Detecting motor unit abnormalities in amyotrophic lateral sclerosis using high-density surface EMG

Title: Detection of Motor Unit in Amyotrophic Lateral Sclerosis Using High-Density Surface Electromyography

Authors' full names

Yuichi Nishikawa, Ph.D.[1*]; Aleš Holobar, Ph.D.[2]; Kohei Watanabe, Ph.D.[3]; Tetsuya Takahashi, Ph.D.[4]; Hiroki Ueno, Ph.D.[5]; Noriaki Maeda, Ph.D.[6]; Hirofumi Maruyama, Ph.D.[7]; Shinobu Tanaka, Ph.D.[1]; and Allison S. Hyngstrom, Ph.D.[8]

Author affiliations

1) Faculty of Frontier Engineering, Institute of Science & Engineering, Kanazawa University, Kanazawa, Japan
2) Faculty of Electrical Engineering and Computer Science, University of Maribor, Maribor, Slovenia
3) Laboratory of Neuromuscular Biomechanics, School of Health and Sport Sciences, Chukyo University, Nagoya, Japan
4) Faculty of Rehabilitation Medicine, Department of Rehabilitation Medicine, Hiroshima International University, Higashi-Hiroshima, Japan
5) Department of Neurology, Hiroshima City Hiroshima Citizens Hospital, Hiroshima, Japan
6) Division of Sports Rehabilitation, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
7) Department of Clinical Neuroscience and Therapeutics, Graduate School of Biomedical and

Health Sciences, Hiroshima University, Hiroshima, Japan

8) Department of Physical Therapy, Marquette University, Milwaukee, WI, United States

Running Title: Quantitative assessment of MU behavior in ALS

Correspondence:

Yuichi Nishikawa, Faculty of Frontier Engineering, Institute of Science & Engineering,

Kanazawa University, Kanazawa, Japan

Kakuma-machi, Kanazawa, 920-1192

Tel. & Fax.: +81-76-234-4760, Email: yuichi@se.kanazawa-u.ac.jp

Keywords

Amyotrophic lateral sclerosis, electromyography, motor unit recruitment

Highlights

- The establishment of a quantitative assessment tool for diagnosis of ALS is an important clinical issue.
- People with ALS had higher motor unit firing rate than age-matched control subjects.
- The motor unit firing rate at recruitment was useful for differentiating people with ALS from control subjects.

Abstract

Objective: The purpose of this study was to detect specific motor unit (MU) abnormalities in people with amyotrophic lateral sclerosis (ALS) compared to controls using high-density surface electromyography (HD-SEMG).

Methods: Sixteen people with ALS and 16 control subjects. The participants performed ramp up and sustained contractions at 30% of their maximal voluntary contraction. HD-SEMG signals were recorded in the vastus lateralis muscle and decomposed into individual MU firing behavior using a convolution blind source separation method.

Results: In total, 339 MUs were detected (people with ALS; n=93, control subjects; n=246). People with ALS showed significantly higher mean firing rate, recruitment threshold, coefficient of variation of the MU firing rate, MU firing rate at recruitment, and motoneurons excitability than those of control subjects (p<0.001). The number of MU, MU firing rate, recruitment threshold, and MU firing rate at recruitment were significantly correlated with disease severity (p<0.001). Multivariable analysis revealed that an increased MU firing rate at recruitment was independently associated with ALS.

Conclusions: These results suggest increased excitability at recruitment, which is consistent with neurodegeneration results in a compensatory increase in MU activity.

Significance: Abnormal MU firing behavior provides an important physiological index for understanding the pathophysiology of ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder of motoneurons, and its prevalence in Japan is reported to be 9.9 per 100,000 people (Doi *et al.*, 2014). ALS is characterized by selective motoneuron death that develops within weeks or months (van Es et al., 2017). However, the mechanisms of neurodegeneration in ALS are not fully understood, and biomarkers reflecting the disease condition have not yet been established. Given the involvement of motoneurons, pathology in motor unit (MU) firing rates is a strong candidate biomarker, as common signs of ALS include increased MU firing rates as well as unstable and polyphasic MUAPs on needle electromyography (EMG) due to a decreased number of MUs (EMG) (de Carvalho *et al.*, 2008).

The MU is the basic functional unit of the neuromuscular system and is composed of motoneurons, including their dendrites and axons, and the muscle fibers innervated by the axons (Duchateau and Enoka, 2011). In ALS, degeneration of lower motoneurons leads to denervation of muscle fibers, resulting in abnormal MU firing behavior (prolonged and polyphasic) (Kiernan *et al.*, 2011). These findings are usually obtained by needle EMG, but this method depends on the experience and skill of the examiner, as the morphology of the MU varies greatly depending on the position of the needle electrode and the effort to get the needle in a good position causes sampling bias. Consequently, inexperienced physicians may produce a false-negative evaluation. Several studies have quantitatively evaluated MUAP

morphology, mobilization pattern, and interference pattern (Fuglsang-Frederiksen and Rønager, 1990; Kurca and Drobný, 2000; Sanders et al., 1996), but no objective quantitative evaluation is available for actual clinical practice. Therefore, it would be useful to establish and disseminate a quantitative EMG method other than needle EMG.

Recently, there have been several papers on noninvasive methods for MU identification in control subjects and in patients with different diseases using high-density surface EMG (HD-SEMG) decomposition (Mazzo et al., 2021; Nishikawa et al., 2021a, 2021b; Nuccio et al., 2021; Watanabe et al., 2021). HD-SEMG with more than 60 surface electrodes allows this technique to increase the number of detectable MUs compared to intramuscular EMG and to investigate detailed MU firing characteristics, such as the relationship of firing rates between MUs with different recruitment thresholds (Holobar et al., 2009; Martinez-Valdes et al., 2017; Merletti et al., 2008). It is possible to accurately analyze how MU populations respond to pathological conditions by employing HD-SEMG (Castronovo *et al.*, 2017). Although a previous study evaluated MU activity by HD-SEMG in people with ALS (Weddell *et al.*, 2021), there are still many unknown factors about the disease-specific abnormalities of MU firing behavior. Weddell et al. reported that in the later stage of ALS, MUs recruited at lower force levels in the biceps brachii muscle adopt a faster phenotype, revealing compensatory changes in MUs (Weddell *et al.*, 2021). Furthermore, an animal study has shown that ALS model mice already exhibit aberrations in motoneuron

function (increased firing rates and persistent inward currents (PICs)) in the early and probably presymptomatic stage of the disease (Jensen et al., 2020). According to the report, the presence of motoneuron aberrations in people with early-stage ALS may indicate an increased MU firing rate. In addition, Piotrkiewicz et al. reported that the duration of the afterhyperpolarization (AHP) in motoneurons was significantly lower in ALS patients than in control subjects as measured by needle EMG (Piotrkiewicz and Hausmanowa-Petrusewicz, 2011). A reduced AHP duration is expected to increase the variability in the MU firing rate because the firing times of motoneurons are sensitive to noise in motor commands. However, the characteristics of abnormal MU activity in people with early-stage ALS compared with control subjects have not been elucidated using HD-SEMG. Furthermore, parameters useful for diagnosis have not been identified.

Our study examined the use of HD-SEMG to detect disease-specific changes in MUs in the vastus lateralis (VL) muscle of people with ALS. We hypothesized that HD-SEMG could be used to detect an abnormal MU firing rate in people with ALS and that abnormalities in MU firing behavior are correlated with metrics of disease severity. The results of this study will support the use of HD-SEMG as a quantitative non-invasive method for the diagnosis and evaluation of ALS disease progression.

2. Materials and methods

2.1. Subjects

Sixteen people with ALS and 16 control subjects were enrolled in this study after signing an informed consent form. The study protocol and procedures were approved by Kanazawa University's Committee on Ethics in Research (approval number No. 77-2) and conformed to the requirements of the Declaration of Helsinki. The inclusion criteria were a diagnosis of ALS, confirmed signs of successful recording from VL motoneurons by needle EMG, and the ability to walk without assistance. The Awaji criteria were used to diagnose ALS (Costa et al., 2012). People with the following conditions were not included: lower extremity injury, dementia, myositis, spinal muscular atrophy, and dystonia. The inclusion criteria for control subjects were consent to participate in the study and appropriate age matching. The exclusion criteria for control subjects were orthopedic diseases and neurological diseases. Each person with ALS underwent an ALS Functional Rating Scale-Revised (ALSFRS-R) test with the same neurologist (H.U.). The ALSFRS-R consists of 12 items (on a 5-point scale ranging from 0 to 4), including speech, swallowing, gait, and other activities. The total symptom severity score ranges from 0 to 48, with lower values indicating more severe symptoms.

2.2. Experimental protocols

Experimental protocols were performed according to the same procedure used in a previous study (Nishikawa *et al.*, 2021*a*). During the examination, all participants were seated

133 in a custom-made dynamometer (TSA-110, Takei Scientific Instruments. Niigata, Japan),
134 with the hip and knee joint fixed at 90° of flexion (full extension at 0°) (Fig. 1A). We
135 captured the force signals using an analog-to-digital converter (Quattrocento, OT
136 Bioelettronica, Turin, Italy) at a frequency of 2048 Hz. All participants were asked to perform
137 the maximal knee extension force in two trials for each limb, with 2 minutes between trials
138 and a warm-up of 10 minutes. In the maximum voluntary contraction (MVC), the participant
139 gradually increased the toque exerted by the knee extensor muscles from zero to maximum
140 over a period of three seconds and held the maximum torque for two seconds (Nishikawa *et*
141 *al.*, 2019). The peak torque was selected as the target torque level of the submaximal ramp-up
142 contraction task. After a rest period of at least 10 minutes from the MVC measurement, all
143 participants underwent EMG measurement. HD-SEMG signals from the VL muscle were
144 recorded on the more severely side (weaker muscle strength) in the participants with ALS and
145 the dominant side (self-reported when asked which leg is used to kick ball) in control
146 participants. Participants were asked to perform the ramp and hold contraction task (ramp up
147 to 30% MVC in 15 sec, sustained contraction for 15 sec, and ramp down in 15 sec: Fig. 1B).
148 The subjects received real-time visual feedback of their force, which was displayed on a
149 monitor.

