First-recruited motor units adopt a faster phenotype in amyotrophic lateral sclerosis

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Key points

- Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disorder of motor neurons, carrying a short survival.
- High-density motor unit recordings permit analysis of motor unit size (amplitude) and firing behaviour (afterhyperpolarization duration and muscle fibre conduction velocity).
- Serial recordings from biceps brachii indicated that motor units fired faster and with greater amplitude as disease progressed.
- First-recruited motor units in the latter stages of ALS developed characteristics akin to fast-twitch motor units, possibly as a compensatory mechanism for the selective loss of this motor unit subset.
- This process may become maladaptive, highlighting a novel therapeutic target to reduce motor unit vulnerability.

Abstract  Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder with a median survival of 3 years. We employed serial high-density surface electromyography (HDSEMG) to characterize voluntary and ectopic patterns of motor unit (MU) firing at different stages of disease. By distinguishing MU subtypes with variable vulnerability to disease, we aimed to evaluate compensatory neuronal adaptations that accompany disease progression. Twenty patients with ALS and five patients with benign fasciculation syndrome (BFS) underwent 1–7 assessments each.

After completing a PhD investigating the electrophysiology of the mammalian cochlea at the University of Brighton, Thomas Weddell retrained in medicine at Imperial College. During post-graduate training he applied previously obtained skills from his PhD on a research project on high-density EMG recordings in ALS at King’s College London. In the future he hopes to work clinically in audiovestibular medicine and collaborate on research to bring about improvements in patient treatments. After graduating from Cambridge University medical school in 2010 and commencing neurology training in 2014, James Bashford now works as a clinical lecturer at King’s College Hospital (London). His PhD focused on the detection of fasciculations in ALS patients, resulting in a novel analytical tool called the Surface Potential Quantification Engine (SPiQE). His research focuses on motor nerve pathophysiology and how the accessibility and versatility of remote home monitoring could expand our understanding of neurological disease.

Thomas Weddell and James Bashford contributed equally to the work.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating progressive neurodegenerative disorder of upper and lower motor neurons with a median survival of just 3 years from symptom onset (Al-Chalabi & Hardiman, 2013; Baumer et al. 2014). The initial events that lead to cell dysfunction and death in ALS have not been fully characterized despite extensive genetic, molecular and electrophysiological investigations in recent years (Al-Chalabi et al. 2014; Lutz, 2018; van den Bos et al. 2019). As a consequence, it has proven difficult to establish appropriate biomarkers for disease progression and identify appropriate targets for therapeutic intervention.

The motor unit (MU) is the final effector output of the motor system. It consists of a lower motor neuron, originating in the ventral horn of the spinal cord, and the muscle fibres it innervates (de Carvalho & Swash, 2016). The MU pool comprises a spectrum of activity that can be categorized into three main subtypes: slow, fatigue-resistant, small MUs (S-type); fast, fatigue-resistant MUs of intermediate size (FR-type); and, fast, fatigable, large MUs (FF-type) (Burke et al. 1971). In simple terms, the smallest MUs in a pool have the lowest threshold for activation and are recruited first, while larger MUs are recruited on increasing force, as set out by Henneman’s size principle (Milner-Brown et al. 1973; Burke, 2011). An important determinant of a lower motor neuron’s firing frequency is its prolonged (tens of milliseconds) post-spike afterhyperpolarization (AHP) (Calvin, 1974; de Carvalho & Swash, 2016). AHP is dependent on multiple potassium conductances and its duration is correlated with the twitch duration of the innervated muscle fibres (Gossen et al. 2003); S-type MUs possess longer AHPs than FF- and FR-type MUs (de Carvalho & Swash, 2016). Piotrkiewicz et al. validated a technique to estimate AHP based on an analysis of MU inter-spike interval (ISI) variability (Piotrkiewicz, 1999; Piotrkiewicz et al. 2001). This technique relies on the premise that inter-spike intervals longer than the AHP are subject to random variation (the so-called occasional spike mode), whereas intervals shorter than the AHP are under tight internal regulation adjustable by inter- and supra-spinal inputs (the so-called rhythmic firing mode) (Piotrkiewicz & Hausmanowa-Petrusewicz, 2011). Another measurable property of the MU is the muscle fibre conduction velocity, whereby more slowly conducting muscle fibres belong to the first-recruited slow-twitch MUs (Del Vecchio et al. 2018).

As lower motor neurons die in ALS, surrounding MUs compensate by reinnervating the orphaned muscle fibres. In electrophysiological recordings, this translates into the emergence of larger, polyphasic MU potentials (Kiernan et al. 2011). Importantly, S-type MUs are the most resistant to disease, maintaining an ability to reinnervate, whereas FF-type MUs die early in the disease course and contribute very little to compensatory reinnervation (Frey et al. 2000; Pun et al. 2006; Hegedus et al. 2008; Saxena et al. 2013).

