

Inability to increase the neural drive to muscle is associated with task failure during submaximal contractions

Martinez Valdes, Eduardo; Negro, Francesco; Falla, Deborah; Dideriksen, Jakob Lund; Heckman, CJ; Farina, Dario

DOI:
[10.1152/jn.00447.2020](https://doi.org/10.1152/jn.00447.2020)

License:
None: All rights reserved

Document Version
Peer reviewed version

Citation for published version (Harvard):
Martinez Valdes, E, Negro, F, Falla, D, Dideriksen, JL, Heckman, CJ & Farina, D 2020, 'Inability to increase the neural drive to muscle is associated with task failure during submaximal contractions', *Journal of Neurophysiology*, vol. 124, no. 4, pp. 1110-1121. <https://doi.org/10.1152/jn.00447.2020>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:
Copyright © 2020, Journal of Neurophysiology
<https://doi.org/10.1152/jn.00447.2020>

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Inability to increase the neural drive to muscle is associated with task failure during submaximal contractions

*Eduardo Martinez-Valdes¹, *Francesco Negro², Deborah Falla¹, Jakob L. Dideriksen³, Charles J. Heckman⁴, Dario Farina⁵

Affiliations

1. Centre of Precision Rehabilitation for Spinal Pain (CPR Spine), School of Sport, Exercise and Rehabilitation Sciences, College of Life and Environmental Sciences, University of Birmingham, Birmingham, UK
2. Department of Clinical and Experimental Sciences, Research Centre for Neuromuscular Function and Adapted Physical Activity "Teresa Camplani", Università degli Studi di Brescia, Brescia, Italy
3. Center for Sensory-Motor Interaction, Department of Health Science and Technology, Aalborg University, 9220, Aalborg, Denmark
4. Department of Physiology, Northwestern University, Chicago, IL, USA
5. Department of Bioengineering, Imperial College London, Royal School of Mines, London, UK

*Authors contributed equally to this work

Correspondence to:

Dario Farina

Department of Bioengineering, Imperial College London, London, UK.

Tel: +44 (0) 20 759 28 41387

Email: d.farina@imperial.ac.uk

Running title: Biphasic motor unit behaviour during fatigue

Keywords: Motor unit, fatigue, high-density surface electromyography, vastus medialis, vastus lateralis

Running title: Biphasic motor unit behaviour during submaximal fatiguing contractions

Competing Interests: The authors declare no competing financial interests.

32 **ABSTRACT**

33 We investigated changes in motor unit (MU) behaviour and vasti-muscle contractile properties during
34 sustained submaximal fatiguing contractions with a new time-domain tracking technique in order to
35 understand the mechanisms responsible for task failure. Sixteen participants performed a non-fatiguing
36 15s isometric knee-extension at 50% of the maximum voluntary torque (MVC), followed by a 30% MVC
37 sustained contraction until exhaustion. Two grids of 64 surface electromyography electrodes were
38 placed over vastus medialis and lateralis. Signals were decomposed into MU discharge-times and the
39 MUs from the 30% MVC sustained contraction were followed until task failure by overlapping
40 decomposition intervals. These MUs were then tracked between 50% and 30% MVC. During the
41 sustained fatiguing contraction, MUs of the two muscles decreased their discharge rate until ~40% of
42 the endurance time, referred to as the reversal time, and then increased their discharge rate until task
43 failure. This reversal in firing behaviour predicted total endurance time and was matched by opposite
44 changes in twitch force (increase followed by a decrease). Despite the later increase in MU firing rates,
45 peak discharge rates at task failure did not reach the frequency attained during a non-fatiguing 50%
46 MVC contraction. These results show that changes in MU firing properties are influenced by adjustments
47 in contractile properties during the course of the contraction, allowing the identification of two phases.
48 Nevertheless, the contraction cannot be sustained possibly due to progressive motoneuron
49 inhibition/decreased excitability, as the later increase in firing rate saturates at a much lower frequency
50 compared to a higher-force non-fatiguing contraction.

51

52

53

54 NEW & NOTEWORTHY

55 Motor unit firing and contractile properties during a submaximal contraction until failure were assessed
56 with a new tracking technique. Two distinct phases in firing behaviour were observed, which
57 compensated for changes in twitch area and predicted time to failure. However, the late increase in
58 firing rate was below the rates attained in absence of fatigue, which points to an inability of the central
59 nervous system to sufficiently increase the neural drive to muscle with fatigue.

60 INTRODUCTION

61 It is commonly assumed that during sustained submaximal contractions, muscle fatigue, defined as a
62 reduction in the maximum force generation capacity of muscles, is a progressive phenomenon which
63 develops from contraction onset and is associated to the same mechanisms during the contraction
64 (Enoka and Duchateau 2008). This conclusion is based on previous observations that showed similar
65 changes in central and peripheral electrophysiological properties during the entire duration of a task.
66 For example, it has often been observed that the discharge rates of motor units progressively decrease
67 from the beginning to the end of submaximal isometric contractions (Carpentier et al. 2001; Garland et
68 al. 1994; McManus et al. 2016; Vila-Cha et al. 2012). Because the progressive decrease in discharge rates
69 is typically paralleled by an increase in surface EMG amplitude, it has been generally assumed that the
70 contraction can be sustained by the recruitment of additional motor units, which is supported by
71 experimental observations (Adam and De Luca 2005; Carpentier et al. 2001). On the other hand, other
72 studies have challenged the observation of a continuous decrease in discharge rate during submaximal
73 fatiguing contractions (Adam and De Luca 2005; Dorfman et al. 1990; Garland et al. 1997; Griffin et al.
74 2000; Kuchinad et al. 2004; Mettler and Griffin 2016). In these studies, an increase in motor unit
75 discharge rates was observed after an initial decrease, and this behaviour was linked with a decrease in
76 whole-muscle single-twitch force (Adam and De Luca 2005). These results implied that neural

77 adjustments might be required to compensate for changes in contractile properties in order to maintain
78 a steady force. This diverse motor unit behaviour across studies may be related to the wide variety of
79 protocols utilized to assess fatigue (Enoka and Duchateau 2008; Hunter et al. 2004), the specific muscle
80 under study and the force level assessed. Regardless of the protocol employed and the target muscle
81 assessed, it has been difficult to track the same motor units during the entire duration of fatiguing
82 contractions using intramuscular EMG recordings, and this difficulty has generated controversy on the
83 actual adaptations in motor unit behaviour with fatigue (Enoka 2019). Identifying the activity of the
84 same motor units during both fatiguing and non-fatiguing contractions is essential to unravel the neural
85 control of fatigue, but, it is extremely challenging due to the different levels of superimposition of motor
86 unit action potentials in the recorded signals. Moreover, the waveforms of the motor unit action
87 potentials (MUAPs) change during the course of different contractions, specifically during contractions
88 until failure, and therefore to track the MUAPs of the same motor units in these recording conditions is
89 not always possible. In this study, we aimed to investigate the changes in motor unit discharge rate and
90 contractile properties of two synergistic muscles by tracking a representative number of motor units
91 during a sustained submaximal isometric contraction until task failure. For this purpose, we developed a
92 novel approach of time-domain motor unit tracking that assures high accuracy in the observed motor
93 unit properties with fatigue. We coupled this tracking approach with a recently developed technique to
94 estimate the characteristics of (fused) motor unit twitches quantified during the course of the sustained
95 contraction (Negro and Orizio 2017). Finally, we compared the firing properties of the same motor units
96 identified at task failure and a higher-force non-fatiguing contraction in order to assess the neural
97 mechanisms responsible for task failure. The results provide a comprehensive characterisation of the
98 neural and contractile adjustments of motor units with fatigue and prove the inability of the central
99 nervous system to sufficiently increase the neural drive to muscle with fatigue.

100

101 **METHODS**

102 Participants

103 Sixteen healthy and physically active men [mean (SD) age: 28 (4) yr, height: 177 (5) cm, mass: 78 (6) kg]
104 without a history of neuromuscular disorders or previous lower limb surgery, were recruited.
105 Participants were asked to avoid any strenuous physical activity 24 h before the measurements. The
106 Ethics Committee of the Universität Potsdam approved the study (approval no. 26/2015), in accordance
107 with the Declaration of Helsinki (2004). All participants gave written, informed consent.