150 “Place Figure 1 around here.”

151 *2.3. EMG recording*

152 An array of 64 multiple electrodes (1 mm in diameter, 8 mm between electrodes,
153 GR08MM1305, OT Bioelettronica) was used to record HD-SEMG signals from the VL
154 muscle using the same procedure used in previous studies (Nishikawa et al., 2017b, 2017a;
155 Watanabe et al., 2012). The positioning of the electrode was determined by previous studies
156 (the center of the electrode was placed at the midpoint between the head of the greater
157 trochanter and the lateral edge of the patella; Fig. 1D) (Nishikawa et al., 2021b, 2018, 2017b).
158 After skin cleaning (80% alcohol), the electrode grid was attached to the muscle surface using
159 a biadhesive sheet (KIT08MM1305, OT Bioelettronica) with conductive paste (Elefix ZV-
160 181E, NIHON KOHDEN, Tokyo, Japan) (Nishikawa *et al.*, 2017*b*). A ground electrode was
161 placed on the patella. The monopolar HD-SEMG signals were recorded using a 16-bit analog-
162 to-digital converter (Quattrocento, OT Bioelettronica, sampling frequency at 2048 Hz),
163 amplified with a gain of 150, and off-line bandpass filtered 10–500 Hz. The force and EMG
164 signals were analyzed by MATLAB software (MATLAB 2021b, Math Works GK, MA,
165 USA).

166 *2.4. Data processing*

167 A validated convolutive blind source separation technique was used to separate the
168 HD-SEMG recordings into individual MU discharge timings (Holobar et al., 2009; Holobar
169 and Zazula, 2004; Merletti et al., 2008). After decomposition analysis, all identified MU spike
170 trains were manually verified by one investigator (Y.N.). Interspike intervals (ISI) < 33.3 or >

250 ms (firing rate > 30 and < 4 Hz, respectively) (Holobar *et al.*, 2009; Watanabe *et al.*, 2016) as well as low-quality MU spike trains (pulse-to-noise ratio < 30 dB corresponding to MU firing identification accuracy < 90%) (Holobar *et al.*, 2014) were excluded. We calculated the instantaneous firing rates (pulse per second, pps) of identified MUs from the time interval between spikes. Afterward, the mean firing rate of the identified MUs was calculated during ramping up and holding of the contraction (30 s in total). We defined the coefficient of variation (CV) of the MU firing rate as the ratio of standard deviations and the mean values of the MU firing rates. Further analysis did not include the MU firing rates with > 30% CV (Fuglevand *et al.*, 1993). The MU recruitment threshold (RT) was defined as the force level (%MVC) at the first firing of each MU (Fig. 2A). Detected MUs were classified into three subgroups for each RT (MU10, <10% MVC; MU20, 10%–20% MVC; and MU30, 20%–30% MVC; Fig. 2B) (Watanabe *et al.*, 2016). We further divided them into two subgroups using the absolute value of knee extension torque to compare the MU firing rate as a function of the actual torque being generated. The two subgroups were divided as follows: MU10Nm group, MUs recruited with less than 10 Nm; MU20Nm group, MUs recruited at forces between 10 Nm and 20 Nm.

"Place Figure 2 around here."

To estimate PICs, we calculated ΔF. ΔF is calculated as the difference between the smooth firing rate of the reference MU (using a 2-s Hanning window) and the recruitment-to-

derecruitment interval of the test MU (Gorassini et al., 2002) (Fig. 3A and B). The inclusion criteria of ΔF values from MUs were that (1) the test MU was recruited at least 1 s after the reference MU to ensure full activation of the PIC, (2) the test MU was derecruited at least 1.5 s before the reference MU in order to prevent overestimation of ΔF, and (3) the duration of the firing of the test unit was greater than 2 s (Hassan et al., 2020, 2021).

We demonstrated the evaluation of motoneuron AHP duration by the analysis of their ISI values. Previous study reported that the ISI, which corresponds to transitions between the two ranges (transition interval, TI), is associated with the duration of AHP (Piotrkiewicz, 1999). The absolute value of each adjacent ISI difference is derived as follows: $Consecutive\ interval\ difference\ (CD)2 = |ISI_{i+1} - ISI_i|$. CD2 was plotted against the mean ISI duration calculated from the same two intervals: $MISI = (ISI_{i+1} + ISI_i)/2$ (Piotrkiewicz and Hausmanowa-Petrusewicz, 2011). Furthermore, we averaged mean CD2 data (CD_m) and MISI values over an interval of 10 ms. An example of a plot of CD_m vs. MISI is shown Fig. 3C. The short- and long-interval sections of TI are estimated based on a previous study (Piotrkiewicz et al., 2007).

"Place Figure 3 around here."

2.5. Statistical analysis

We performed all statistical analyses using Stata version 17 (Stata Corp LLC, Texas, USA). All data were checked for normality of distribution using the Shapiro–Wilk test. An

209 unpaired t-test was used to compare participants with ALS and control subjects based on age,
210 height, body mass, thickness of subcutaneous tissue, knee extension torque, knee extension
211 torque/body mass, RT, CV of the MU firing rate, MU firing rate at recruitment, ΔF, and TI.
212 Analysis of covariance was performed to compare the number of MUs between participants
213 with ALS and control subjects; the dependent variable was the number of MUs, the fixed
214 factor was the group (ALS or control), and the covariate was the thickness of the
215 subcutaneous tissue. An analysis of the mean MU firing rate was conducted using a mixed-
216 effect model with a random intercept and a random slope. There were two explanatory
217 variables: the group (ALS or control) and MU subgroups (e.g., MU10, MU20, MU30,
218 MU10Nm, and MU20Nm). The association between the mean MU firing rate and the RT
219 and/or absolute torque in participants with ALS and healthy control subjects were computed
220 using Pearson's correlation coefficient. Furthermore, in the ALS group, Pearson's correlation
221 coefficient was used to determine the relationship between ALSFRS-R scores or knee
222 extension torque/body mass and the mean MU firing rate and the MU firing rate at
223 recruitment. The association between the ALSFRS-R and the number of MUs was analyzed
224 using partial correlation coefficients with the thickness of the subcutaneous tissue as a control
225 variable. To clarify factors related to the diagnosis of ALS, multiple logistic regression
226 analysis was conducted with ALS diagnosis and control status as the dependent variables and
227 outcomes that showed significant differences in the univariate analysis as the independent

variables. Furthermore, the outcome selected by multiple logistic regression analysis was calculated as the area under the curve (AUC), specificity, and sensitivity of the outcome by receiver operating characteristic (ROC) curve, and the Youden's index was used to calculate the cutoff value. The significance level was set at a $p\ value < 0.05$.

3. Results

3.1. Participant characteristics

The characteristics of the participants are shown in Table 1. The age, height, and thickness of the subcutaneous tissue of the participants did not differ between the groups ($p = 0.0585$, $p = 0.1051$, and $p = 0.6888$, respectively), while weight, knee extension torque, and knee extension torque/body mass were significantly lower in people with ALS than in control subjects ($p = 0.0072$, $p < 0.0001$, and $p < 0.0001$, respectively).

"Place Table 1 around here."

3.2. MU decomposition

A total of 339 MUs were accepted for data processing (people with ALS; $n = 93$, control subjects; $n = 246$). The detected number of MUs was significantly lower in people with ALS than in control subjects according to analysis of covariance ($p < 0.0001$). The number of MUs detected in the MU subgroups was as follows: MU10, $n = 60$ (people with ALS; $n = 16$, control subjects; $n = 44$), MU20, $n = 156$ (people with ALS; $n = 27$, control

247 subjects; n = 129), MU30, n = 123 (people with ALS; n = 50, control subjects; n = 73),
248 MU10Nm, n = 114 (people with ALS; n = 65, control subjects; n = 49), MU20Nm, n = 155
249 (people with ALS; n = 26, control subjects; n = 129) (Fig. 3).

250 "Place Figure 4 around here."

251 *3.3. Properties of MUs*

252 In people with ALS, the values of RT, CV of the MU firing rate, MU firing rate at
253 recruitment, and ΔF were significantly higher than in control subjects, whereas TI was
254 significantly reduced in people with ALS ($p < 0.01$ for each comparison; Fig. 4 and 5).
255 Analysis of the mean MU firing rate at the MU subgroups (MU10, MU20, MU30, MU10Nm,
256 and MU20Nm) showed that people with ALS had a significantly higher MU firing rate than
257 control subjects ($p < 0.0001$, respectively, Fig. 6). Furthermore, the firing rate of the MUs in
258 the MU30 group of control subjects was significantly lower than the rates of those in the
259 MU10 and MU20 groups ($p < 0.0001$ and $p = 0.0021$, respectively), and the firing rate of the
260 MUs in the MU20 group was significantly lower than that of those in the MU10 group ($p =$
261 0.0009, Fig. 6A). The MU firing rate was also significantly lower in the MU20Nm group than
262 in the MU10Nm group ($p < 0.0001$, Fig. 6B). On the other hand, significant differences
263 among the MU subgroups were not observed in people with ALS (Fig. 6A and B).

264 "Place Figure 5 around here."

265 "Place Figure 6 around here."

The mean MU firing rate was significantly related to the RT in the control group (r = −0.6381, p < 0.0001) but not people with ALS (r = −0.1356, p = 0.1949, Fig. 7A and B). Absolute torque was significantly related to the mean MU firing rate in control subjects (r = −0.6788, p < 0.0001) but not people with ALS (r = −0.1781, p = 0.0877, Fig. 7C and D). In people with ALS, ALSFRS-R scores were significantly related to the number of MUs (r = 0.6708, p = 0.0062), RT (r = −0.5653, p = 0.0225), mean MU firing rate (r = −0.6369, p = 0.0080), and MU firing rate at recruitment (r = −0.5034, p = 0.0468, Fig. 8). The knee extension torque/body mass did not significantly correlate with the number of MUs (r = −0.08527, p = 0.7535), RT (r = −0.1728, p = 0.5221), mean MU firing rate (r = 0.1732, p = 0.5212), or MU firing rate at recruitment (r = 0.3210, p = 0.2254).

“Place Figure 7 around here.”

“Place Figure 8 around here.”