Changes in MU excitability are fundamental to ALS disease progression (de Carvalho & Swash, 2016). Peripheral excitability studies have highlighted axonal ion channel dysfunction of the lower motor neuron (Bostock et al. 1995). ALS mouse models have demonstrated...
alterations in MU excitability, which differ amongst MU subtypes (Pieri et al. 2003; Kuo et al. 2005; Martin et al. 2013; Martinez-Silva et al. 2018). Some of these results have been confirmed in ALS patients (Piotrkiewicz & Hausmanowa-Petrusewicz, 2011). Fasciculations are brief spontaneous muscle fibre contractions and are one of the earliest clinical signs of ALS, reflecting pathological MU hyperexcitability (de Carvalho & Swash, 2016). A number of studies have demonstrated that fasciculating MUs are unresponsive to voluntary recruitment (Trojaborg & Buchthal, 1965; Janko et al. 1989), while others have identified a subgroup that can be recruited voluntarily (Conradi et al. 1982; Guiloff & Modarres-Sadeghi, 1992; de Carvalho & Swash, 1998; de Carvalho & Swash, 2017). The contrasting results may mirror progressive changes in the fasciculation generator site. In early disease, fasciculating MUs that are driven proximal to the terminal arborization remain recruitable, whereas in late disease fasciculating MUs with generator sites distal to the terminal arborization lose their functionality (de Carvalho & Swash, 2017).

High-density surface electromyography (HDSEMG) has become of particular interest in recent years due to the significant advantages it offers over the more widely available needle EMG (Bashford et al. 2020a). HDSEMG gives spatial and temporal information, which, when combined with advances in computing power and signal processing, allows decomposition of highly complex superimposed MU firing trains. This provides information on MU firing, amplitude and morphology (Bashford et al. 2020a). One such automated tool (progressive FastICA peel-off technique) employs independent component analysis to decompose the HDSEMG signal into its component MU spike train. This approach has been validated with both simulated and experimental data with high degrees of accuracy (Chen & Zhou, 2016; Chen et al. 2018a,b).

In this study, we characterized ectopic and voluntary MU firing patterns from biceps HDSEMG recordings during a 14-month longitudinal study of ALS patients and BFS controls (Bashford et al. 2019). We observed pathophysiological MU adaptations that may become maladaptive over time, warranting further focus as a potential route for therapeutic intervention.

Methods

Ethical approval

Patients with ALS were diagnosed according to the revised El Escorial Criteria (Brooks et al. 2000) within 24 months of symptom onset. Ethical approval was obtained from the East Midlands (Nottingham 1) Research Ethics Service (Ref: 17/EM/0221). Patients were recruited from the King’s College Hospital Motor Nerve Clinic between July 2017 and February 2018 and provided informed written consent before participation according to the Declaration of Helsinki. The study was registered at ClinicalTrials.gov (NCT03809845).

Data collection

All assessments took place in the Academic Neuroscience Centre, King’s College Hospital, London, UK. Baseline demographic data were documented on the first visit. Muscle power scores according to the Medical Research Council scale were documented from each recorded muscle by a single assessor (JB). At each assessment, 30 min of resting muscle and 1 min of light voluntary activity were recorded from biceps brachii bilaterally. Patients were asked to relax on the examination couch with forearms prone and an elbow angle of 90–120°. Before sensor placement, the skin overlying biceps was lightly scrubbed with an abrasive gel and a 70% alcohol wipe. The sensor had 64 circular electrodes (8 × 8 grid; electrode diameter 4.5 mm; inter-electrode distance 8.5 mm) and signals were amplified by the Refa-64 EMG Recording System (Twente Medical Systems International BV, The Netherlands). After 30 min of resting muscle activity, the patient was asked to initiate slight muscle contraction of biceps brachii and hold this contraction at a steady state without resistance for 1 min. Patients were aided by a real-time visual display of MU output comprising all 64 channels. In addition, the investigator (JB) provided real-time verbal feedback, instructing the patient to contract more or less strongly, in an attempt to maintain the activity of 3–4 MUs. The raw HDSEMG data were stored as a proprietary Polybench file at a sampling rate of 2048 Hz per channel.

High-density motor unit decomposition

For this and all subsequent steps of the MU parameter analysis, the two investigators (JB and TW) were blinded to the disease group of the recording (achieved through the random allocation of a 7-character scrambled alpha-numeric code to all recordings). A 10–500 Hz band-pass filter was applied to HDSEMG data. Channels that were either null or had a root mean square > 3 standard deviations away from the mean of all channels were automatically excluded. A manual checking procedure was in place to ensure appropriate exclusion of channels. The progressive FastICA peel-off technique was employed in MATLAB to decompose segments of HDSEMG data into their constituent MU firing trains (Chen & Zhou, 2016; Chen et al. 2018a,b).