108 Experimental Protocol

109 The participants performed submaximal and maximal knee extension (dominant leg) contractions on an
110 isokinetic dynamometer (CON-TREX MJ; PHYSIOMED, Regensdorf, Switzerland). All isometric
111 contractions were exerted with the knee flexed to 90° and hip flexed to 80° (chair was reclined by 10°).
112 The participant's torso, waist and contralateral leg were strapped to the chair in order to prevent
113 compensatory movements throughout the trial. After placement of the surface EMG electrodes (see
114 Data Acquisition), subjects performed three maximal voluntary contractions (MVC) of knee extension for
115 5 s and each of these trials was separated by 2 min of rest. The highest MVC value was used as a
116 reference to define the submaximal torque level. After 5 min of rest, and following a few familiarization
117 trials, subjects performed a submaximal isometric knee extension contraction at 50% MVC by following
118 a target that had a trapezoidal waveform presented in a computer screen (5 s ramp-up and ramp-down
119 contraction with a 15 s hold-phase). Then, after 15 min of rest, the participants performed a contraction
120 at 30% MVC until exhaustion. This contraction level was selected as participants typically reach knee-
121 extension task failure after a few minutes (Martinez-Valdes et al. 2017a; Vila-Cha et al. 2012) and the
122 intensity is well below the force level corresponding to full motor unit recruitment in both investigated
123 muscles (Enoka and Fuglevand 2001). In each of these trials, the participants also received visual

124 feedback of the torque applied by the leg to the dynamometer. Time to task failure was defined as the
125 time instant when the subject exerted a force 10% MVC below the target force for a 2 s interval of time
126 (Castronovo et al. 2015). Another MVC was performed at the end of the sustained contraction to verify
127 the presence of fatigue.

128 Data Acquisition

129 Surface EMG signals were recorded from the vastus medialis (VM) and vastus lateralis (VL) in monopolar
130 derivation with 2-dimensional adhesive grids (Spes Medica, Salerno, Italy) of 13 (rows) x 5 (columns)
131 equally spaced electrodes (1-mm diameter, interelectrode distance of 8 mm). EMG signals were initially
132 recorded during a brief voluntary contraction during which a linear dry electrode array of 8 silver-bar
133 electrodes (1-mm diameter, 5-mm length, 5-mm interelectrode distance; SA 8/5; OT Bioelettronica,
134 Torino, Italy) was moved over the skin to detect the location of the innervation zone and fibre alignment
135 (Martinez-Valdes et al. 2016). After the skin was shaved and cleansed with abrasive paste and water, the
136 electrode cavities of the grids were filled with conductive paste (Spes Medica). High-density surface
137 EMG (HDsEMG) grids were positioned between the proximal and distal tendons of the VM and VL
138 muscles with the electrode columns oriented along the muscle fibers as previously described (Martinez-
139 Valdes et al. 2018a; Martinez-Valdes et al. 2018b). Reference electrodes were positioned over the
140 malleoli and patella of the dominant leg. EMG and torque signals were sampled at 2048 Hz and
141 converted to digital data by a 12-bit analog-to-digital converter (3 dB, bandwidth 10–500 Hz, 256-
142 channel EMG amplifier; EMG-USB 2; OT Bioelettronica). EMG signals were amplified by a factor of 500
143 for the 50% MVC contractions and a factor of 1000 for the sustained 30% MVC contractions. Data were
144 analyzed offline using MATLAB 2017b (The Math-Works, Natick, MA). The 64-monopolar EMG channels
145 were re-referenced offline to form 59 bipolar channels as the differences between adjacent electrodes
146 in the muscle fibres direction.

147 Signal Analysis: motor unit decomposition and tracking

148 The EMG signals recorded during the submaximal contractions were decomposed offline with a method
149 that has been extensively validated (Negro et al. 2016). Since EMG amplitude increases and MUAP
150 morphology changes throughout the time-course of a sustained submaximal fatiguing contraction (Beck
151 et al. 2005), it is not possible to apply the decomposition algorithm for the full duration of the
152 contraction. The algorithm indeed relies on the stationarity of the action potential waveforms,
153 something that is not satisfied in fatiguing contractions. Therefore, for the 30% MVC contraction, the
154 HDsEMG signals were divided into 30 s intervals, with an overlap of 15 s between intervals. Each interval
155 was then decomposed individually and the accuracy of the decomposition was tested with the
156 silhouette (SIL) measure for each motor unit. SIL is the difference between the within and between-
157 cluster sums of point-to-centroid distances, normalized dividing by the maximum of the two values. SIL
158 is a normalized accuracy index for EMG decomposition that can be directly associated to the accuracy of
159 the decomposition (Negro et al. 2016). Only motor units with a SIL greater than 0.90 were considered
160 for further analysis (Martinez-Valdes et al. 2017a; Negro et al. 2016). Then, the decomposition accuracy
161 was improved by manual editing of consecutive discharges separated from each other by <33.3 ms
162 or >200 ms and the iterative estimation of the motor unit extraction filters. This semi-automatic
163 approach was used to iteratively improve the accuracy of the automatic decomposition algorithm by the
164 visual interaction with an expert operator (Boccia et al. 2019; Martinez-Valdes et al. 2020). Following the
165 decomposition, the motor units were tracked across the decomposition intervals by direct cross-
166 correlation of the discharge times (time domain analysis) identified during the overlapping time portions
167 of the 30 s processing intervals. In these overlapped portions, discharges were considered to belong to
168 the same motor unit when at least 70% of the discharge times were identified by the two independent
169 decompositions in the two 30 s intervals within a delay of 5 ms (**Figure 1**). In this way, the tracking of the
170 motor units did not depend on the shape of the action potential waveforms, which varied over time.

171 Moreover, since the decomposition of the 30 s intervals were all performed independently, the
172 identification of the same discharge pattern for the identified sources (motor units) can only be
173 associated to the detection of the same motor units. With this approach, motor units could be tracked
174 over time with the only assumption that their action potential waveforms were stable within each of the
175 30 s intervals.

176 The modulation in discharge rates for each of the tracked motor units was filtered and smoothed (400
177 ms Hanning window) and then correlated to the torque signal to compare common fluctuations in
178 torque and discharge rate. Since EMG signals during sustained submaximal fatiguing contractions
179 become highly interferential, we verified that the fluctuations in firing rate were highly correlated to the
180 torque signal (see Results) to further support the accuracy of the decomposition (Enoka 2019; Negro et
181 al. 2009; Thompson et al. 2018). Any decrease in this correlation may be due to the erroneous
182 identification of the discharge times (Enoka 2019; Nawab et al. 2008).

183 As an additional validation step, the two-dimensional representation of the MUAPs (Martinez-Valdes et
184 al. 2017b), was extracted by spike-triggered averaging from the first and last 50 firings of each motor
185 unit (beginning and end of the contraction). Changes in MUAP shape are expected during the time-
186 course of a fatiguing contraction (i.e. MUAP size and duration), due to adjustments in peripheral
187 properties (e.g., conduction velocity) (Beck et al. 2005). This was the reason for developing the tracking
188 method which is not based on MUAP waveforms. Yet, based on previous studies (Carpentier et al. 2001;
189 Garland et al. 1997), it was expected that the changes in MUAP shapes would be progressive and that
190 they would not completely disrupt the initial spatial representations of MUAPs. Therefore, it was
191 assumed that the MUAP waveforms estimated at the beginning and end of the contraction would not be
192 completely uncorrelated. Based on this assumption, only motor units with MUAP waveforms which
193 were correlated by > 0.8 at the end with respect to the beginning of the contraction were considered for

194 further analysis (Boccia et al. 2019). This indirect criterion for decomposition accuracy is based on the
195 observation that discharge times were identified by independent decompositions of the 30 s intervals
196 and, therefore, it would be highly unlikely that spike-triggered averaging on these estimated discharges
197 would lead to similar MUAP waveforms if the decomposition was incorrect. Moreover, since the
198 procedure for tracking over time did not involve the MUAP waveforms, this validation measure was
199 independent from the tracking criterion. An example of this procedure for one motor unit from the VM
200 can be seen in **Figure 2**.

201 Finally, and in order to check the effects of fatigue on discharge rate at task failure, motor units were
202 matched between contractions at 50% MVC and the end of 30% MVC to study the behaviour of later
203 recruited motor units just before task failure (cross correlation level of 2D MUAP waveforms >0.8)
204 (Martinez-Valdes et al. 2017b). In addition, we matched motor units from the contractions at 50% MVC
205 with the beginning and end of the 30% MVC contractions (2D MUAP cross-correlation >0.8), in order to
206 compare the level of discharge rate between motor units identified at 50% MVC and earlier recruited
207 units at 30% MVC during task failure (Martinez-Valdes et al. 2017b).

208 Motor unit and torque signal analysis

209 Mean discharge rate, the coefficient of variation for the interspike interval (CoVisi) and the coefficient of
210 variation of torque (CoV torque) from early recruited motor units (motor units that were recruited at
211 the beginning of the contraction), were calculated from the stable torque region of the sustained
212 contraction until task failure in 1 s epochs. The motor unit recruitment threshold was defined as the
213 knee extension torque (%MVC) at the time when the motor unit began discharging action potentials.