In the univariate analysis, significant differences between participants with ALS and controls were found for the MU firing rate at recruitment and RT values (p = 0.007 and p = 0.042, respectively), which were used as dependent variables in the multivariable logistic regression analysis. Multivariable analysis revealed that an increased MU firing rate at recruitment was independently associated with ALS (odds ratio = 23.26, 95% CI = 1.44–376.53, p = 0.027). When the probability of ALS positivity is P, the model equation by logistic regression can be written as follows.

$$ln\,{}^{P}/_{1-P} = 2.25146 \times (MU\ firing\ rate\ at\ the\ RT) - 16.20322$$

The cutoff value of the MU firing rate at recruitment calculated from the ROC curve was 7.368 (sensitivity = 81.25%, specificity = 93.75%, AUC = 0.9258, Fig. 9). The positive predictive value using this cutoff value was 87.5%.

"Place Figure 9 around here."

4. Discussion

This study investigated the detection of disease-specific abnormal MU behavior in people with ALS using the HD-SEMG method. We found that people with ALS exhibited (1) a small number of detected MUs, (2) overactive MUs at each contraction level, and (3) high variability in MU firing behavior compared with control subjects. These findings confirm that HD-array data are consistent with those obtained with needle EMG. Furthermore, it was found that the MU firing rate at recruitment could be a useful index for detecting ALS. This finding may reflect the existence of ALS-specific MU abnormalities, which is the main novel result of this study. These findings supported our hypothesis that the HD-SEMG method can noninvasively detect disease-specific MU dysfunction in people with ALS. In general, abnormal MU behavior detected by needle EMG in people with ALS is characterized by a reduced number of MUs and a large MUAP amplitude, which can be used only to

304 differentiate the disease from myogenic diseases; additionally, this method is invasive and
305 therefore causes significant pain to the patient. Consequently, this study focused on the
306 identification of abnormalities in MU firing behavior using HD-SEMG, which is a
307 noninvasive method that can be applied to people with ALS without requiring them to endure
308 any pain.

309 Fast-twitch MUs are especially vulnerable to degeneration in people with ALS, while
310 slow-twitch MUs are more affected by compensatory reinnervation of denervated MUs (Frey
311 *et al.*, 2000; Pun *et al.*, 2006). Therefore, a decrease in the number of MUs is a known
312 electrophysiological finding in ALS with neurogenic degeneration (van Es et al., 2017). The
313 HD-array showed that the number of MUs detected was significantly lower in people with
314 ALS than in control subjects, consistent with these previous findings. However, it should be
315 noted that protocol of the present study uses only 30% MVC and therefore does not
316 encompass all MUs.

317 We found that the firing rates of MUs in all MU subgroups were significantly higher
318 in people with ALS than in control subjects. Notably, people with ALS are known to have a
319 reduced number of MUs due to degeneration of motoneurons, such that the unaffected
320 motoneurons compensatively increase their firing rate (Shiga *et al.*, 2000). The results of this
321 study showed that people with ALS exhibited a significantly small number of MUs. The
322 number of MUs and their firing rate are important factors in exerting muscle force (Heckman

323 and Enoka, 2012), and the results of this study may reflect the result of compensating for the
324 decrease in the number of MUs by increasing the firing rate. Importantly, our results also
325 showed a higher MU firing rate at a low RT (recruited at < 10% MVC and < 10 Nm) in
326 people with ALS compared with control subjects. Previous studies reported that slow-twitch
327 MUs are the most resistant to disease, maintaining an ability to reinnervate (Frey et al., 2000;
328 Hegedus et al., 2008; Pun et al., 2006), suggesting the involvement of a mechanism other than
329 degeneration of motoneurons. In addition to motoneuron degeneration, there are several
330 reports of increased motor cortex excitability in people with ALS (Vucic et al., 2013; Vucic
331 and Kiernan, 2006). According to previous research, increased cortical excitability can be a
332 result of changes in neurons' intrinsic properties, dysfunctional inhibitory mechanisms, and/or
333 structural changes leading to behavioral deficits (Sankaranarayani *et al.*, 2014). Furthermore,
334 SOD1 mice show increased PICs in motoneurons during the early and possibly
335 presymptomatic stages of disease (Jensen et al., 2020). These findings suggest that there could
336 be changes in the intrinsic electrical properties of the motoneurons as well as changes in
337 cortex. We considered that these findings support the results of this study, which confirmed
338 the overactivity of MUs regardless of the firing threshold.
339 When lower motoneurons die in ALS, fast-twitch MUs are preferentially
340 degenerated, and surrounding slow-twitch MUs compensate by reinnervating (Frey et al.,
341 2000; Hegedus et al., 2008; Pun et al., 2006). We found that people with ALS exhibited a

342 significant increase in RT compared with control subjects. This increase is consistent with a
343 greater degree of rate modulation in MUs that have relatively larger forces due to the onset of
344 reinnervation. AHPs and PICs are known to be important factors that affect the firing
345 frequency of motoneurons and the excitability of spinal motoneurons (Jensen et al., 2020;
346 Kuo et al., 2004). Previous studies reported that an increase in PICs is responsible for the
347 increase in motoneurons excitability (Kuo et al., 2004), while a decrease in AHPs is
348 responsible for the increase in firing frequency (de Carvalho and Swash, 2016). In this study,
349 the TI and ΔF methods were used to estimate AHPs and PICs, respectively, and the results
350 showed that people with ALS exhibited greater ΔF and lower TI than control subjects. These
351 findings suggested that the mechanism of greater rate modulation in MUs consisted of greater
352 current-to-frequency gain in motoneurons, caused by a reduction in AHPs and/or an increase
353 in PICs. Furthermore, a reduction in AHP amplitudes would contribute to the increased CV of
354 the firing rate, as a shorter AHP duration would make motoneuron firing times more sensitive
355 to noise in motor commands (Matthews, 1996). This finding is in accordance with the results
356 of the present study, showing that people with ALS exhibit a greater CV of the mean MU
357 firing rate than control subjects. Although there has been a report of progressive
358 hyperexcitability followed by hypoexcitability of the spinal motoneurons until the onset of
359 denervation (Martínez-Silva et al., 2018), and there is controversy over the excitability level
360 of spinal motoneurons in the early stage of ALS, our results support the findings of Huh et al.

showing that excitability increases in onset of denervation period in ALS model mice (Huh et al., 2021). Huh et al. also report a decrease in spinal cord excitability as symptoms progress (Huh et al., 2021). Although our study only included subjects in the early stages of disease onset, we consider that it would be important to include subjects at different stages of disease in the future studies to examine serial changes in spinal excitability.

Motoneuron firing rates and thresholds are correlated at any specified force level; thus, the RT is determined by the input resistance of the cell and number of leak channels (De Luca and Hostage, 2010). The MU firing behavior in the control group exemplifies the neural control scheme referred to as the "onion skin" control scheme (De Luca and Erim, 1994; De Luca and Hostage, 2010; de Souza et al., 2018), which states that early recruited MUs have a higher mean MU firing rate than later recruited MUs and maintain that higher MU firing rate over time. On the other hand, the MU firing behavior in the ALS group did not follow the onion skin scheme, and there was no relationship between the MU firing rate and the RT. The MU firing rate was significantly higher in people with ALS in all MU subgroups, confirming a tendency for later recruited MUs to be more active, as shown in Fig. 7B. A previous study attempted to examine the RT of MUs in people with ALS, but visual inspection was performed, and the RT could not be used as a useful index (de Carvalho *et al.*, 2012). It is difficult to visually confirm the recruitment of MUs, but in this study, the RTs of MUs can be easily identified by synchronization with force data. The RT of an MU is an important

physiological indicator in people with ALS, especially in muscles with predominantly fast-twitch MUs (Frey *et al.*, 2000; Pun *et al.*, 2006), because it reflects the characteristics of the MU activation properties (e.g., slow-twitch or fast-twitch MUs) (Gregory and Bickel, 2005). Our findings reaffirm the importance of the RT of MUs as one of the indicators of abnormal MU firing behavior in people with ALS.

The comparison between the ALSFRS-R score and several MU outcomes (e.g., mean MU firing rate, RT, CV of the MU firing rate, and MU firing rate at recruitment) revealed that the MU firing rate is significantly influenced by disease severity. ALS is a rapidly progressive neurodegenerative disease (van Es et al., 2017). It has been shown in rat models of ALS that the proportion of muscle fiber types changes from time to time as neurodegeneration progresses (Kryściak *et al.*, 2014). The correlation of neurodegeneration with abnormal MU firing patterns is consistent with these observations. On the other hand, there was no correlation between muscle weakness and abnormal MU firing behavior. This finding indicates that there is no relationship between the progression of muscle weakness and abnormal MU firing behavior and suggests that abnormalities in MUs can be detected in the early phase of ALS. The assessment of MU firing behavior in people with ALS is often used for diagnostic purposes and rarely for the purpose of tracking long-term changes in the disease condition. However, our findings suggest that abnormalities in MU firing behavior directly reflect the pathogenesis of ALS and may be an important biomarker for

399 understanding the progression of signs and symptoms. In particular, our results revealed that
400 the MU firing rate at recruitment is useful for differentiating disease by multiple logistic
401 regression analysis. The cutoff value for the MU firing rate at recruitment was calculated to
402 be > 7.660, and the ALS detection rate was 87.5% when this cutoff value was used. This
403 finding was inferred from only one parameter, and we consider that its discriminative
404 accuracy is relatively high and useful for diagnosis. In addition to pain, an inherent
405 disadvantage of conventional needle EMG is that the position of the needle electrode must be
406 changed every time to search for a small electrode region, which could decrease the reliability
407 of measurement. Furthermore, needle EMG causes the shape of the MUAP to change with the
408 position of the needle electrode, resulting in the examiner focusing on lesion detection and
409 resulting sampling bias. By contrast, HD-SEMG can analyze MU firing behavior over a wide
410 range within a muscle at once, which may reduce sampling bias.
411 Our study has several limitations. First, since this study was a cross-sectional study
412 and did not examine neurodegeneration longitudinally, it only predicts the process of
413 neurodegeneration based on differences in the severity of symptoms. In the future,
414 longitudinal follow-up studies of MU firing behavior in the same patients will clarify how
415 neural reinnervation due to neurodegeneration affects MUs. This information will provide a
416 more detailed understanding of the pathogenesis of ALS and provide useful data for clarifying
417 pathology and new treatment methods. Second, although this study only included subjects