In stage 1 of the analysis, we randomly selected 90 recordings, representing a cross-section of the serial dataset. The progressive FastICA peel-off technique was
used to analyse 10 s segments of voluntary contraction data from 3 subject groups (Fig. 1A): (a) ALS-STRONG: recordings from ALS individuals where the muscle was 5/5 power; (b) ALS-WEAK: recordings from ALS individuals where the muscle was 4+/5 power or less; and (c) BFS. From each group, 30 recordings were selected by a random-number generator in MATLAB.

In stage 2 (sections A–D) of the analysis, we took advantage of the longitudinal nature of the dataset. The progressive FastICA peel-off technique was applied to 30 s segments of voluntary contraction data taken from the same muscle during the same visit (Fig. 1B). This approach was applied to four muscle groups: (a) ALS-PRE: ALS muscles that achieved 5/5 power at every assessment; (b) ALS-STRONG: ALS muscles that transitioned from 5/5 power to 4+/5 power or less during the course of the study; (c) ALS-POST: ALS muscles that were weak (4+/5 or less) at the start of the study; (d) BFS. From each group, five muscles were randomly selected.

Segments of data were displayed and selected using specifically customized scripts in MATLAB. The operator ensured the chosen resting segment maximized the number and amplitude range of fasciculations within the 30 s time window. The voluntary contraction data often contained an initial period of unstable contraction, which was avoided. If decomposition returned zero MUs for a recording, two further attempts were performed (varying the chosen section each time).

**Calculation of inter-spike interval (stage 1)**

Based on timestamps provided by the decomposition tool regarding the firing of individual MUs (Fig. 1A), an ISI was calculated as the time interval between two successive MU potentials. ISIs >250 ms were excluded. Only MUs

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**Figure 1. Methodological approach**

A. regular firing of three MUs under light voluntary contraction for 10 s, as derived from the decomposition tool. Each tick represents a single MU potential. B. illustrative firing patterns of three types of MU after concatenating 30 s of resting data and 30 s of light contraction data from the same muscle: type 1 = VOL; type 2 = VOL-FASC; type 3 = FASC. C. morphological MU template across the 64-channel grid, as derived from the decomposition tool. Note the channel number in the top left corner, the peak–peak amplitude in the bottom left corner and checkboxes for manual inclusion/exclusion of each channel. Only checked channels were used to compute the median amplitude. D. example of the method (Piotrkiewicz et al. 2011) to estimate the AHP duration, as derived from the transition interval (TI). Note that the red diamonds correspond to the mean CD2 values (CD2<sub>m</sub>) for each 10 ms bin of MISI2 (shifted every 5 ms). E. corresponding plot of the instantaneous firing frequency (1/ISI) for over 300 consecutive ISIs (ISI = inter-spike interval). Note the stable baseline firing of 10–11 Hz with random peaks representative of fasciculations. [Colour figure can be viewed at wileyonlinelibrary.com]
that produced at least 40 potentials were included in the inter-group comparison.

**Categorization of the MU firing pattern (stage 2A)**

By observing the MU firing pattern during rest and light contraction (Fig. 1B), the following categorization was made: type 1 (VOL) = a MU that was only active during light voluntary contraction; type 2 (VOL-FASC) = a MU that was active during light contraction and produced fasciculations during rest; type 3 (FASC) = a MU that only produced fasciculations. Any spikes occurring at the very start of the analysis were discarded as these were considered artefactual.

**Analysis of MU potential duration, amplitude and area (stage 2B)**

In MATLAB, the template morphology of each decomposed MU potential was visualized across all 64 channels simultaneously (Fig. 1C). Channels that displayed characteristic features of a MU potential (at least two phases with clear deflections away from the baseline) were included. The onset and offset of the MU potential were manually identified from the channel with the greatest peak–peak amplitude. The following parameters were computed: MU potential duration, median peak–peak amplitude and median area under the curve (AUC).

**Estimation of the afterhyperpolarization duration (stage 2C)**

An analytical technique developed by Piotrkiewicz *et al.* (2011) was applied to each decomposed MU firing train to give an estimation of the AHP duration (Fig. 1D–E). A detailed explanation of the physiological basis and application of the technique can be found in (Piotrkiewicz & Hausmanowa-Petrusewicz, 2011). In summary, the absolute differences between each pair of adjacent ISIs (CD2 = |ISI\textsubscript{i+1} – ISI\textsubscript{i}|) for a MU firing train were calculated and plotted against the mean ISI of the corresponding ISI pairs (MISI2 = (ISI\textsubscript{i+1} + ISI\textsubscript{i})/2). Mean CD2 values (CD2\textsubscript{m}) for each 10 ms bin of MISI2 (shifted every 5 ms) were plotted against the mid-bin MISI2 value. The resultant MISI2-CD2\textsubscript{m} plot demonstrated a distinctive pattern (Fig. 1D): a short interval range (flat/slow rising section) with low variability and a long interval range (rapid rising section) with greater variability (Calvin, 1974; Matthews, 1996). The ISI at the transition between these two ranges (transition interval) correlates with the AHP duration. This estimate provides a relative measure of 'faster' (shorter AHP) and 'slower' (longer AHP) MU subtypes. Identification of the transition interval for each MU was performed both manually and automatically. The manual method involved visual inspection of the MISI2-CD2\textsubscript{m} plot. Two assessors (TW and JB) independently estimated the transition interval, applying the following rules:

1. At least seven CD2\textsubscript{m} values were required: three either side of the transition interval.
2. A clear increase in the gradient of CD2\textsubscript{m} values should be observed to the right of the transition interval compared with the left side.
3. The CD2\textsubscript{m} values to the left of the transition interval should lie on a near-horizontal line.
4. Excessively deviated CD2\textsubscript{m} values for MISI2 bins <75 ms were ignored as these represented mains interference or fasciculations.
5. The MISI2 at the transition interval should be taken as the AHP estimate.