214 The values from the 1 s epochs were then averaged over time-normalized intervals with a duration of 10%
215 of the time to task failure (i.e., 10-100% of time to task failure in increments of 10%). Cross-correlation
216 between low-frequency (<5 Hz) fluctuations in motor unit discharges and torque was also computed for

217 each of the 10 segments. This comparison was made between single motor units and torque (each spike
218 train was correlated individually to the torque signal) to decrease the chance of high cross-correlation
219 values due to the averaging of larger populations of motor units (Negro et al. 2009; Thompson et al.
220 2018).

221 For statistical reasons, the discharge behaviour and the properties of MUAPs were analysed only for the
222 motor units active for the entire duration of the contraction. The discharge rates of motor units that
223 were identified after the beginning of the contraction were analysed separately. Since both the early
224 and later recruited motor units showed a time point after which discharge rates increased following the
225 initial decrease (see Results), this time point was compared across all identified motor units (see
226 Statistics).

227 Interference EMG

228 The root mean square amplitude (RMS) obtained from the sustained submaximal contractions was
229 averaged over all channels of the electrode grid for each muscle (59 bipolar channels). As with motor
230 unit variables, the RMS was computed from the HDsEMG signals in intervals of 1 s and also normalized
231 in 10 segments as defined above. Differences in RMS amplitude were also compared between the non-
232 fatiguing 50% MVC contraction and the last epoch before task failure (30% MVC, epoch 10). For 50%
233 MVC, RMS values were determined from the steady-torque part of the contraction (average RMS value
234 of ten 1 s windows).

235 Motor unit contractile properties

236 Motor unit twitch torque during the course of the contraction (fused twitches) was determined by a
237 recently proposed technique based on the deconvolution of the torque signal using identified discharge
238 times of a population of motor units (Negro and Orizio 2017). In this study, a simplified model of twitch

239 profile characterized only by two parameters (peak amplitude and time to peak) was used (Fuglevand et
240 al. 1993). Briefly, a large range of peak amplitude and time to peak values were explored. The
241 reconstruction of the force profile was performed using motor unit firing data and twitch waveforms. A
242 band-pass filter (0.75-10 Hz) was applied to both reconstructed and original force profiles for each
243 twitch parameter combination in order to limit the influence of unidentified motor units. The squared
244 Euclidean distance was used as a measure of similarity between the two signals (original and
245 reconstructed force) and the twitch parameters providing minimum distance were used as the final
246 estimation. Since both approaches may result in multiple minima, we used the area of the estimated
247 twitch profile as a measure of contractile properties. To obtain accurate estimates, this analysis requires
248 a large number of motor units (>10) identified over long time intervals (>20s) (Negro and Orizio, 2017),
249 therefore it was applied only to the VM, for which a larger number of motor units was usually identified
250 compared to VL (see Results), and for longer epochs (20, 40, 60, 80 and 100% of total endurance time)
251 than those used for the other analyses. Despite these choices, the assumptions for this analysis were
252 met only by the data from 4 participants [mean (SD) age: 27 (6) yr, height: 178 (5) cm, mass: 77 (5) kg].
253 The average motor unit twitch force was estimated from these participants only and characterised by its
254 area.

255 Statistical Analysis

256 All results are expressed as mean and standard deviation (SD). All variables were tested for normality
257 using the Shapiro–Wilk test and statistical significance was set at $p < 0.05$. MVCs performed at the
258 beginning and end of the sustained contractions were compared by t-tests. Piecewise regressions and
259 polynomial models (2nd order) were used to fit the variables extracted for motor units (early recruited)
260 and torque variability. The model leading to the highest correlation coefficient with the original data was
261 used to fit the data (Loscher et al. 1996b). The piecewise regression analyses showed two distinct

262 phases of the contraction for all variables analysed (see Results). Thus, the intersection point of the two
263 regression lines [piecewise regression (Beaver et al. 1985)] was defined as the reversal time instant (i.e.,
264 transition from a positive/null to a negative/null slope or viceversa). The time instants (seconds) of
265 reversal for discharge rate, CoVisi and CoV for torque were analysed by two-way repeated measures
266 ANOVA with factors the variable (discharge rate, CoVisi, CoV torque) and muscle (VM and VL). Since
267 from this analysis, the reversal points were not different between variables or muscles (see Results), we
268 averaged the reversal time instants across muscles and variables. This averaged value will be referred in
269 the following to as motor unit behaviour reversal point as it defines the time instants where central
270 motor unit properties (i.e. discharge rate, recruitment and CoVisi) and contractile (peripheral motor unit
271 properties) variables investigated, changed their behaviour. For this reason, the motor unit behaviour
272 reversal point separates the contraction in two phases, which are characterized by different adaptations.
273 The association between the motor unit behaviour reversal point and the total endurance time was
274 analysed by linear correlation. Piecewise linear regressions were used to fit the discharge rate data from
275 later recruited motor units (i.e., motor units not active from the beginning of the task) and the break
276 point between the two lines was considered the reversal time instant for these motor units and
277 analysed by two way repeated measures ANOVA to compare it with the reversal time for early recruited
278 units (factors: later/early recruited units; and muscle VM/VL). The number of later recruited units that
279 were activated before and after the motor unit behaviour reversal point (phase 1 vs phase 2) was also
280 compared by two-way repeated measures ANOVA (factors: phase 1/phase 2 and muscle VM/VL). Two-
281 way repeated measures ANOVA (factors of time: 10 epochs and muscle: VM and VL) was used to
282 compare each of the normalized variables, which consisted of 10 segments of RMS amplitude, discharge
283 rate, CoV isi, single-motor unit/torque cross-correlation and CoV of torque. The firing behaviour of the
284 common units identified at 50% MVC and 30% MVC preceding task failure (for both early and later
285 recruited units) was compared by three-way repeated measures ANOVA with factors muscle (VM and

286 VL), force (50% and 30% MVC) and recruitment (early vs late). Finally, RMS amplitude between 50%
287 MVC and 30% MVC immediately before task failure was compared by two-way repeated measures
288 ANOVA (factors: muscle and force level). Bonferroni corrected t-tests were performed when ANOVA was
289 significant.

290 **RESULTS**

291 Endurance time and maximal torque generation capacity

292 Participants sustained the 30% MVC knee extension contraction for 122.8 (33.3) s and MVC peak torque
293 significantly decreased by 18.1 (7.5)% immediately following the contraction (from 198.0 (28.7) Nm to
294 162.1 (25.6) Nm, $p < 0.001$).

295 Motor unit identification and tracking during sustained contraction until failure (30% MVC)

296 Motor units could not be tracked in two of the 16 subjects due to poor signal quality and therefore the
297 results are presented for 14 participants [mean (SD) age: 29 (2) yr, height: 177 (6) cm, mass: 78 (7) kg].

298 An average of 5 (3) and 4 (3) motor units could be tracked for the entire contraction duration per subject
299 for VM and VL, respectively. The MUAP waveforms of these motor units were similar at the beginning
300 and end of the contraction (cross-correlation coefficient of 0.81 (0.07) and 0.80 (0.04) for VM and VL,
301 respectively). The average recruitment threshold of these units was 22.6 (4) and 21.7 (6.3) % MVC for
302 VM and VL respectively ($p = 0.22$). Finally, an average of 4 (2) and 3 (3) motor units per subject (values
303 were rounded to the closest integer) were recruited later and were followed until the end of the
304 contraction for the VM and VL, respectively. The number of later recruited units was significantly larger
305 during the second phase of the contraction (after the motor unit behaviour reversal point; see next
306 section) as on average, we could identify the recruitment of 1 (1) motor unit in phase one and 2 (2)
307 motor units in phase two (per participant), in both muscles (phase effect: $p < 0.0001$, $\eta^2 = 0.64$).

308 Motor Unit Behaviour

309 The representative behavior of VM motor units from one participant can be seen in **Figure 3**. In this
310 participant, 10 motor units could be followed from the beginning of the contraction and a total number
311 of 16 motor units were identified throughout the entire course of the contraction. In this example, early
312 recruited units showed an initial decrease in discharge rate until approximately 50% of endurance time,
313 and then increased their discharge frequency until the end of the contraction. Later recruited motor
314 units followed the same behavior of early recruited units, with their discharge rates increasing after 50%
315 of the endurance time, irrespective of the time of recruitment. Motor units that were recruited after 50%
316 of the endurance time increased their discharge rates during the entire duration of their activation. All
317 motor units showed a discharge rate at the end of the contraction that was similar or slightly higher
318 than the discharge rate at the beginning of their activation. This representative behaviour was
319 consistent for the group of participants. At the group level, the time instant of reversal in discharge rate
320 was not different between early and later (recruited before and after the deflection point) recruited
321 motor units, nor between muscles (VM early: 66.7 (18.2) s vs. VM later: 65.3 (20) s, VL early: 66.4 (21.8)
322 s vs. VL later: 65.8 (21) s, $p > 0.78$ for main effects). The 2nd order polynomials explained 61.1 (16.3)% and
323 63.4 (18.1)% of the variance in discharge rate during the sustained contractions for VM and VL,
324 respectively (**Figure 4**). Only one subject showed a continuous decrease in firing rates (bright-green dots
325 in **Figure 4**). Across all participants, the mean discharge rates decreased significantly until approximately
326 40% of the normalized endurance time ($p = 0.039$, 95% CI of difference between 10% and 40% endurance
327 time: 0.02 to 2.1 Hz) and subsequently increased until task failure, where discharge rates were
328 significantly higher than those at the beginning of the contraction ($p < 0.001$, 95% CI difference: -2.5 to -
329 0.5 Hz) (**Figure 5A**). There was no difference in mean firing rates between muscles (interaction: muscle x
330 time, $p = 0.27$, $\eta^2 = 0.09$). This increase in discharge rate from ~40% of endurance time was also concurrent
331 with a significant increase in discharge rate variability (CoVisi, time effect: $p < 0.0001$, $\eta^2 = 0.55$) (**Figure 5B**)

332 and CoV torque (time effect: $p < 0.0001$, $\eta^2 = 0.55$) (**Figure 5C**), which were both constant over time before
333 this time instant. Additionally, the correlation between torque and firing rate fluctuations of single
334 motor units also increased after 40% of endurance time (time effect: $p < 0.0001$, $\eta^2 = 0.35$) (**Figure 5D**).