418 who could walk independently, the included patients had significantly lower muscle strength
419 than the control subjects. Detecting abnormalities in MU firing behavior in the very early
420 stages of neurodegeneration in ALS, when muscle strength is still preserved, would be a more
421 useful tool for the early detection and diagnosis of the disease. Finally, we did not perform
422 comparisons with needle EMG. However, HD-SEMG has been validated extensively in
423 comparison with needle EMG in previous work, and the usefulness of the HD-SEMG
424 methodology has already been verified (Holobar et al., 2014, 2010; Marateb et al., 2011).
425 Although most of the people with ALS in this study were evaluated by needle EMG at the
426 time of diagnosis, clinical needle examinations of decomposition were not performed, and the
427 EMG waveforms were visually evaluated to confirm pathophysiology. Therefore, to reduce
428 the physical pain of the people with ALS, we did not perform additional needle EMG
429 measurements, and only HD-SEMG measurements were performed in this study.
430

431 ## 5. Conclusion

432 We identified disease-specific MU firing behavior in people with ALS using HD-
433 SEMG. The people with ALS exhibited overactivity in all investigated types of MUs
434 compared to control subjects, indicating the usefulness of this new method for the
435 noninvasive assessment of neuromuscular degeneration in people with ALS. Abnormalities in

MU firing behavior were significantly correlated with disease severity, providing an important physiological index for understanding the pathophysiology of ALS.

Data availability statement

All primary data reported here are available from the corresponding author (Y.N.) upon reasonable request.

Conflict of Interest Statement

The authors declare no competing interests.

Author contributions

Conception or design of the work: Y.N., A.H., K.W., and T.T.; Acquisition or analysis or interpretation of data for the work: Y.N., A.H., K.W., T.T., H.U., N.M., H.M., S.T., and A.S.H.; Drafting the work or revising it critically for important intellectual content: Y.N., A.H., K.W., T.T., H.U., N.M., H.M., S.T., and A.S.H. All authors have read and 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 related to the accuracy or integrity of any part of the work are appropriately

454 investigated and resolved. All persons designated as authors qualify for authorship, and all
455 those who qualify for authorship are listed.
456

457 ## Acknowledgements

458 Yuichi Nishikawa, Kohei Watanabe, and Aleš Holobar were supported by the
459 Bilateral Program of JSPS (JPJSBP-82626). Aleš Holobar was supported by the Slovenian
460 Research Agency (project J2-1731 and program funding P2-0041).

References

Castronovo M, Mrachacz-Kersting N, Landi F, Jørgensen HR, Severinsen K, Farina D. Motor Unit Coherence at Low Frequencies Increases Together with Cortical Excitability Following a Brain-Computer Interface Intervention in Acute Stroke Patients. Converging Clinical and Engineering Research on Neurorehabilitation II, Springer International Publishing; 2017, p. 1001–5.

Costa J, Swash M, de Carvalho M. Awaji criteria for the diagnosis of amyotrophic lateral sclerosis:a systematic review. Arch Neurol 2012;69:1410–6.

De Luca CJ, Erim Z. Common drive of motor units in regulation of muscle force. Trends Neurosci 1994;17:299–305.

De Luca CJ, Hostage EC. Relationship between firing rate and recruitment threshold of motoneurons in voluntary isometric contractions. J Neurophysiol 2010;104:1034–46.

de Carvalho M, Dengler R, Eisen A, England JD, Kaji R, Kimura J, et al. Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol 2008;119:497–503.

de Carvalho M, Swash M. Lower motor neuron dysfunction in ALS. Clin Neurophysiol 2016;127:2670–81.

de Carvalho M, Turkman A, Swash M. Motor unit firing in amyotrophic lateral sclerosis and other upper and lower motor neurone disorders. Clin Neurophysiol 2012;123:2312–8.

Doi Y, Atsuta N, Sobue G, Morita M, Nakano I. Prevalence and incidence of amyotrophic lateral sclerosis in Japan. J Epidemiol 2014;24:494–9.

Duchateau J, Enoka RM. Human motor unit recordings: origins and insight into the integrated motor system. Brain Res 2011;1409:42–61.

Frey D, Schneider C, Xu L, Borg J, Spooren W, Caroni P. Early and selective loss of neuromuscular synapse subtypes with low sprouting competence in motoneuron diseases. J Neurosci 2000;20:2534–42.

Fuglevand AJ, Winter DA, Patla AE. Models of recruitment and rate coding organization in motor-unit pools. J Neurophysiol 1993;70:2470–88.

Fuglsang-Frederiksen A, Rønager J. EMG power spectrum, turns-amplitude analysis and motor unit potential duration in neuromuscular disorders. J Neurol Sci 1990;97:81–91.

Gorassini M, Yang JF, Siu M, Bennett DJ. Intrinsic activation of human motoneurons: possible contribution to motor unit excitation. J Neurophysiol 2002;87:1850–8.

Gregory CM, Bickel CS. Recruitment patterns in human skeletal muscle during electrical stimulation. Phys Ther 2005;85:358–64.

Hassan A, Thompson CK, Negro F, Cummings M, Powers RK, Heckman CJ, et al. Impact of parameter selection on estimates of motoneuron excitability using paired motor unit analysis. J Neural Eng 2020;17:016063.

497 Hassan AS, Fajardo ME, Cummings M, McPherson LM, Negro F, Dewald JPA, et al.
498 Estimates of persistent inward currents are reduced in upper limb motor units of older adults.
499 J Physiol 2021;599:4865–82.
500 Heckman CJ, Enoka RM. Motor unit. Compr Physiol 2012;2:2629–82.
501 Hegedus J, Putman CT, Tyreman N, Gordon T. Preferential motor unit loss in the SOD1
502 G93A transgenic mouse model of amyotrophic lateral sclerosis. J Physiol 2008;586:3337–51.
503 Holobar A, Farina D, Gazzoni M, Merletti R, Zazula D. Estimating motor unit discharge
504 patterns from high-density surface electromyogram. Clin Neurophysiol 2009;120:551–62.
505 Holobar A, Minetto MA, Botter A, Negro F, Farina D. Experimental analysis of accuracy in
506 the identification of motor unit spike trains from high-density surface EMG. IEEE Trans
507 Neural Syst Rehabil Eng 2010;18:221–9.
508 Holobar A, Minetto MA, Farina D. Accurate identification of motor unit discharge patterns
509 from high-density surface EMG and validation with a novel signal-based performance metric.
510 J Neural Eng 2014;11:016008.
511 Holobar A, Zazula D. Correlation-based decomposition of surface electromyograms at low
512 contraction forces. Med Biol Eng Comput 2004;42:487–95.
513 Huh S, Heckman CJ, Manuel M. Time Course of Alterations in Adult Spinal Motoneuron
514 Properties in the SOD1(G93A) Mouse Model of ALS. ENeuro 2021;8.
515 https://doi.org/10.1523/ENEURO.0378-20.2021.

516 Jensen DB, Kadlecova M, Allodi I, Meehan CF. Spinal motoneurones are intrinsically more
517 responsive in the adult G93A SOD1 mouse model of amyotrophic lateral sclerosis. J Physiol
518 2020;598:4385–403.
519 Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, et al. Amyotrophic
520 lateral sclerosis. Lancet 2011;377:942–55.
521 Kryściak K, Grieb P, Celichowski J. Changes in motor unit properties in SOD1 (G93A) rats.
522 Muscle Nerve 2014;50:577–86.
523 Kuo JJ, Schonewille M, Siddique T, Schults ANA, Fu R, Bär PR, et al. Hyperexcitability of
524 cultured spinal motoneurons from presymptomatic ALS mice. J Neurophysiol 2004;91:571–5.
525 Kurca E, Drobný M. Four quantitative EMG methods and theirs individual parameter
526 diagnostic value. Electromyogr Clin Neurophysiol 2000;40:451–8.
527 Marateb HR, McGill KC, Holobar A, Lateva ZC, Mansourian M, Merletti R. Accuracy
528 assessment of CKC high-density surface EMG decomposition in biceps femoris muscle. J
529 Neural Eng 2011;8:066002.
530 Martínez-Silva M de L, Imhoff-Manuel RD, Sharma A, Heckman CJ, Shneider NA, Roselli
531 F, et al. Hypoexcitability precedes denervation in the large fast-contracting motor units in two
532 unrelated mouse models of ALS. Elife 2018;7. https://doi.org/10.7554/eLife.30955.

533 Martinez-Valdes E, Negro F, Laine CM, Falla D, Mayer F, Farina D. Tracking motor units
534 longitudinally across experimental sessions with high-density surface electromyography. J
535 Physiol 2017;595:1479–96.
536 Matthews PB. Relationship of firing intervals of human motor units to the trajectory of post-
537 spike after-hyperpolarization and synaptic noise. J Physiol 1996;492 (Pt 2):597–628.
538 Mazzo MR, Weinman LE, Giustino V, Mclagan B, Maldonado J, Enoka RM. Changes in
539 neural drive to calf muscles during steady submaximal contractions after repeated static
540 stretches. J Physiol 2021;599:4321–36.
541 Merletti R, Holobar A, Farina D. Analysis of motor units with high-density surface
542 electromyography. J Electromyogr Kinesiol 2008;18:879–90.
543 Nishikawa Y, Watanabe K, Holobar A, Maeda N, Maruyama H, Tanaka S. Identification of
544 the laterality of motor unit behavior in female patients with parkinson's disease using high-
545 density surface electromyography. Eur J Neurosci 2021a;53:1938–49.
546 Nishikawa Y, Watanabe K, Holobar A, Takahashi T, Maeda N, Maruyama H, et al.
547 Association between the Degree of Pre-Synaptic Dopaminergic Pathway Degeneration and
548 Motor Unit Firing Behavior in Parkinson's Disease Patients. Sensors 2021b;21.
549 https://doi.org/10.3390/s21196615.
550 Nishikawa Y, Watanabe K, Kawade S, Takahashi T, Kimura H, Maruyama H, et al. The
551 effect of a portable electrical muscle stimulation device at home on muscle strength and