Where estimates between the two assessors disagreed by <20%, an average of the two values was taken. Otherwise, the two assessors re-examined the data together and formed a consensus. The automated method was based on the intersection of a bilinear fit curve, whereby the x-coordinate of the intersection was recorded as the AHP estimate.

**Calculation of muscle fibre conduction velocity (stage 2D)**

A customized script was created in MATLAB for the calculation of muscle fibre conduction velocity (MFCV). Based on the morphological template of each MU determined by decomposition, six double-differential signals were computed for each of eight columns of channels, whereby each column ran parallel to the muscle fibre orientation (Fig. 2A). Cross-correlation coefficients (CC) were calculated between all double-differential signals in each column, and only signals with CC > 0.7 were included (Fig. 2B). Visual inspection of the double-differential signals ensured appropriate inclusion according to the following rules:

1. A minimum of one column was required to calculate the MFCV.
2. At least three double-differential signals must be chosen for inclusion of each column.
3. The chosen signals must either include the innervation zone (the channel with the earliest signal) or all be on the same side of the innervation zone.

The inter-electrode distance was 8.5 mm and the time delay between signals was computed by the cross-correlation coefficient function. For each column, linear regression of the distance–time graph produced...
a slope, the gradient of which was taken as the MFCV estimate for that column (Fig. 2C). Only regression models with an R-squared \( \geq 0.5 \) were included. Finally, after exclusion of clear outliers, a mean MFCV was calculated from the remaining columns.

**Statistical analysis**

The Mann–Whitney test was used to assess any difference in ages between ALS and BFS patients. For all other analyses, linear mixed-effect models were employed in R using the 'lme4' package to avoid the pseudo-replication bias that is inherent to a serial dataset. The following template formula (in R notation) for the linear mixed-effect model was used:

\[
\text{lmer} \left( \text{depVar} \sim \text{fixed} + (\text{fixed}|\text{subject}), \text{data} \right)
\]

Where \( \text{lmer} \) was the linear mixed-effect regression function, \( \text{depVar} \) was the dependent variable in question (e.g. amplitude), \( \text{fixed} \) was the categorical fixed effect (i.e. disease group or firing pattern) and \( \text{fixed}|\text{subject} \) modelled a random intercept and a random slope. The random effect, \( \text{subject} \), was the patient study identifier. The Shapiro–Wilk test confirmed non-normal residual distributions for ISI, MU potential amplitude, MFCV and AHP, therefore log transformations were employed in the \( \text{lmer} \) models for these parameters. In contrast, a normal residual distribution for MU potential duration avoided the need for transformation of this parameter. A slight modification was made for the analysis of MU firing patterns, whereby these count data were more appropriately analysed with a Poisson mixed-effect model using the \( \text{glmer} \) model (assigning \( \text{family} = \text{poisson} \)).

Results from the \( \text{lmer/glmer} \) models are presented as: mean (95% confidence interval).

In order to calculate a \( P \) value, a comparison model was created that omitted the fixed effect. An ANOVA test was performed comparing the addition of the fixed effect, which produced a \( P \) value according to the Chi-squared test. For multiple comparison testing, the \( \text{glht} \) function (‘Generalised linear hypotheses’), which is part of the \( \text{multcomp} \) package (v1.4–10), was used in R. A \( P \) value < 0.05 was interpreted as statistical significance. A major benefit of using mixed-effect models was that missing values did not invalidate the model.

**Results**

**Patient recruitment**

Twenty patients with ALS and five patients with BFS each underwent up to seven assessments at intervals of 2 months (Table 1) (Bashford et al. 2020b). The BFS cohort was younger (median = 38 years, inter-quartile range (IQR) = 37–48 years, \( n = 5 \)) than the ALS cohort (median = 63 years, IQR = 57–71 years, \( n = 20; \ P = 0.0003 \)). There was an over-representation of males in both groups (ALS: 18M, 2F; BFS: 5M, 0F).

**Stage 1**

A total of 192 MUs were obtained from 78 recordings. Twelve recordings returned zero MUs. After exclusion of MUs with <40 ISIs, we identified 56 MUs from the ALS-STRONG group, 62 MUs from the ALS-WEAK group and 54 MUs from the BFS group.