335 Associations between motor unit discharge rate and endurance time

336 The association between the reversal time points in discharge rate and CoVisi in VM and VL motor units
337 and CoV torque can be seen for a representative participant in **Figure 6**. These variables changed their
338 trends at similar instants in time. On a group level, the reversal points were not significantly different
339 among the variables analysed and between muscles (for discharge rate, CoV force and CoVisi: 66.8 (24.9)
340 s, 66.5 (23) s and 64.4 (25.3) s for VM, and 64.6 (23.1), 65.9 (22.6) and 68.9 (29) s for VL; $P = 0.945$). Thus,
341 any of these instants represented the change in central (i.e. discharge rate) and peripheral (i.e. twitch
342 area) motor unit behaviour during the course of the fatiguing contraction. For this reason, we averaged
343 these time instants and obtained the motor unit behaviour reversal point. The motor unit behaviour
344 reversal point accurately predicted total endurance time ($r = 0.93$, $R^2 = 0.87$, $p < 0.0001$, **Figure 7**).

345 Comparison of motor unit discharge rate between non-fatiguing 50% MVC contractions and 30% MVC
346 contractions at task failure

347 The discharge rate for both earlier and later recruited units during 30% MVC during the last seconds
348 before task failure (last epoch) was lower than the average discharge rate of the same motor units when
349 activated at 50% MVC. The representative behaviour of a VL motor unit that was tracked between 50%
350 MVC and 30% MVC just before task failure can be seen in **figure 8**. A total of 3 (2) and 2 (2) motor units
351 could be matched per subject between 50% MVC and 30% MVC few seconds before task failure (late
352 recruited units) for VM and VL, respectively [average cross correlation coefficient of 0.87 (0.06) and 0.86
353 (0.05)] . Additionally, 2 (1) and 2 (1) motor units per subject could be matched between 50% MVC and
354 the beginning and end of the 30% MVC contraction (behaviour of earlier recruited at task failure) for VM

355 and VL, respectively [average cross-correlation coefficient of 0.9 (0.03) and 0.83 (0.02)]. As expected,
356 earlier recruited units had a higher discharge rate (at both 50% MVC and 30% MVC) compared to later
357 recruited units (recruitment effect: $p=0.005$, $\eta^2=0.46$), **figure 9**. However, both earlier and later recruited
358 units always had a firing frequency significantly higher at 50% MVC (force effect: $p<0.0001$, $\eta^2=0.79$),
359 **figure 9**.

360 Interference EMG

361 RMS amplitude increased linearly throughout the sustained contraction (time effect: $p<0.0001$, $\eta^2=0.74$)
362 in both muscles (**Figure 10a**). There was a significant interaction between VM and VL, as VM showed a
363 greater increase in RMS amplitude throughout the course of the contraction (interaction: muscle x time,
364 $p<0.0001$, $\eta^2=0.65$). RMS amplitude was similar between the 50% MVC contraction and the 30% MVC
365 contraction at failure in both muscles (force effect: $p=0.44$, $\eta^2=0.05$, **figure 10b**).

366 Assessment of contractile properties

367 Twitch area and its relationship with discharge rate for one participant can be observed in **Figure 11**. For
368 this participant, the twitch area increased over time until approximately 40% of the endurance time and
369 then decreased, reaching its minimum value at 100% of total endurance time. Even though we could not
370 directly compare statistically the reversal points in neural properties and twitch force (as the twitch had
371 to be estimated in a different time interval), we could observe that the point where neural adjustments
372 reversed their behaviour approximately coincided with the point of maximum twitch force (blue line).
373 This behavior was also observed in the other three participants for which the average twitch could be
374 estimated. Overall, twitch area declined in this group of subjects by 35.3% (range from 0% to 61.8%) at
375 failure compared to baseline (20% of endurance time).

376 DISCUSSION

377 Using a new time-domain technique of motor unit tracking during sustained submaximal fatiguing
378 contractions we were able to follow the firing behavior of a representative number of units until task
379 failure and compare them with the same motor units identified during a higher-force non-fatiguing
380 contraction. The results show two distinct phases in motor unit firing and contractile properties,
381 characterized by different motor unit behaviour. In the first phase, motor unit discharge rates decreased,
382 and interspike interval and force variabilities remained constant. At a well defined time instant, which
383 marked the beginning of the second phase, these three variables began to increase until task failure. The
384 time point marking the separation of the two phases predicted the time to failure. The contractile motor
385 unit properties also changed differently in the two phases, with an increase in twitch area in the first
386 phase and a decrease in the second phase. Finally, the later increase in discharge rate, peaked at a
387 slower firing frequency compared to a non-fatiguing 50% MVC contraction, revealing possible saturation
388 of the motor unit pool due to strong central inhibition or lack of additional excitation. Taken
389 together, these results show that both motor unit central and peripheral processes during sustained
390 submaximal contractions until failure, in the vasti muscles, do not progress similarly throughout the
391 contraction but are rather characterized by at least two distinct stages. Since the beginning of the
392 second phase determines the time of task failure, the adjustments needed in the second phase are
393 presumably the limiting factor of the neuromuscular system in maintaining the task.

394 *Trends of motor unit discharge rate over time*

395 Although it is generally accepted that motor unit discharge rate decreases linearly during submaximal
396 fatiguing contractions until failure (Enoka and Duchateau 2008), many of the studies analysing motor
397 unit firing properties during these contractions have reported an initial decrease and later increase in
398 firing frequency (Adam and De Luca 2005; Bigland-Ritchie et al. 1986; Castronovo et al. 2015; Dorfman
399 et al. 1990; Garland et al. 1997; Griffin et al. 2000; Kuchinad et al. 2004; Mettler and Griffin 2016).

400 Although the divergence in motor unit behaviour could be likely related to differences in the tasks
401 performed to study fatigue (Enoka and Duchateau 2008), it is important to note that all previous studies
402 used decomposition methods based on MUAP template-matching to follow motor units throughout the
403 contractions with a high probability of erroneous identification of discharges. In this study, we have
404 proposed an approach that does not rely on tracking MUAP waveforms, which allowed us to successfully
405 track a more representative number of motor units during the entire duration of a sustained contraction
406 until failure. Moreover, using a recent deconvolution algorithm (Negro and Orizio 2017) we could
407 monitor changes in motor unit twitch areas from a subset of participants during the course of the
408 contraction. This particular method, can provide a more accurate assessment of motor unit twitch force
409 compared to single-twitch whole-muscle force measurements (Adam and De Luca 2005) and spike-
410 triggered averaging techniques (Carpentier et al. 2001). Therefore, utilizing these approaches, we
411 identified a biphasic behaviour in the neuromuscular processes underlying fatigue and associated this
412 behaviour with endurance time.