552 activation patterns in locomotive syndrome patients: A randomized control trial. J
553 Electromyogr Kinesiol 2019;45:46–52.
554 Nishikawa Y, Watanabe K, Takahashi T, Hosomi N, Orita N, Mikami Y, et al. Sex
555 differences in variances of multi-channel surface electromyography distribution of the vastus
556 lateralis muscle during isometric knee extension in young adults. Eur J Appl Physiol
557 2017a;117:583–9.
558 Nishikawa Y, Watanabe K, Takahashi T, Kimura H, Maruyama H. The effect of medication
559 on vastus lateralis muscle activation patterns in Parkinson's disease patients. J Electromyogr
560 Kinesiol 2018;42:66–73.
561 Nishikawa Y, Watanabe K, Takahashi T, Orita N, Kimura H, Matsumoto M, et al. Spatial
562 electromyography distribution pattern of the vastus lateralis muscle during ramp up
563 contractions in Parkinson's disease patients. J Electromyogr Kinesiol 2017b;37:125–31.
564 Nuccio S, Del Vecchio A, Casolo A, Labanca L, Rocchi JE, Felici F, et al. Deficit in knee
565 extension strength following anterior cruciate ligament reconstruction is explained by a
566 reduced neural drive to the vasti muscles. J Physiol 2021;599:5103–20.
567 Piotrkiewicz M. An influence of afterhyperpolarization on the pattern of motoneuronal
568 rhythmic activity. J Physiol Paris 1999;93:125–33.
569 Piotrkiewicz M, Hausmanowa-Petrusewicz I. Motoneuron afterhyperpolarisation duration in
570 amyotrophic lateral sclerosis. J Physiol 2011;589:2745–54.

571 Piotrkiewicz M, Kudina L, Mierzejewska J, Jakubiec M, Hausmanowa-Petrusewicz I. Age-
572 related change in duration of afterhyperpolarization of human motoneurones. J Physiol
573 2007;585:483–90.
574 Pun S, Santos AF, Saxena S, Xu L, Caroni P. Selective vulnerability and pruning of phasic
575 motoneuron axons in motoneuron disease alleviated by CNTF. Nat Neurosci 2006;9:408–19.
576 Sanders DB, Stålberg EV, Nandedkar SD. Analysis of the electromyographic interference
577 pattern. J Clin Neurophysiol 1996;13:385–400.
578 Sankaranarayani R, Raghavan M, Nalini A, Laxmi TR, Raju TR. Reach task-associated
579 excitatory overdrive of motor cortical neurons following infusion with ALS-CSF. J Neural
580 Transm 2014;121:49–58.
581 Shiga Y, Onodera H, Shimizu H, Kimura I, Ohnuma A, Itoyama Y. Two consecutive
582 fasciculation potentials having different motor unit origins are an electromyographically
583 pathognomonic finding of ALS. Electromyogr Clin Neurophysiol 2000;40:237–41.
584 de Souza LML, Cabral HV, de Oliveira LF, Vieira TM. Motor units in vastus lateralis and in
585 different vastus medialis regions show different firing properties during low-level, isometric
586 knee extension contraction. Hum Mov Sci 2018;58:307–14.
587 van, Es, Michael A, Hardiman O, Chio A, Al-Chalabi A, Pasterkamp RJ, Veldink JH, et al.
588 Amyotrophic lateral sclerosis. Lancet 2017;390:2084–98.

589 Vucic S, Kiernan MC. Novel threshold tracking techniques suggest that cortical
590 hyperexcitability is an early feature of motor neuron disease. Brain 2006;129:2436–46.
591 Vucic S, Ziemann U, Eisen A, Hallett M, Kiernan MC. Transcranial magnetic stimulation and
592 amyotrophic lateral sclerosis: pathophysiological insights. J Neurol Neurosurg Psychiatry
593 2013;84:1161–70.
594 Watanabe K, Holobar A, Kouzaki M, Ogawa M, Akima H, Moritani T. Age-related changes
595 in motor unit firing pattern of vastus lateralis muscle during low-moderate contraction. Age
596 2016;38:48.
597 Watanabe K, Holobar A, Uchida K, Mita Y. Fish Protein Ingestion Induces Neural, but Not
598 Muscular Adaptations, Following Resistance Training in Young Adults. Front Nutr
599 2021;8:645747.
600 Watanabe K, Kouzaki M, Moritani T. Task-dependent spatial distribution of neural activation
601 pattern in human rectus femoris muscle. J Electromyogr Kinesiol 2012;22:251–8.
602 Weddell T, Bashford J, Wickham A, Iniesta R, Chen M, Zhou P, et al. First-recruited motor
603 units adopt a faster phenotype in amyotrophic lateral sclerosis. J Physiol 2021;599:4117–30.

Figure legends

Fig. 1. Experimental setting.

All subjects were seated on a custom-made dynamometer with their hip and knee fixed at 90° and performed the motor task (A). A 30% maximal voluntary contraction task was performed as a submaximal voluntary contraction task (B). A 2D grid of 64 electrodes (C) was attached to the vastus lateralis muscle (D).

Fig. 2. Definition of recruitment threshold (RT) and motor unit (MU) subgroups.

The RT was calculated from the spike train detected at the beginning of each MU (the white circles indicate the RT, and the solid line indicates torque data) in control subject (A) and ALS individual (C). Three MU subgroups were defined with reference to the RT in control subject (B) and ALS individual (D): the MU10 group includes MUs recruited with less than 10% maximal voluntary contraction (MVC), the MU20 group includes MUs recruited at forces between 10% MVC and 20% MVC, and the MU30 group includes MUs recruited at forces higher than 20% MVC.

Fig. 3. Estimation of ΔF and transition interval.

Example data from one subject (control subject, age = 59 years, male) showing the instantaneous discharge rate profiles of two concurrently active motor units expressed in

623 pulses per second (pps). The first recruited motor unit was classified as the reference motor
624 unit (B), and the second recruited motor unit was the test motor unit (A). ΔF was estimated as
625 $F_{start} - F_{end}$. Relationship between the consecutive interval difference (CD) and mean
626 interspike interval (MISI) (C). Arrow, transition interval.
627
628 **Fig. 4. Comparison of motor unit (MU) properties between the people with amyotrophic**
629 **lateral sclerosis (ALS) and control subjects.**
630 People with ALS showed significantly higher recruitment thresholds (A), MU firing rates at
631 recruitment (B), and CV values of the MU firing rate (C) than control subjects.
632
633 **Fig. 5. Comparison of ΔF and transition interval (TI) between people with amyotrophic**
634 **lateral sclerosis (ALS) and control subjects.**
635 People with ALS showed significantly higher values of Δ*F*, and significantly lower values of
636 TI than control subjects.
637
638 **Fig. 6. Comparison of the mean motor unit (MU) firing rate in control subjects and**
639 **people with amyotrophic lateral sclerosis (ALS) among the MU10, MU20, MU30,**
640 **MU10Nm, and MU20Nm groups.**

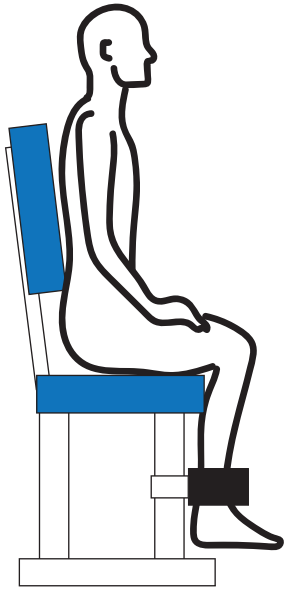
641 There were significant differences between the control subjects and people with ALS in each
642 MU subgroup (e.g., MU10, MU20, MU30 (A), and MU10Nm, MU20Nm (B)). Furthermore,
643 control subjects showed a higher mean MU firing rate for the early-recruited MUs than for the
644 later-recruited MUs, whereas no such difference was observed in people with ALS. * $p <$
645 0.0001, † $p < 0.0001$ compared with control subjects.
646
647 **Fig. 7. Relationship of the recruitment threshold (RT) and/or absolute torque and with**
648 **the motor unit (MU) firing rate in control subjects and people with amyotrophic lateral**
649 **sclerosis (ALS).**
650 Significant correlations of the Rt and/or absolute torque with the MU firing rate were
651 observed in control subjects (A and C) but not in people with ALS (B and D).
652
653 **Fig. 8. Relationship between the amyotrophic lateral sclerosis (ALS) Functional Rating**
654 **Scale-Revised (ALSFRS-R) score and motor unit (MU) properties in people with ALS.**
655 Significant correlations were observed between the ALSFRS-R scores and number of MUs
656 (A), RT (B), mean MU firing rate (C), and MU firing rate at recruitment (D).
657
658 **Fig. 9. The receiver operating characteristic (ROC) curve of the motor unit (MU) firing**
659 **rates at recruitment.**

660 The ROC curve was calculated from the data of people with amyotrophic lateral sclerosis (ALS)

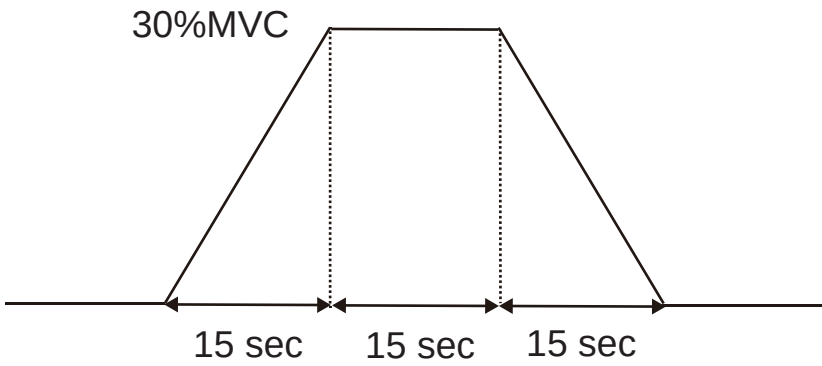
661 and control subjects. The cutoff value for detecting ALS was calculated to be > 7.6, and the

662 sensitivity and specificity were 81.25% and 90.63%, respectively.