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**Figure 2. Estimation of muscle fibre conduction velocity (MFCV)**

*Panel A*, six double-differential amplitude signals derived from a column of eight surface electrodes, showing the spread of conduction along the muscle fibres. *Panel B*, cross-correlation coefficients (CC) for the six signals, whereby a CC > 0.7 indicates sufficient morphological similarity for inclusion in the MFCV estimation. *Panel C*, a distance–time plot of the six signals, whereby the slope of the best-fit linear regression model provides an estimate of the MFCV. [Colour figure can be viewed at wileyonlinelibrary.com]
Table 1. Patient demographics and recording details

| Patient No. | Age (years) | Gender | Right Group | Left Group | Number of recordings successfully decomposed (corresponding number of identified motor units) |
|-------------|-------------|--------|-------------|------------|-----------------------------------------------------------------------------------------------|
|             |             |        | Biceps Weakness |            | Stage 1: 10 s voluntary activity | Stage 2: 30 s resting and 30 s voluntary activity |
| 1           | 72          | M      | ALS-PERI     | ALS-PERI   | 7 3 (9) 3 (9)                     | 7 (11) 6 (13) |
| 2           | 62          | M      | ALS-PRE      | ALS-PRE    | 1 0 0                           | 0 0          |
| 3           | 60          | M      | ALS-PRE      | ALS-PRE    | 7 1 (1) 2 (6)                    | 0 5 (13)     |
| 4           | 55          | M      | ALS-PRE      | ALS-PRE    | 4 0 0                           | 0 0          |
| 5           | 51          | M      | ALS-PRE      | ALS-PRE    | 7 1 (2) 0                       | 7 (11) 0     |
| 6           | 68          | M      | ALS-PRE      | ALS-POST   | 5 1 (2) 3 (6)                    | 0 0          |
| 7           | 57          | M      | ALS-PRE      | ALS-PRE    | 1 0 1 (1)                       | 0 0          |
| 8           | 64          | M      | ALS-POST     | ALS-POST   | 4 1 (3) 2 (6)                    | 0 4 (8)      |
| 9           | 66          | F      | ALS-PRE      | ALS-PRE    | 4 0 1 (1)                       | 0 0          |
| 10          | 64          | M      | ALS-POST     | ALS-PERI   | 7 3 (12) 2 (5)                  | 7 (15) 7 (12) |
| 11          | 66          | M      | ALS-PRE      | ALS-PRE    | 7 1 (1) 1 (1)                   | 5 (5) 0      |
| 12          | 61          | M      | ALS-PRE      | ALS-PRE    | 3 2 (4) 1 (4)                   | 0 0          |
| 13          | 79          | M      | ALS-POST     | ALS-PERI   | 4 1 (4) 2 (4)                   | 0 4 (7)      |
| 14          | 48          | M      | ALS-PRE      | ALS-PRE    | 6 1 (2) 1 (2)                   | 0 0          |
| 15          | 80          | M      | ALS-PRE      | ALS-PRE    | 6 1 (5) 0                       | 0 0          |
| 16          | 61          | M      | ALS-PERI     | ALS-PRE    | 7 0 2 (8)                       | 6 (11) 0     |
| 17          | 77          | F      | ALS-PRE      | ALS-PRE    | 7 3 (7) 1 (2)                   | 0 0          |
| 18          | 52          | M      | ALS-PRE      | ALS-PRE    | 5 4 (10) 1 (3)                  | 5 (10) 0     |
| 19          | 58          | M      | ALS-POST     | ALS-POST   | 7 2 (4) 4 (11)                  | 6 (12) 7 (17) |
| 20          | 71          | M      | ALS-POST     | ALS-PRE    | 6 1 (1) 1 (1)                   | 3 (4) 1 (1)  |
| 21          | 41          | M      | BFS          | BFS        | 7 2 (2) 3 (8)                   | 0 7 (10)     |
| 22          | 38          | M      | BFS          | BFS        | 2 1 (2) 1 (2)                   | 0 0          |
| 23          | 36          | M      | BFS          | BFS        | 6 2 (5) 3 (9)                   | 3 (4) 0      |
| 24          | 55          | M      | BFS          | BFS        | 7 5 (13) 2 (4)                  | 0 5 (7)      |
| 25          | 38          | M      | BFS          | BFS        | 7 2 (5) 3 (5)                   | 7 (8) 2 (2)  |

The biceps weakness group was classified as follows: A, ALS-PRE: ALS muscles that achieved 5/5 power at every assessment; B, ALS-PERI: ALS muscles that transitioned from 5/5 power to 4+/5 power (or less) during the course of the study; C, ALS-POST: ALS muscles that were weak (4+/5 or less) at the start of the study; D, BFS. ALS, amyotrophic lateral sclerosis; BFS, benign fasciculation syndrome.

Inter-spike interval

Mean ISIs across the groups (Fig. 3) were: ALS-STRONG = 96.0 ms (73.5–125.4); ALS-WEAK = 82.7 ms (65.5–104.4); BFS = 95.3 ms (78.8–115.2). The ISI mean in the ALS-WEAK group was significantly lower than both the ALS-STRONG group (P = 0.00919) and the BFS group (P = 0.0039).

