction block contribute to variability in fasciculation potential wave shape. Fasciculation potential discharge rate increases as amyotrophic lateral sclerosis evolves and DSFPs then become more prominent, suggesting the axonal membrane abnormality is also more marked.

Supplementary material

Supplementary material is available at Brain online.

References