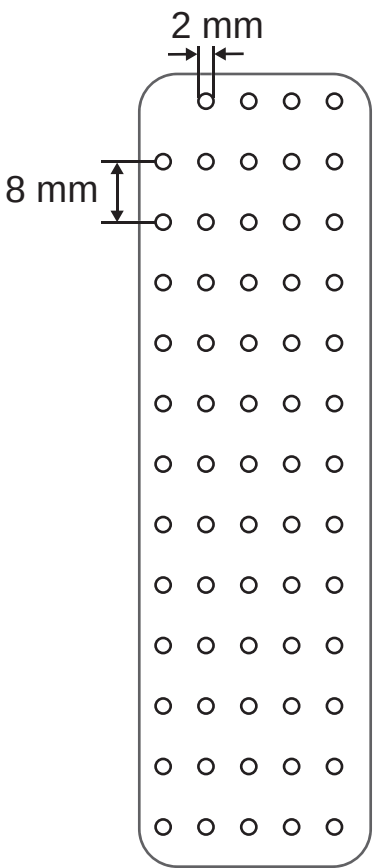
A



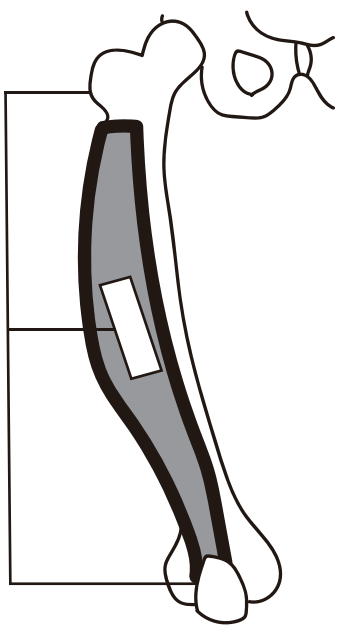
B

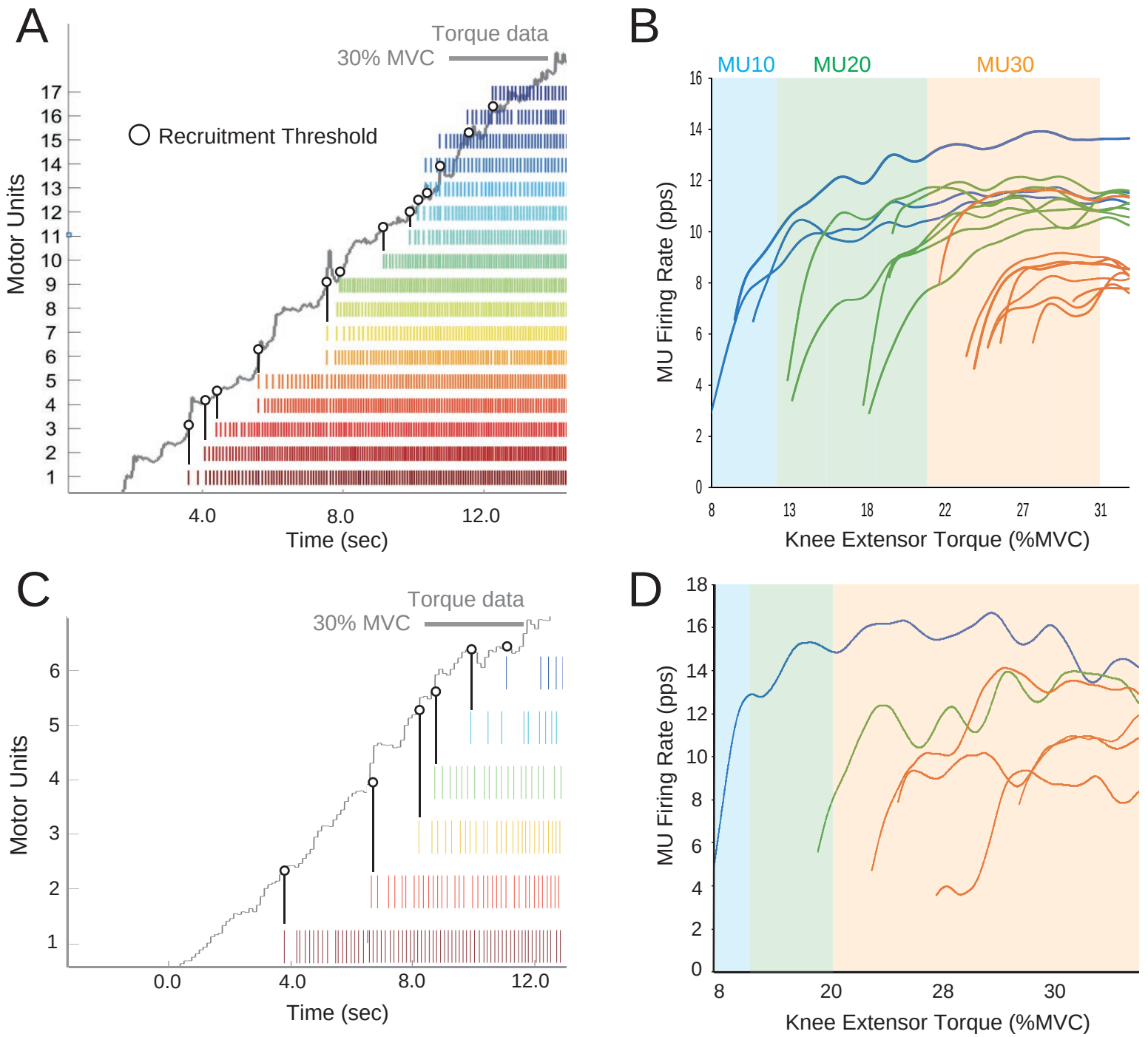


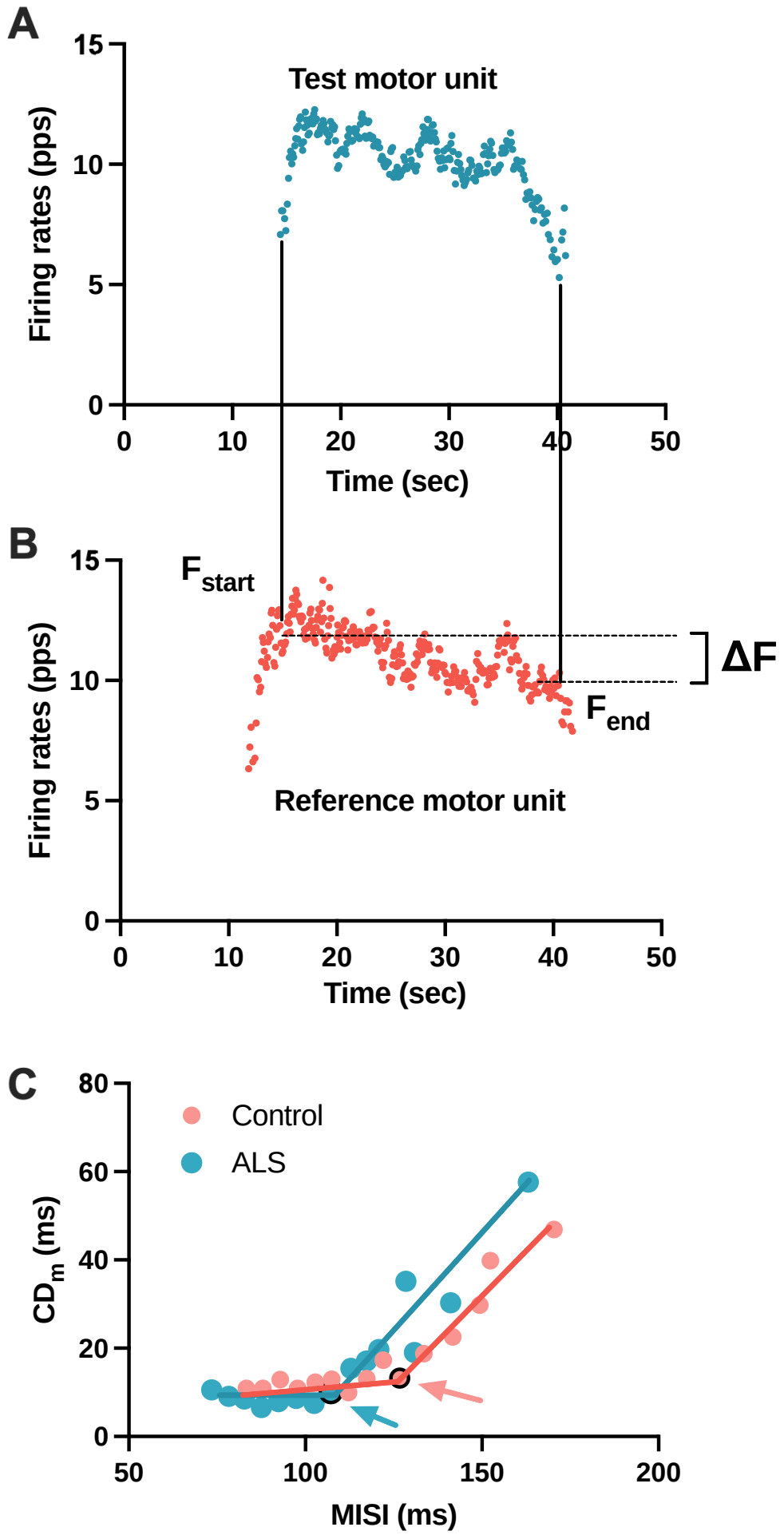
C

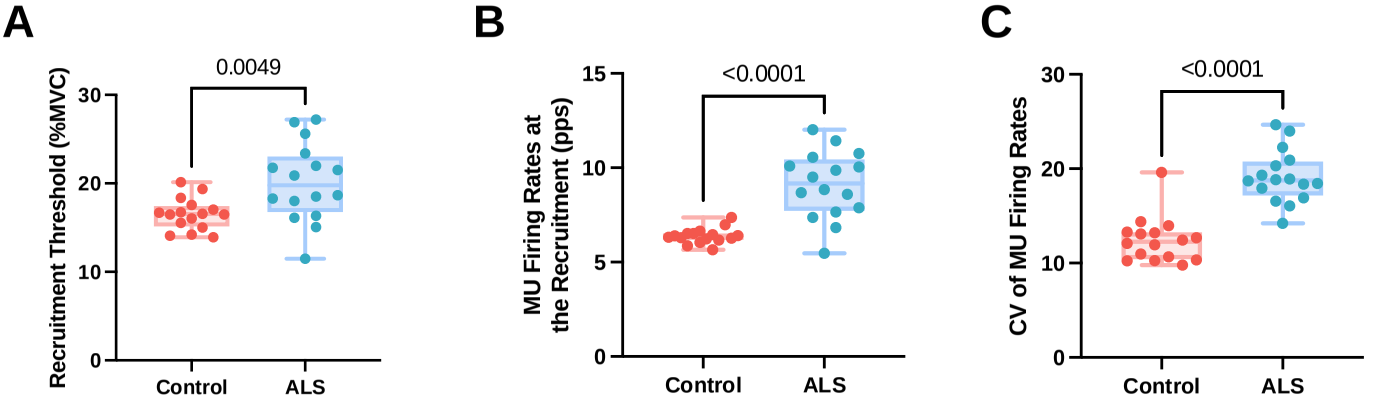


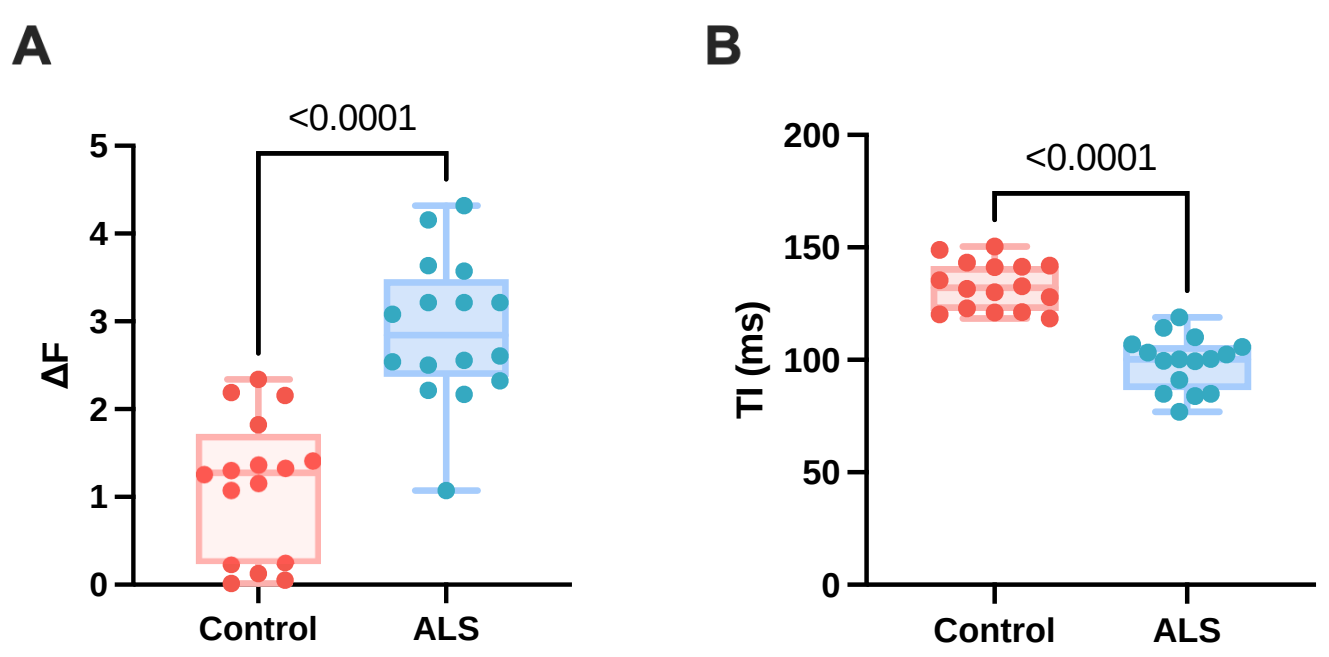
D

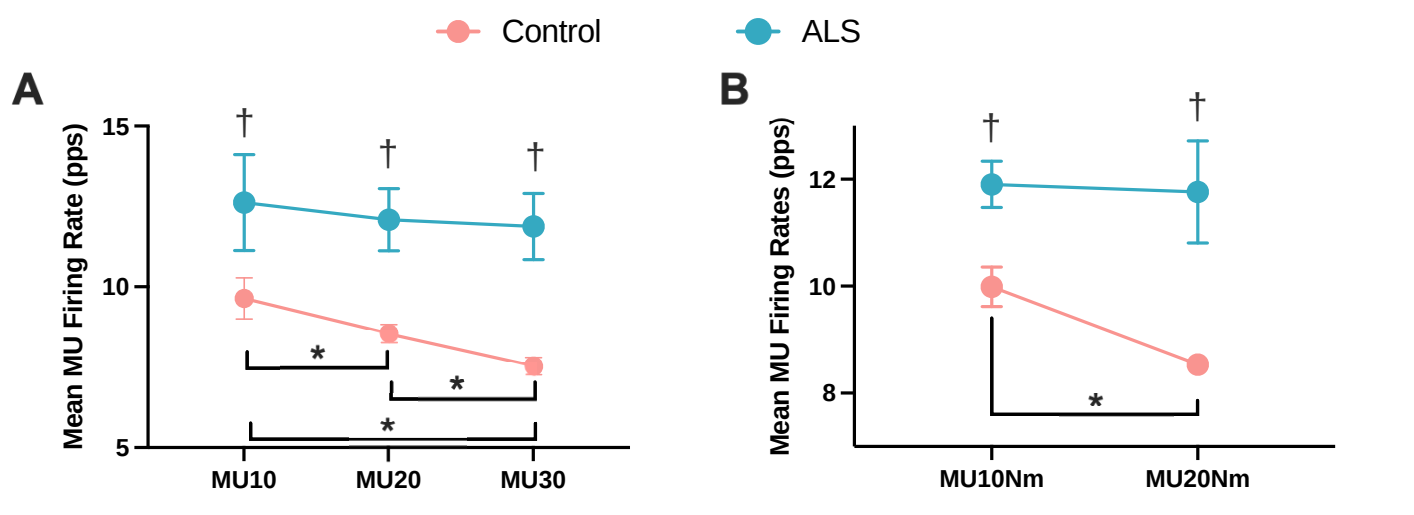