Stage 2

A total of 181 MUs from 104 recordings were identified from the following disease groups: ALS-PRE (n = 40), ALS-PERI (n = 54), ALS-POST (n = 56) and BFS (n = 31). The remaining 25 recordings returned zero MUs. Separate categorization of the ALS subgroups into their observed MU firing patterns was as follows: VOL (n = 59), VOL-FASC (n = 79) and FASC (n = 12).

MU firing pattern (stage 2A)

There was a trend for more MUs with a pure voluntary pattern (VOL) in the ALS-PRE group (1.1 per recording (0–2.4)) than in the ALS-PERI group (0.4 per recording (0–1.2); P = 0.0504; Fig. 4), but not when compared with the ALS-POST group (0.8 per recording (0–1.5)). There were no significant differences between the disease groups in the number of VOL-FASC MUs per recording (ALS-PRE = 0.6 (0–2.0); ALS-PERI = 1.2 (0.4–2.0); ALS-POST = 1.0 (0.3–1.6)) or in the number of FASC MUs per recording (ALS-PRE = 0 (0–0); ALS-PERI = 0.1 (0–0.4); ALS-POST = 0.3 (0–0.7)).
MU potential duration, amplitude and area (stage 2B)

Reliable estimates for MU potential duration and amplitude were obtained for all decomposed MUs \((n = 181)\). The number of channels selected on visual inspection was \(60 \pm 2.5\) (mean \(\pm\) standard deviation). MU potential durations did not significantly differ between the disease groups: ALS-PRE = 21.9 ms (17.8–26.0 ms); ALS-PERI = 25.2 ms (21.2–29.2 ms); ALS-POST = 22.9 ms (19.5–26.3 ms); BFS = 25.0 ms (22.1–27.9 ms), although there were trends for greater durations in the ALS-PERI \((P = 0.0894)\) and BFS \((P = 0.0531)\) groups when compared with the ALS-PRE group.

Compared with the BFS group \((17.8 \mu V (10.3–30.6))\), peak-peak amplitudes were greater in the ALS-PERI

![Figure 3. Inter-spike interval (ISI) histograms](image)

ISIs were computed based on MU decomposition results from 10 s voluntary recordings (ALS-STRONG = 56 MUs, ALS-WEAK = 62, BFS = 54). *Mean ISI was lower in ALS-WEAK muscles (82.7 ms (65.5–104.4)) than in ALS-STRONG muscles (96.0 ms (73.5–125.4); \(P = 0.00919)\) and BFS muscles (95.3 ms (78.8–115.2); \(P = 0.0039)\), as determined by linear mixed-effect regression. [Colour figure can be viewed at wileyonlinelibrary.com]

![Figure 4. Identification of distinct motor unit (MU) firing patterns over time](image)

Combined resting (30 s) and light voluntary (30 s) longitudinal recordings from 15 ALS muscles identified three patterns of MU firing: VOL was only active during light voluntary contraction; VOL-FASC produced fasciculations and was also active during light voluntary contraction; FASC produced only fasciculations. ALS subgroups: PRE, MUs from muscles that remained strong throughout the study; PERI, MUs from muscles that became weak during the study; POST, MUs from muscles that were weak at the start of the study. Number of months elapsed into the study are shown. The total numbers of MUs in each category are displayed. [Colour figure can be viewed at wileyonlinelibrary.com]
(66.2 μV (27.5–159.4); P < 0.0001) and ALS-POST (103.8 μV (37.0–290.6); P < 0.0001) groups. There was a trend for a greater peak–peak amplitude in the ALS-PRE group (43.9 μV (9.3–206.9); P = 0.0693). Similarly, MU potential area was greater in the ALS-PERI (AUC: 376.4 μV ms (163.3–867.3); P < 0.0001) and ALS-POST (487.5 μV ms (172.5–1377.8); P < 0.0001) groups than in the BFS group (98.7 μV ms (54.2–179.8); Fig. 5A). Again, there was a trend for a greater MU potential area in the ALS-PRE group (240.9 μV ms (55.7–1041.7)). There were no significant differences between any of the ALS subgroups. Purely fasciculating MUs (AUC: 679.5 μV ms (162.4–2844.0)) had a greater MU potential area than MUs in the VOL-FASC (232.4 μV ms (75.8–713.1);