413 Besides methodological constraints, other factors such as contraction duration (sustained contraction
414 for a fixed period of time vs sustained contraction until failure), force level and muscle studied, might be
415 behind the differences in results across studies. For instance, all studies that have identified a non-linear
416 firing response have performed submaximal contractions until task failure (Adam and De Luca 2005;
417 Bigland-Ritchie et al. 1986; Castronovo et al. 2015; Dorfman et al. 1990; Garland et al. 1997; Griffin et al.
418 2000; Kuchinad et al. 2004; Mettler and Griffin 2016), while those reporting monotonic decreases in
419 discharge rate have been performed for either a fixed duration (Mottram et al. 2005; Pascoe et al. 2014;
420 Riley et al. 2008) or until failure (Carpentier et al. 2001; Dalton et al. 2010; Garland et al. 1994;
421 McManus et al. 2016). In the specific case of the studies from Carpentier et al. and Garland et al., the
422 authors showed that motor unit behaviour could be related to the recruitment-threshold. In those
423 studies, motor units with lower recruitment thresholds showed a linear decrease in discharge rate while

424 higher threshold units showed maintained or increased firing frequency. Considering that the average
425 recruitment threshold of motor units that could be tracked for most of the sustained contraction was
426 relatively high in the present study (22% MVC), the results are to be compared with those on the higher-
427 threshold motor units of these previous studies. The next factor explaining the divergence in results
428 across studies could be related to the exerted force level. Most of the studies reporting non-linear
429 changes in discharge rate have used relatively low-force contractions, ranging from 20-30%MVC, while
430 the studies reporting monotonic decreases have usually employed higher-force contractions, at 50%
431 MVC (Carpentier et al. 2001; Enoka et al. 1989), from 10 to 64%MVC (Garland et al. 1994) or even 75%
432 MVC (Dalton et al. 2010). The behaviour observed in the present study is likely specific for contractions
433 at low to moderate forces. At very high forces, the rapid increase in excitation to the motor neuron pool
434 may occur very early in the contraction and just induce a linear decrease in firing frequency, as
435 commonly observed during maximal voluntary contractions (Bigland-Ritchie et al. 1983). Changes in
436 discharge rate can also be muscle-specific. For instance, Adam and De Luca observed the same
437 behaviour as reported in the present study for vastus lateralis (Adam and De Luca 2005) at 30%MVC,
438 while monotonic reductions in discharge rate have been consistently reported for the first dorsal
439 interosseous muscle (Carpentier et al. 2001; Enoka et al. 1989; McManus et al. 2016), regardless of the
440 force level (30 or 50% MVC). These observations suggest that motor unit adjustments to fatigue may
441 depend on muscle-specific properties, such as the upper limit of recruitment, and therefore the findings
442 obtained from individual muscles cannot be generalized. Finally, it is also important to mention that
443 most of previous studies relied on pooled firing rate data which considers each motor unit as an
444 independent observation (even when belonging to the same subject). This can provide equivocal
445 estimates of motor unit behaviour (Adam and De Luca 2005; Tenan et al. 2014) and therefore it is
446 important to assess motor unit firing responses on a subject by subject basis, as done in the present
447 study.

448 *Changes in contractile properties*

449 Our results showed that an increase in discharge rate is needed to compensate for the decline in the
450 total area of the motor unit twitch force (**Fig. 11**). Early increases in twitch area (and concomitant
451 decreases in discharge rate) can be explained by post-activation potentiation (Fowles and Green 2003)
452 and twitch contraction time elongation, while later decreases in twitch area might be due to greater
453 accumulation of metabolic by-products, such as potassium, hydrogen ion, inorganic phosphate and
454 reactive oxygen and nitrogen species, among others affecting muscle fibre-contractile capacity and thus
455 twitch amplitude (McKenna et al. 2008). Consequently, changes in central mechanisms (motor unit
456 discharge rate and recruitment) should compensate for a decline in contractile properties in order to
457 maintain torque output, as shown in the present study.

458 *Common Synaptic input and changes in motoneuron excitability*

459 In 15 out of 16 subjects, all motor units followed the same biphasic behaviour (**Figure 3**), likely reflecting
460 the strong common synaptic input received by the motor unit pool as the contraction progressed
461 (Castronovo et al. 2015). This increase in common input is also typically accompanied by higher torque
462 and discharge rate variability, and by stronger correlations between fluctuations in muscle force and
463 discharge rate (Castronovo et al. 2018; Castronovo et al. 2015). Consistent with these observations, our
464 data shows that CoV of torque and CoVisi, and the correlation between fluctuations in motor unit
465 discharge rates and torque, increased from 40% of the endurance time until task failure (**Figure 5**).
466 Moreover, a greater number of motor units were recruited during the second phase of the contraction,
467 in agreement with an increase in drive to motoneurons. This mechanism is possibly required to
468 compensate for the gradual decrease in twitch area and the increased inhibition experienced by the
469 motoneuron pool during this phase of the contraction. Motoneuron inhibition may occur due to a
470 number of mechanisms, including decreased muscle spindle activity (Brunetti et al. 2003), increased

471 firing of type III and IV afferents (Martinez-Valdes et al. 2020), and decreased recurrent inhibition (Taylor
472 et al. 2016). However, these inhibitory inputs could be compensated by increasing supraspinal excitatory
473 voluntary drive, as previously shown in studies assessing corticospinal excitability during sustained
474 submaximal contractions until failure (Levenez et al. 2008; Sacco et al. 1997; Taylor et al. 1996). Thus,
475 the increased discharge rate observed in the second phase might be due to an increased supraspinal
476 input into the motoneuron pool. Nevertheless and like motor unit recordings, not all studies have
477 reported an increase in corticospinal excitability during fatigue, which may be related to differences in
478 protocols to measure motoneuron excitability, in particular contraction duration (contractions sustained
479 for a fixed period of time vs contractions until failure). Most studies informing an increase in
480 corticospinal excitability have employed sustained submaximal contractions until failure (Levenez et al.
481 2008; Sacco et al. 1997), while those reporting decreases have usually employed sustained (or
482 intermittent) contractions for a fixed duration (i.e. (Finn et al. 2018; McNeil et al. 2011; Sogaard et al.
483 2006), which did not reach full exhaustion. Therefore, future studies should aim to combine motor unit
484 recordings with measures of spinal and cortico-spinal excitability during sustained contractions until
485 failure, in order to understand the relationship between the biphasic motor unit firing behaviour
486 observed in the present study and motoneuron excitability.

487 *Motor unit saturation at task failure*

488 Despite the increase in discharge rates in the second stage, these firings did not reach values attainable
489 at greater forces in absence of fatigue (**Figures 8 and 9**). This finding provides unique evidence to further
490 support a primary role of central rather than peripheral mechanisms for task failure, as the 40-50%
491 decline in twitch force observed by the end of the contraction could have been still compensated by
492 increasing discharge rates. Consistent with these findings, previous studies have shown that EMG
493 activity just before task failure is lower than that observed during an MVC, even when subject's exert

494 maximal effort (Fuglevand et al. 1993). Our findings confirm these observations, as RMS only reached
495 the level of a 50% MVC contraction (**Figure 10B**). Although EMG combines information on both central
496 and peripheral factors, these findings can be interpreted as an inability to drive the muscle further at the
497 point of failure (Loscher et al. 1996a). It is evident that even when participants exert maximal effort, this
498 increase in firing frequency (and probably motor unit recruitment) would not be sufficient to maintain
499 the task. The descending drive generated by maximal efforts could be opposed by an increase in
500 inhibition to motoneurons or higher centers, or reductions in motoneuron excitability. Previous studies
501 (Kennedy et al. 2014; 2013) found evidence for increased inhibition of central drive via fatigue-induced
502 activation of high threshold afferents. An additional possibility is a decrease in intrinsic excitability of
503 motoneurons. During prolonged activation, motoneurons exhibit spike frequency adaptation (Kernell
504 and Monster 1982; Sawczuk et al. 1995), although this mechanism does not induce the severe
505 saturation seen in the present study. The intrinsic excitability of motoneurons is in fact primarily
506 controlled by neuromodulatory inputs that originate in the brainstem and act via axons releasing the
507 monoamines serotonin and norepinephrine. A seminal study by Fornal et al. (Fornal et al. 2006) showed
508 that, as cats ran to exhaustion on a treadmill, firing rates in serotonergic neurons in the caudal raphe
509 nuclei initially increased but then began to fall. The caudal raphe is the primary source of serotonergic
510 axons that project to motoneurons and the actions of this serotonergic projection on motoneuron
511 excitability are profound, increasing the efficacy of synaptic inputs as much as 3-5 fold (Heckman and
512 Enoka 2012; Heckmann et al. 2005). Consequently a decrease in this neuromodulatory drive could
513 greatly decrease the efficacy of descending motor commands. While this speculation is reasonable, the
514 work in the cat was carried out for long periods of locomotion, a different motor task than in the
515 present study. In the case of sustained submaximal contractions in humans, a recent study has shown
516 that increased availability of serotonin influences central fatigue, particularly increasing perceptions of
517 fatigue and shortening the silent period (Thorstensen et al. 2020). These adaptations did not influence

518 motor pathways nor motor performance, and therefore the authors concluded that serotonergic
519 neurotransmission influence supraspinal processes without altering motor pathways during low-force
520 sustained muscle contractions. Nevertheless, it is important to mention that the authors did not
521 perform a contraction until task failure. Therefore, it could be possible that serotonin decreases the
522 excitability of spinal motoneurons during submaximal contractions at task failure in a similar extent to
523 what has been reported during maximal fatiguing contractions (Kavanagh et al. 2019). However, this
524 needs to be confirmed in future investigations. Taken together, we can suggest that a combination of
525 increased inhibition and decreased intrinsic excitability of motoneurons seems a plausible mechanism
526 for firing rate saturation in the fatigue task performed in this study.