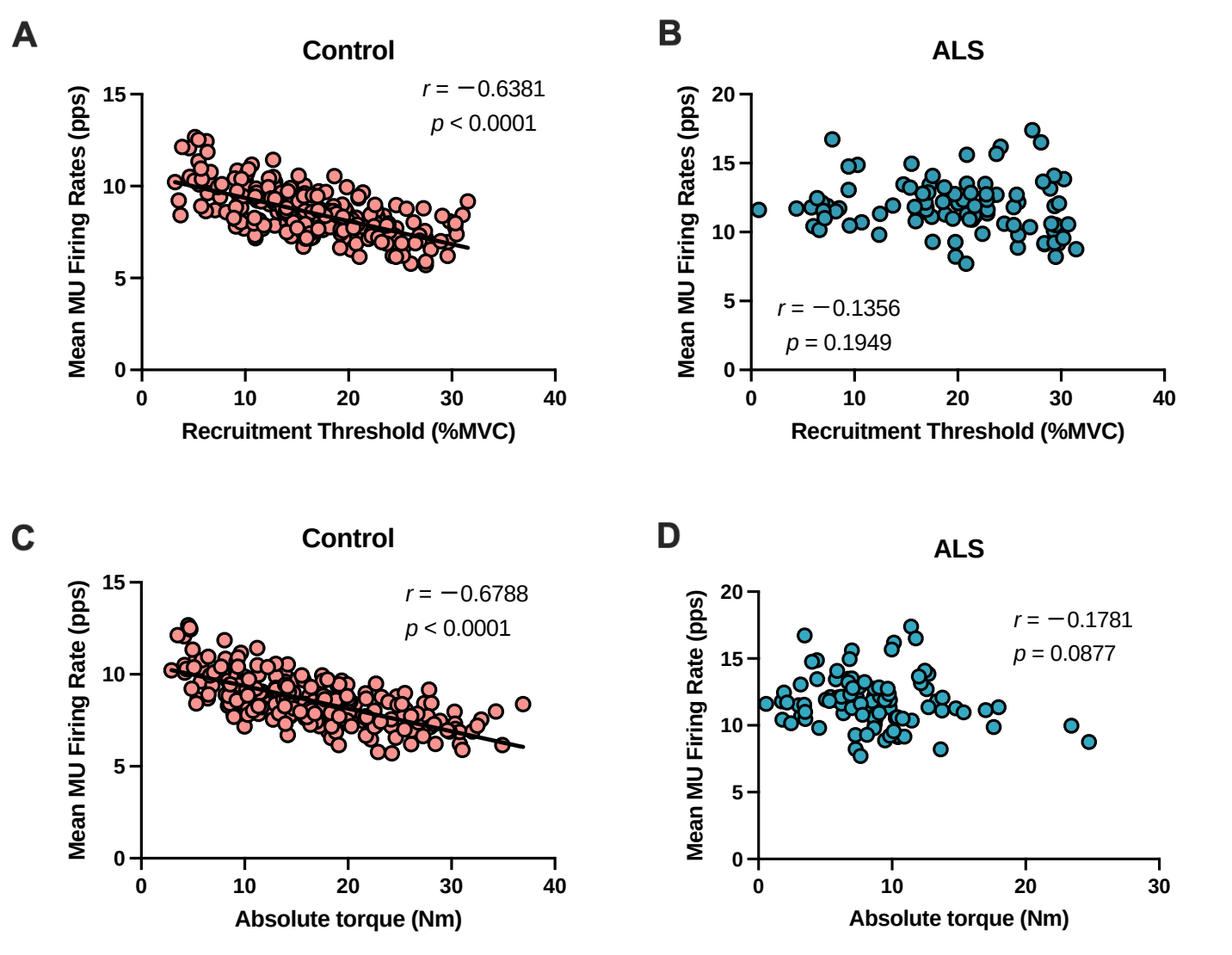

A
Torque data
30% MVC
Recruitment Threshold
Motor Units
Time (sec)
B
MU10
MU20
MU30
MU Firing Rate (pps)
Knee Extensor Torque (%MVC)
C
Torque data
30% MVC
Motor Units
Time (sec)
D
MU Firing Rate (pps)
Knee Extensor Torque (%MVC)