Figure 5. MU potential area under the curve (AUC) (A+B), afterhyperpolarization (AHP) estimates (C+D) and muscle fibre conduction velocity (MFCV) estimates (E+F) according to disease group (a,c and e) and motor unit firing pattern (b, d and f). Reliable estimates for MU potential AUC, AHP and MFCV were available for 181, 89 and 157 MUs, respectively. Note logarithmic scale of y-axis for MU potential AUC. Means and 95% confidence intervals from the linear mixed-effect regression models are shown. Only significant differences are shown. Groups: PRE, MUs from muscles that remained strong throughout the study; PERI, MUs from muscles that became weak during the study; POST, MUs from muscles that were weak at the start of the study; VOL, MUs under voluntary control without producing fasciculations; VOL-FASC, MUs under voluntary control and able to produce fasciculations; FASC, MUs that only produced fasciculations. ALS = amyotrophic lateral sclerosis; BFS = benign fasciculation syndrome. [Colour figure can be viewed at wileyonlinelibrary.com]
that did not, were indistinguishable based on motor unit amplitude, MFCV and estimated AHP duration. Most significantly of all, we demonstrated that the first-recruited motor units altered their firing properties during the course of disease, showing features more consistent with a faster MU subtype (greater amplitude and MFCV) once clinical weakness was established (Fig. 6). We suggest this may occur as a compensatory mechanism within a motor neuron pool selectively bereft of vulnerable fast MUs. In turn, we hypothesize that these compensatory adaptations may be detrimental to the longevity of the compensating motor neurons, as they acquire the same vulnerability to disease as their late predecessors. By exploring and possibly halting the molecular mechanisms that drive this phenotypic drift, a novel therapeutic strategy might emerge. Such an approach would be amenable to testing in ALS patients through the investigative tools set out in this study.

We focused on the first-recruited MUs, which, by definition, are those with the lowest threshold for voluntary activation during light force. These fatigue-resistant S-type MUs assume baseline characteristics that differ from their FF-type counterparts, such as a relatively small amplitude on EMG, a longer AHP and a slower MFCV (de Carvalho & Swash, 2016). We sought deviations from these baseline characteristics across three stages of disease, defined in relation to the onset of weakness in individual muscles. It is important to note that MU amplitude is dependent on several key factors: (1) Baseline MU phenotype: FF-type MUs are bigger than S-type, allowing them to exert a

![Figure 6. Combining MU potential area under the curve (AUC) and muscle fibre conduction velocity (MFCV) to distinguish disease groups](https://example.com/figure6.png)

**Figure 6.** Combining MU potential area under the curve (AUC) and muscle fibre conduction velocity (MFCV) to distinguish disease groups. Summary of the proposed adaptive changes as a consequence of ALS disease progression compared with BFS control subjects. Mean values for each parameter shown. ALS subgroups: PRE, MUs from muscles that remained strong throughout the study; PERI, MUs from muscles that became weak during the study; POST, MUs from muscles that were weak at the start of the study. ALS = amyotrophic lateral sclerosis; BFS = benign fasciculation syndrome. [Colour figure can be viewed at wileyonlinelibrary.com]
greater force; (2) Degree of reinnervation: a sprouting MU in the context of ALS produces a greater amplitude on EMG than baseline – this compensatory behaviour is the domain of S-type and FR-type MUs; (3) Depth of MU: it is unknown whether this changes significantly during disease progression, although it is likely that cachexia and muscular atrophy would have a similar effect on all MU subtypes.

It is widely appreciated that fast-twitch motor units are most vulnerable to disease in mouse models of ALS, whereas slow-twitch motor units are the most amenable to compensatory reinnervation of denervated muscle fibres (Frey et al. 2000; Pun et al. 2006; Kaplan et al. 2014). It has been suggested that the reinnervating MUs may take on similar neuronal properties to the ones that they are compensating for. Adding weight to this concept from a molecular standpoint, the expression of matrix metalloproteinase 9 (MMP-9; a conventional marker of FF-type MUs (Kaplan et al. 2014)), re-emerged in sprouting, reinnervating motor units after its initial disappearance secondary to the early loss of vulnerable FF-type MUs (Spiller et al. 2016). In a separate study, the co-expression of the S-type MU marker, osteopontin, with MMP-9 identified remodelled slow-twitch MUs in an SOD1 (G93A) mouse model (Morisaki et al. 2016). Interestingly, MMP-9 was absent from naturally disease-resistant neurons in oculomotor and Onuf’s nuclei (Kaplan et al. 2014). As MMP-9 has been shown to drive neuronal degeneration and the endoplasmic reticulum stress response (Kaplan et al. 2014), this may become maladaptive in previously disease-resistant motor neurons, possibly contributing to greater fatigue and impaired spinal microcircuit homeostasis in ALS (Brownstone & Lancelin, 2018).

Circulating MMP-9 levels have been shown to be dysregulated in mouse models of ALS (Soon et al. 2010), while raised serum (but not cerebrospinal fluid) MMP-9 levels in human ALS patients appears to be a non-specific marker of denervation and neuronal remodelling (Beuche et al. 2000). Conversely, fewer osteopontin-positive neurons (normalized according to overall motor neuron loss) were present in the ventral horn of ALS patients compared with controls (Yamamoto et al. 2017). We did not measure MMP-9 or osteopontin serum levels; however, this would be a useful follow-up study to explore the interaction between molecular and neurophysiological parameters at different stages of disease. Multimodal assessment will undoubtedly hold greater power when it comes to monitoring such a complex disease and may prove vital in identifying therapies that preserve the functionality and natural resistance of specific MU subtypes.