527 *Limitations*

528 One limitation of this study is that the results on contractile properties could only be obtained from four
529 participants, because of constraints of the analysis method used to estimate twitch force. Additionally,
530 the results have been obtained for only one submaximal force level (30% MVC). A previous study
531 (without motor unit tracking) reported similar increases in CoV force, CoVisi and RMS amplitude
532 between the beginning and end of submaximal contractions at 20 and 50% MVC, but not at 75% MVC
533 (Castronovo et al. 2015). Therefore, it is important to confirm the observed behaviour at different force
534 levels. Task-dependent effects of fatigue, need to be also studied in order to assess whether the same
535 mechanisms observed in the current study can be transferred to other tasks. Finally, the analysis of the
536 rate of motor unit recruitment during the contraction is limited by the small proportion of motor units
537 identified with respect to the total number of active motor units. Future methods combining HDsEMG
538 and thin-film multi-channel intramuscular electrodes (Muceli et al. 2015) could aid to sample larger
539 populations of motor units and directly determine the number of units active during submaximal
540 sustained contractions until failure.

541 *Conclusion*

542 During sustained submaximal knee-extension contractions until failure, the behaviour of motor units is
 543 characterized by two distinct phases. The time of transition from the first to the second phase marks the
 544 beginning of an increase in excitatory drive to motor neurons and is a predictor of task failure. Despite
 545 of the increase in excitatory drive during the second phase, the contraction cannot be sustained possibly
 546 due to progressive motor neuron inhibition or reduced level of excitation as the later increase in firing
 547 rate saturates at a much slower discharge rate compared to the one recorded at a higher-force non-
 548 fatiguing contraction. This finding provides strong evidence suggesting that central rather than
 549 peripheral mechanisms are the main determinants for task failure.

550 **REFERENCES**

- 551 **Adam A, and De Luca CJ.** Firing rates of motor units in human vastus lateralis muscle during fatiguing
 552 isometric contractions. *J Appl Physiol* (1985) 99: 268-280, 2005.
- 553 **Beaver WL, Wasserman K, and Whipp BJ.** Improved detection of lactate threshold during exercise using
 554 a log-log transformation. *J Appl Physiol* (1985) 59: 1936-1940, 1985.
- 555 **Beck RB, O'Malley MJ, Stegeman DF, Houtman CJ, Connolly S, and Zwarts MJ.** Tracking motor unit
 556 action potentials in the tibialis anterior during fatigue. *Muscle Nerve* 32: 506-514, 2005.
- 557 **Bigland-Ritchie B, Cafarelli E, and Vollestad NK.** Fatigue of submaximal static contractions. *Acta Physiol*
 558 *Scand Suppl* 556: 137-148, 1986.
- 559 **Bigland-Ritchie B, Johansson R, Lippold OC, Smith S, and Woods JJ.** Changes in motoneurone firing
 560 rates during sustained maximal voluntary contractions. *J Physiol* 340: 335-346, 1983.
- 561 **Boccia G, Martinez-Valdes E, Negro F, Rainoldi A, and Falla D.** Motor unit discharge rate and the
 562 estimated synaptic input to the vasti muscles is higher in open compared with closed kinetic chain
 563 exercise. *J Appl Physiol* (1985) 127: 950-958, 2019.
- 564 **Brunetti O, Della Torre G, Lucchi ML, Chiochetti R, Bortolami R, and Pettorossi VE.** Inhibition of muscle
 565 spindle afferent activity during masseter muscle fatigue in the rat. *Exp Brain Res* 152: 251-262, 2003.
- 566 **Carpentier A, Duchateau J, and Hainaut K.** Motor unit behaviour and contractile changes during fatigue
 567 in the human first dorsal interosseus. *J Physiol* 534: 903-912, 2001.
- 568 **Castronovo AM, Mrachacz-Kersting N, Stevenson AJT, Holobar A, Enoka RM, and Farina D.** Decrease in
 569 force steadiness with aging is associated with increased power of the common but not independent
 570 input to motor neurons. *J Neurophysiol* 120: 1616-1624, 2018.
- 571 **Castronovo AM, Negro F, Conforto S, and Farina D.** The proportion of common synaptic input to motor
 572 neurons increases with an increase in net excitatory input. *J Appl Physiol* (1985) 119: 1337-1346, 2015.
- 573 **Dalton BH, Harwood B, Davidson AW, and Rice CL.** Recovery of motoneuron output is delayed in old
 574 men following high-intensity fatigue. *J Neurophysiol* 103: 977-985, 2010.
- 575 **Dorfman LJ, Howard JE, and McGill KC.** Triphasic behavioral response of motor units to submaximal
 576 fatiguing exercise. *Muscle Nerve* 13: 621-628, 1990.

- 577 **Enoka RM.** Physiological validation of the decomposition of surface EMG signals. *J Electromyogr Kinesiol*
578 46: 70-83, 2019.
- 579 **Enoka RM, and Duchateau J.** Muscle fatigue: what, why and how it influences muscle function. *J Physiol*
580 586: 11-23, 2008.
- 581 **Enoka RM, and Fuglevand AJ.** Motor unit physiology: Some unresolved issues. *Muscle & Nerve* 24: 4-17,
582 2001.
- 583 **Enoka RM, Robinson GA, and Kossev AR.** Task and fatigue effects on low-threshold motor units in
584 human hand muscle. *J Neurophysiol* 62: 1344-1359, 1989.
- 585 **Finn HT, Rouffet DM, Kennedy DS, Green S, and Taylor JL.** Motoneuron excitability of the quadriceps
586 decreases during a fatiguing submaximal isometric contraction. *J Appl Physiol (1985)* 124: 970-979, 2018.
- 587 **Fornal CA, Martin-Cora FJ, and Jacobs BL.** "Fatigue" of medullary but not mesencephalic raphe
588 serotonergic neurons during locomotion in cats. *Brain Res* 1072: 55-61, 2006.
- 589 **Fowles JR, and Green HJ.** Coexistence of potentiation and low-frequency fatigue during voluntary
590 exercise in human skeletal muscle. *Can J Physiol Pharmacol* 81: 1092-1100, 2003.
- 591 **Fuglevand AJ, Zackowski KM, Huey KA, and Enoka RM.** Impairment of neuromuscular propagation
592 during human fatiguing contractions at submaximal forces. *J Physiol* 460: 549-572, 1993.
- 593 **Garland SJ, Enoka RM, Serrano LP, and Robinson GA.** Behavior of motor units in human biceps brachii
594 during a submaximal fatiguing contraction. *J Appl Physiol (1985)* 76: 2411-2419, 1994.
- 595 **Garland SJ, Griffin L, and Ivanova T.** Motor unit discharge rate is not associated with muscle relaxation
596 time in sustained submaximal contractions in humans. *Neurosci Lett* 239: 25-28, 1997.
- 597 **Griffin L, Ivanova T, and Garland SJ.** Role of limb movement in the modulation of motor unit discharge
598 rate during fatiguing contractions. *Exp Brain Res* 130: 392-400, 2000.
- 599 **Heckman CJ, and Enoka RM.** Motor unit. *Compr Physiol* 2: 2629-2682, 2012.
- 600 **Heckmann CJ, Gorassini MA, and Bennett DJ.** Persistent inward currents in motoneuron dendrites:
601 implications for motor output. *Muscle Nerve* 31: 135-156, 2005.
- 602 **Hunter SK, Duchateau J, and Enoka RM.** Muscle fatigue and the mechanisms of task failure. *Exerc Sport*
603 *Sci Rev* 32: 44-49, 2004.
- 604 **Kavanagh JJ, McFarland AJ, and Taylor JL.** Enhanced availability of serotonin increases activation of
605 unfatigued muscle but exacerbates central fatigue during prolonged sustained contractions. *J Physiol*
606 597: 319-332, 2019.
- 607 **Kennedy DS, McNeil CJ, Gandevia SC, and Taylor JL.** Fatigue-related firing of distal muscle nociceptors
608 reduces voluntary activation of proximal muscles of the same limb. *J Appl Physiol (1985)* 116: 385-394,
609 2014.
- 610 **Kennedy DS, McNeil CJ, Gandevia SC, and Taylor JL.** Firing of antagonist small-diameter muscle
611 afferents reduces voluntary activation and torque of elbow flexors. *J Physiol* 591: 3591-3604, 2013.
- 612 **Kernell D, and Monster AW.** Time course and properties of late adaptation in spinal motoneurons of
613 the cat. *Exp Brain Res* 46: 191-196, 1982.
- 614 **Kuchinad RA, Ivanova TD, and Garland SJ.** Modulation of motor unit discharge rate and H-reflex
615 amplitude during submaximal fatigue of the human soleus muscle. *Exp Brain Res* 158: 345-355, 2004.
- 616 **Levenez M, Garland SJ, Klass M, and Duchateau J.** Cortical and spinal modulation of antagonist
617 coactivation during a submaximal fatiguing contraction in humans. *J Neurophysiol* 99: 554-563, 2008.
- 618 **Loscher WN, Cresswell AG, and Thorstensson A.** Central fatigue during a long-lasting submaximal
619 contraction of the triceps surae. *Exp Brain Res* 108: 305-314, 1996a.
- 620 **Loscher WN, Cresswell AG, and Thorstensson A.** Excitatory drive to the alpha-motoneuron pool during a
621 fatiguing submaximal contraction in man. *J Physiol* 491 (Pt 1): 271-280, 1996b.
- 622 **Martinez-Valdes E, Falla D, Negro F, Mayer F, and Farina D.** Differential Motor Unit Changes after
623 Endurance or High-Intensity Interval Training. *Med Sci Sports Exerc* 49: 1126-1136, 2017a.