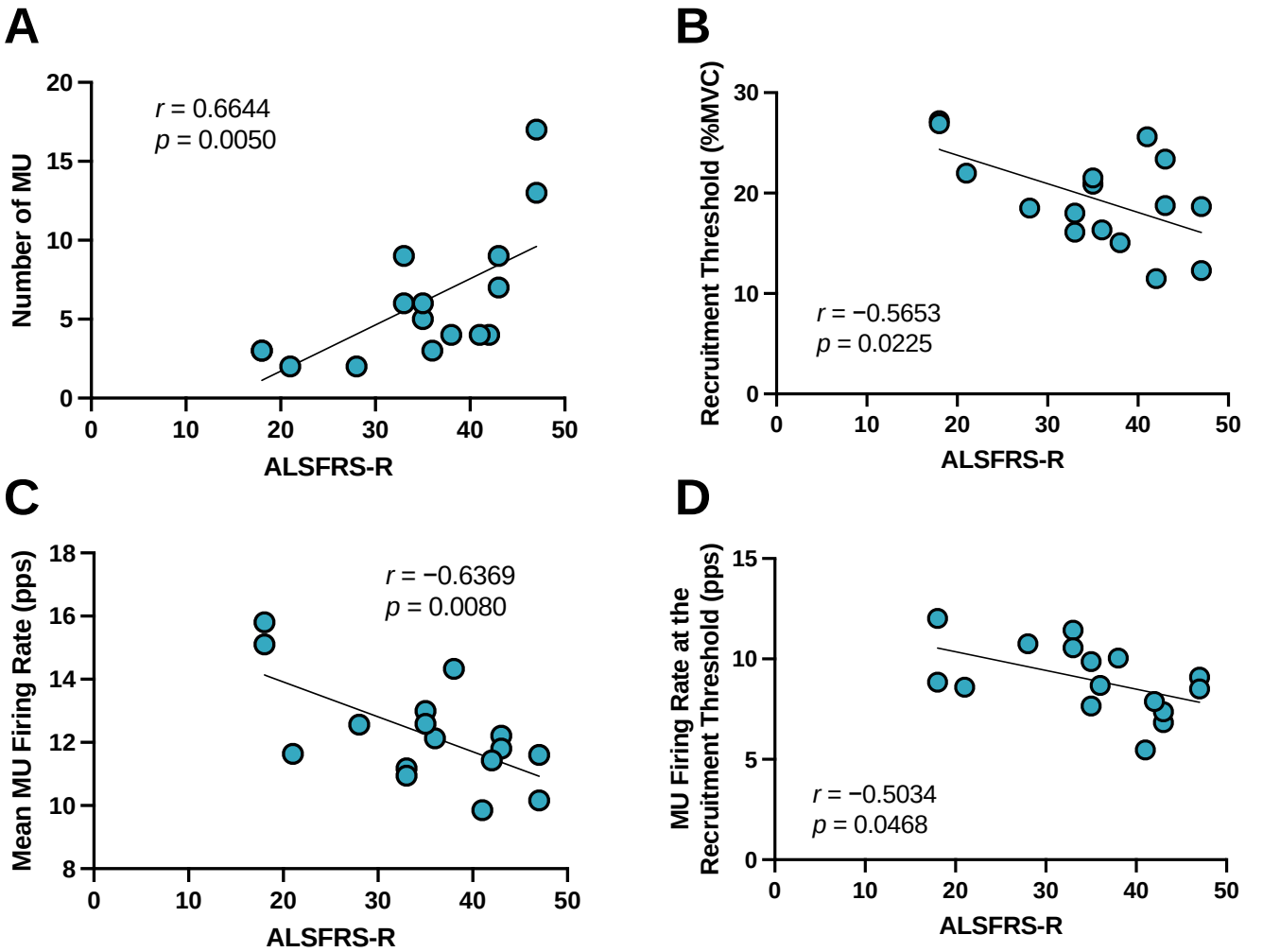

A
Test motor unit
Firing rates (pps)
15
10
5
0
0
10
20
30
40
50
Time (sec)
B
F start
ΔF
F end
Reference motor unit
Firing rates (pps)
15
10
5
0
0
10
20
30
40
50
Time (sec)
C
Control
ALS
CDm (ms)
80
60
40
20
0
50
100
150
200
MISI (ms)

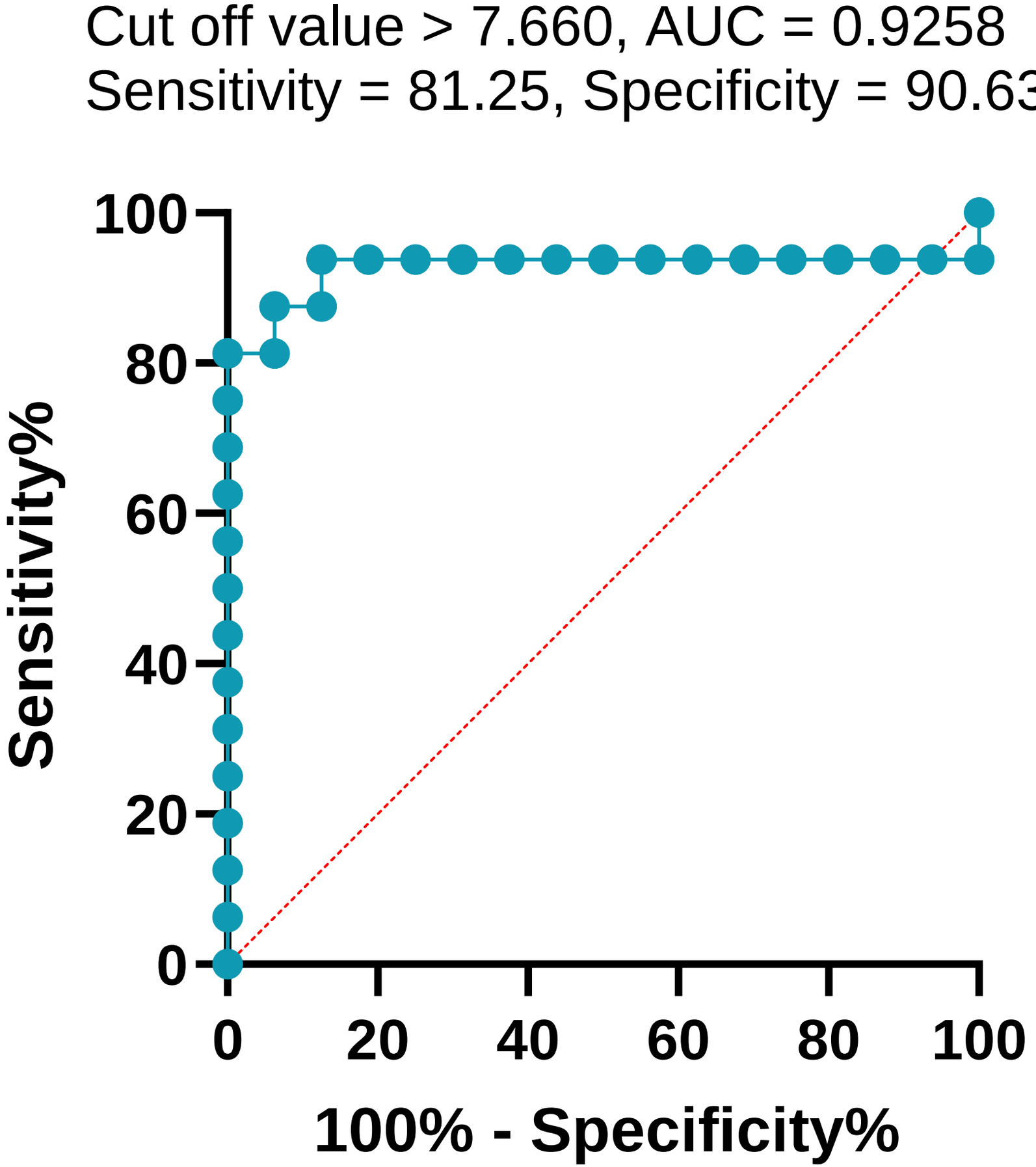

A
Recruitment Threshold (%MVC)
0.0049
30
20
10
0
Control
ALS
B
MU Firing Rates at the Recruitment (pps)
<0.0001
15
10
5
0
Control
ALS
C
CV of MU Firing Rates
<0.0001
30
20
10
0
Control
ALS


A
<0.0001
5
4
3
2
1
0
ΔF
Control
ALS
B
200
150
100
50
0
TI (ms)
<0.0001
Control
ALS


Control
ALS
A
Mean MU Firing Rate (pps)
15
10
5
†
†
†
*
*
*
MU10
MU20
MU30
B
Mean MU Firing Rates (pps)
12
10
8
†
†
*
MU10Nm
MU20Nm


A
Control
r = −0.6381
p < 0.0001
Mean MU Firing Rates (pps)
Recruitment Threshold (%MVC)
B
ALS
r = −0.1356
p = 0.1949
Mean MU Firing Rates (pps)
Recruitment Threshold (%MVC)
C
Control
r = −0.6788
p < 0.0001
Mean MU Firing Rate (pps)
Absolute torque (Nm)
D
ALS
r = −0.1781
p = 0.0877
Mean MU Firing Rate (pps)
Absolute torque (Nm)


A
Number of MU
ALSFRS-R
r = 0.6644
p = 0.0050
B
Recruitment Threshold (%MVC)
ALSFRS-R
r = −0.5653
p = 0.0225
C
Mean MU Firing Rate (pps)
ALSFRS-R
r = −0.6369
p = 0.0080
D
MU Firing Rate at the Recruitment Threshold (pps)
ALSFRS-R
r = −0.5034
p = 0.0468


Cut off value > 7.660, AUC = 0.9258
Sensitivity = 81.25, Specificity = 90.63
100
80
60
40
20
0
Sensitivity%
0
20
40
60
80
100
100% - Specificity%