An important contributing factor to the results observed in this study relates to the effect of muscle inactivity as ALS progresses. In rat models, it is known that weightlessness and hindlimb suspension induce a switch from slow- to fast-twitch motor unit behaviour, particularly in lower limb muscles with a greater proportion of slow-twitch motor units (e.g. soleus) (Gardiner et al. 2006; Baldwin et al. 2013). Therefore, it will be informative to explore the behaviour of lower limb muscles in ALS in future studies, while controlling for the reduction in muscle activity brought about by progressive disability.

Considering the practical feasibility of short, non-invasive EMG recordings, this technique could potentially be adapted for use away from the hospital setting. Coupled with the scientific relevance of the results in relation to biomarker development, remote monitoring in patients’ homes has the potential to significantly expand the dataset in a greater number of patients. While assessments done in this way could be performed more regularly (thereby increasing sampling frequency), geography and disability would no longer present the practical barriers to assessment that they do currently. The increased accessibility of a home-based approach would make it an appealing option in the design of clinical drug trials.

We interpreted the MU firing patterns in the following way: the VOL group was most indicative of slow MUs with normal excitability; the VOL-FASC group was most indicative of hyperexcitable, slow MUs; and the FASC group, having produced amplitudes that were greater than the other two groups, was most indicative of fast/intermediate MUs. The very existence of a separate VOL-FASC group suggests a proximal origin for the detected fasciculations, as an origin distal to the terminal arborization would lead to such variability amongst the resultant MU potentials that they would escape categorization as a single MU. The results support earlier studies that suggest a proportion of fasciculating MUs remain recruitable to the motor pool with a firing generator site proximal to the terminal arborization (de Carvalho & Swash, 2017). Our study’s methodology was unable to establish whether the purely fasciculating MUs of larger amplitude (presumably indicating a faster subtype) remained recruitable to the motor pool. The progressive FastICA peel-off technique is capable of decomposing MU firing at much higher muscle contraction levels, which would be an interesting future avenue of investigation. The complex interplay between spinal cord inter-connections in ALS and how they adapt and may indeed drive maladaptive processes is an increasing focus of research. It would be interesting to examine the effects of sensory input (e.g. vibration) on the VOL-FASC and FASC groups, as these inter-spinal interactions from afferent pathways might be expected to influence the former group but not the latter (de Carvalho et al. 2016). The fact that we could not distinguish the VOL and VOL-FASC groups based on amplitude, AHP
and MFCV suggests that these disease-related adaptations are universal to all slow-twitch MUs and not just a hyperexcitable subset.

We recognize several methodological limitations. The BFS control group was not age-matched to the ALS cohort, whereby BFS patients were 25 years younger on average. There was a significant male predominance in both the ALS and BFS cohorts, therefore the generalisability of these results to female patients would need further exploration. While 100% (181/181) of identified MUs produced a reliable amplitude and 87% (157/181) of the MUs produced a reliable MFCV estimate, only 49% (89/181) produced a reliable AHP estimate. It is likely that this significantly hampered our ability to detect differences in AHP between the disease stages and that the analysis of longer recordings (>30 s) in future studies should improve the reliability of AHP estimation. However, it is acknowledged that the physiological mechanisms determining amplitude, AHP and MFCV are different and one should not necessarily expect interdependence between these parameters.

Conclusion

This study highlights the utility of high-density MU decomposition to dissect the activity of a spectrum of MU subtypes. We have demonstrated compensatory changes in key MU properties, showing that the MUs recruited at low force levels in biceps adopted a faster phenotype in the latter stages of ALS. Although this may be beneficial at first to maintain a diverse motor unit pool, this may become maladaptive, conferring disease susceptibility to naturally resistant motor neurons. Exploration of the mechanisms underlying this phenotypic drift may bring about novel strategies to enhance motor neuron survival.

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Additional information

Data availability statement

The data that support the findings of this study are openly available in Mendeley Data at http://doi.org/10.17632/phc6grmg45.2

Competing interests

The authors have no competing interests to declare.

Author contributions

All data acquisition took place in King's College Hospital, London Neurology clinic and further analysis and preparation of the paper took place within UK Dementia Research Institute, Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience, King's College London. T. W. and J. B. were involved with conceptualization of the project, formal analysis of data, designing the methodology, creation and presentation of published work, and writing the original draft and its further review and editing during the publication process. Additionally, J. B. collected the data. M. C. and P. Z. provided expertise on the motor unit decomposition technique. A. W., R. I., M. C., P. Z., E. D., M. B., K. M. and C. S. were involved in the conception of the project, interpretation of the data and critical revision of the manuscript. All authors approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

afterhyperpolarization, amyotrophic lateral sclerosis, decomposition, motor unit, surface electromyography

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Statistical Summary Document
Peer Review History