- 624 **Martinez-Valdes E, Farina D, Negro F, Del Vecchio A, and Falla D.** Early Motor Unit Conduction Velocity
 625 Changes to High-Intensity Interval Training versus Continuous Training. *Med Sci Sports Exerc* 50: 2339-
 626 2350, 2018a.
- 627 **Martinez-Valdes E, Guzman-Venegas RA, Silvestre RA, Macdonald JH, Falla D, Araneda OF, and**
 628 **Haichelis D.** Electromyographic adjustments during continuous and intermittent incremental fatiguing
 629 cycling. *Scand J Med Sci Sports* 26: 1273-1282, 2016.
- 630 **Martinez-Valdes E, Negro F, Falla D, De Nunzio AM, and Farina D.** Surface electromyographic amplitude
 631 does not identify differences in neural drive to synergistic muscles. *J Appl Physiol (1985)* 124: 1071-1079,
 632 2018b.
- 633 **Martinez-Valdes E, Negro F, Farina D, and Falla D.** Divergent response of low- versus high-threshold
 634 motor units to experimental muscle pain. *J Physiol* 2020.
- 635 **Martinez-Valdes E, Negro F, Laine CM, Falla D, Mayer F, and Farina D.** Tracking motor units
 636 longitudinally across experimental sessions with high-density surface electromyography. *J Physiol* 595:
 637 1479-1496, 2017b.
- 638 **McKenna MJ, Bangsbo J, and Renaud JM.** Muscle K⁺, Na⁺, and Cl disturbances and Na⁺-K⁺ pump
 639 inactivation: implications for fatigue. *J Appl Physiol (1985)* 104: 288-295, 2008.
- 640 **McManus L, Hu X, Rymer WZ, Suresh NL, and Lowery MM.** Muscle fatigue increases beta-band
 641 coherence between the firing times of simultaneously active motor units in the first dorsal interosseous
 642 muscle. *J Neurophysiol* 115: 2830-2839, 2016.
- 643 **McNeil CJ, Giesebrecht S, Gandevia SC, and Taylor JL.** Behaviour of the motoneurone pool in a fatiguing
 644 submaximal contraction. *J Physiol* 589: 3533-3544, 2011.
- 645 **Mettler JA, and Griffin L.** Muscular endurance training and motor unit firing patterns during fatigue. *Exp*
 646 *Brain Res* 234: 267-276, 2016.
- 647 **Mottram CJ, Jakobi JM, Semmler JG, and Enoka RM.** Motor-unit activity differs with load type during a
 648 fatiguing contraction. *J Neurophysiol* 93: 1381-1392, 2005.
- 649 **Muceli S, Poppendieck W, Negro F, Yoshida K, Hoffmann KP, Butler JE, Gandevia SC, and Farina D.**
 650 Accurate and representative decoding of the neural drive to muscles in humans with multi-channel
 651 intramuscular thin-film electrodes. *J Physiol* 593: 3789-3804, 2015.
- 652 **Nawab SH, Wotiz RP, and De Luca CJ.** Decomposition of indwelling EMG signals. *J Appl Physiol (1985)*
 653 105: 700-710, 2008.
- 654 **Negro F, Holobar A, and Farina D.** Fluctuations in isometric muscle force can be described by one linear
 655 projection of low-frequency components of motor unit discharge rates. *J Physiol* 587: 5925-5938, 2009.
- 656 **Negro F, Muceli S, Castronovo AM, Holobar A, and Farina D.** Multi-channel intramuscular and surface
 657 EMG decomposition by convolutive blind source separation. *J Neural Eng* 13: 026027, 2016.
- 658 **Negro F, and Orizio C.** Robust estimation of average twitch contraction forces of populations of motor
 659 units in humans. *J Electromyogr Kinesiol* 37: 132-140, 2017.
- 660 **Pascoe MA, Holmes MR, Stuart DG, and Enoka RM.** Discharge characteristics of motor units during
 661 long-duration contractions. *Exp Physiol* 99: 1387-1398, 2014.
- 662 **Riley ZA, Maerz AH, Litsey JC, and Enoka RM.** Motor unit recruitment in human biceps brachii during
 663 sustained voluntary contractions. *J Physiol* 586: 2183-2193, 2008.
- 664 **Sacco P, Thickbroom GW, Thompson ML, and Mastaglia FL.** Changes in corticomotor excitation and
 665 inhibition during prolonged submaximal muscle contractions. *Muscle Nerve* 20: 1158-1166, 1997.
- 666 **Sawczuk A, Powers RK, and Binder MD.** Spike frequency adaptation studied in hypoglossal
 667 motoneurons of the rat. *J Neurophysiol* 73: 1799-1810, 1995.
- 668 **Sogaard K, Gandevia SC, Todd G, Petersen NT, and Taylor JL.** The effect of sustained low-intensity
 669 contractions on supraspinal fatigue in human elbow flexor muscles. *J Physiol* 573: 511-523, 2006.
- 670 **Taylor JL, Amann M, Duchateau J, Meeusen R, and Rice CL.** Neural Contributions to Muscle Fatigue:
 671 From the Brain to the Muscle and Back Again. *Med Sci Sports Exerc* 48: 2294-2306, 2016.

672 **Taylor JL, Butler JE, Allen GM, and Gandevia SC.** Changes in motor cortical excitability during human
673 muscle fatigue. *J Physiol* 490 (Pt 2): 519-528, 1996.
674 **Tenan MS, Marti CN, and Griffin L.** Motor unit discharge rate is correlated within individuals: a case for
675 multilevel model statistical analysis. *J Electromyogr Kinesiol* 24: 917-922, 2014.
676 **Thompson CK, Negro F, Johnson MD, Holmes MR, McPherson LM, Powers RK, Farina D, and Heckman**
677 **CJ.** Robust and accurate decoding of motoneuron behaviour and prediction of the resulting force output.
678 *J Physiol* 596: 2643-2659, 2018.
679 **Thorstensen JR, Taylor JL, Tucker MG, and Kavanagh JJ.** Enhanced serotonin availability amplifies
680 fatigue perception and modulates the TMS-induced silent period during sustained low-intensity elbow
681 flexions. *J Physiol* 598: 2685-2701, 2020.
682 **Vila-Cha C, Falla D, Correia MV, and Farina D.** Adjustments in motor unit properties during fatiguing
683 contractions after training. *Med Sci Sports Exerc* 44: 616-624, 2012.

684

685

686

687 **FIGURE CAPTIONS**

688 **Figure 1. Procedure for motor unit tracking during sustained-submaximal fatiguing contractions.**

689 A knee extension contraction at 30% of the maximal voluntary torque (MVC) until failure is shown from
690 a representative subject. Surface EMG and torque signals were cut into 30s segments (blue and orange
691 arrows) overlapped by 15s. Surface EMG signals from each of these segments was then decomposed
692 independently to reveal the firing activity of individual motor units (MU). Overlapped segments were
693 then used to cross-correlate MU spike trains from each 30s-segment identified MU and then merged to
694 reconstruct the activity of individual MU throughout the contraction. In addition to this, twitch force
695 was also estimated from the active motor units (fused twitches) in 5 segments corresponding to 20, 40,
696 60, 80 and 100% of total endurance time.

697 **Figure 2. Spatial 2D Cross-correlation of motor unit action potentials (MUAPs)**

698 The 50 initial (beginning, blue) and 50 final (task failure, red) firings belonging to each tracked motor
699 unit were used to trigger HDsEMG signals to build the 2D spatial representation of MUAPs (59 bipolar
700 channels). These MUAPs were then matched by cross correlation (above the figure). Note the increase
701 in MUAP amplitude and length at task failure.

702 **Figure 3. Representative motor unit firing behaviour of vastus medialis (VM) from one participant.**

703 Firing behaviour of tracked motor units (coloured lines) and torque signal (black line, 30% of the
704 maximum voluntary contraction torque, MVC) are presented in 1s epochs. 16 motor units were
705 identified during the course of the contraction and 10 motor units could be followed once the target
706 was reached (30% MVC) until failure. These 10 motor units had an average recruitment threshold of
707 21.0% MVC. The first active motor units (blue and red lines) were recruited at 7.3% and 13.4% MVC
708 respectively. Regardless of when the motor units were recruited, they all follow the same behaviour of
709 the earlier recruited ones, that is, they decrease discharge rate until 60s of the contraction (motor unit
710 behaviour reversal point) and then increase discharge rate from 60s of the contraction until task failure.
711 Two motor units were recruited before the motor unit behaviour reversal point and four additional
712 motor units were recruited after the motor unit behaviour reversal point. Note the common oscillations
713 between motor unit discharge rate and the torque signal.

714 **Figure 4. Motor unit non-linear firing behaviour from the full group of participants**

715 Instantaneous discharge rate from early-recruited motor units was averaged for each participant (1s
716 windows) during the sustained contraction for vastus medialis and vastus lateralis. 13 out of the 14
717 participants showed a nonlinear behaviour in DR, where firing frequency decreased and then increased
718 at a certain point during the contraction. Only one participant depicted in bright green, showed a linear
719 decrease in DR.

720 **Figure 5. Motor unit firing properties, torque and cross-correlation of firing times and torque**

721 Changes in discharge rate (A), coefficient of variation for the interspike interval (CoVisi, B), coefficient of
722 variation of Torque (CoV Torque, C) and cross-correlation between fluctuations in torque and discharge
723 rate of single motor units (D) is presented for the group of participants in vastus medialis and lateralis
724 (VM and VL, respectively). Data was normalized according to total endurance time in 10 epochs.
725 Significant decrease from baseline, $\Psi p < 0.05$). Significant increase from baseline, $\# p < 0.05$ and $* p < 0.01$.

726 **Figure 6. Discharge rate, interspike interval variability and torque variability breakpoints.**

727 Reversal points in discharge rate (DR) (A), coefficient of variation for the inter-spike interval (CoVisi) (B)
728 and coefficient of variation of torque (CoV torque) (C), are presented for one representative
729 participant's vastus medialis and lateralis (VM and VL) muscles. Data represent the average behaviour of
730 3 VM and 3 VL motor units that could be tracked from target torque until failure. Data is averaged in 1s
731 epochs which were fitted by bi-linear regression (piecewise regression). The point in time where the
732 regression lines intersected was used as a breakpoint to mark the change in motor unit firing and torque
733 behaviour. The breakpoint in VM and VL discharge rate, CoVisi and CoV torque happened at a similar
734 time during the contraction (range: 77.5s to 81.5s).

735 **Figure 7. Correlation between motor unit behaviour reversal point and endurance time.**

736 As reversal points in discharge rate, coefficient of variation for the inter-spike interval (CoVisi) and
737 coefficient of variation of torque (CoV torque) happened at similar time instants. Their values were
738 averaged to represent a single point during the contraction. This specific time-instant was called motor
739 unit behaviour reversal point and was able to predict the time to task failure.

740 **Figure 8. Discharge rate for the same motor unit identified during a non-fatiguing 50% MVC**
741 **contraction and a 30% MVC contraction at task failure**

742 A VL motor unit identified during the non-fatiguing 50% MVC contraction was tracked at task failure (30%
743 MVC) by cross-correlation of the 2D-spatial MUAPs (59 bipolar channels). Despite of the increase in
744 discharge rate at task failure with respect to the beginning of the contraction (30% MVC, figure 6A),
745 discharge rate for this unit did not reach the firing frequency observed during a non-fatiguing 50% MVC
746 contraction.

747 **Figure 9. Differences in discharge rate for motor units that were tracked during 50% MVC and 30%**
748 **MVC at task failure**

749 Differences in discharge rate between 50% MVC and discharge rate at task failure for early (active from
750 the beginning of the 30% contraction, A) and late (active at the end or close to the end of the 30% MVC
751 contraction, B) recruited motor units for VM and VL muscles during the 30% MVC contraction.
752 Significant effect of force, $*p < 0.001$. Significant effect of recruitment (early vs late), $\#p < 0.01$.

753 **Figure 10. Interference EMG**

754 Changes in root mean square amplitude of interference EMG signal averaged across 59 bipolar channels
755 is presented for the group of participants for the vastus medialis and lateralis (VM and VL, respectively).
756 A) Data was normalized according to total endurance time in 10 epochs. B) RMS values are compared
757 between 50% MVC and 30% MVC at task failure (TF). Significant interaction between muscles, $*p < 0.001$.
758 Main effect of time, $\#p < 0.05$.

759 **Figure 11. Changes in vastus medialis twitch force during the course of the contraction**

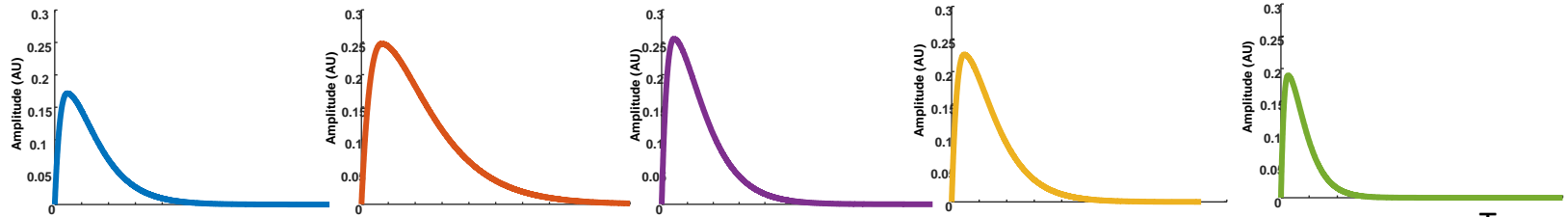
760 Twitch force results and its relation with discharge rate from one representative subject are shown in
761 the figure. Twitch areas are expressed in arbitrary units (AU).

762

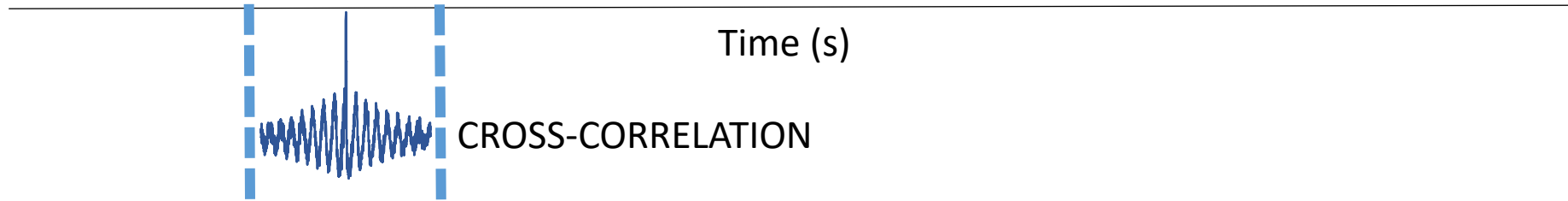
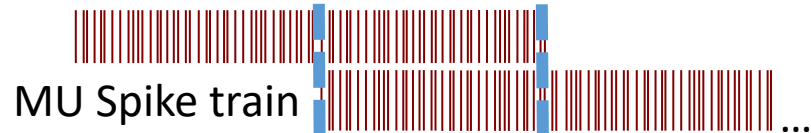
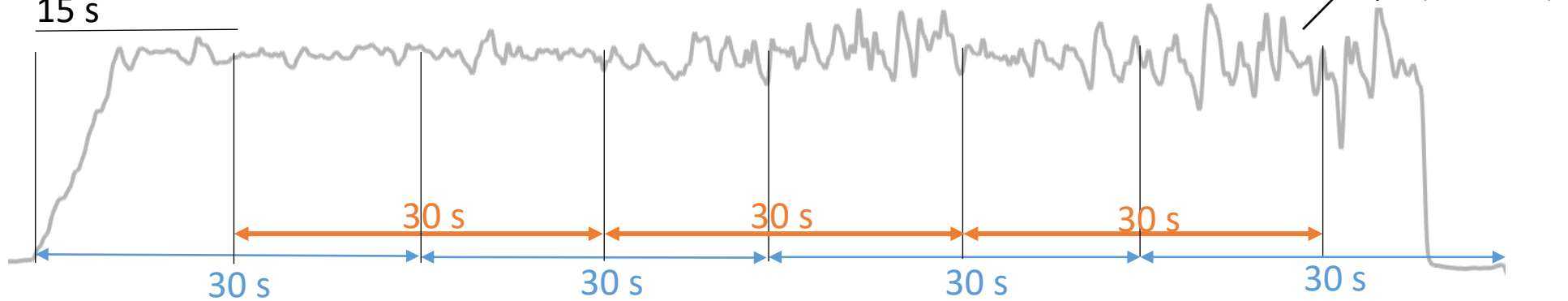
SEMG



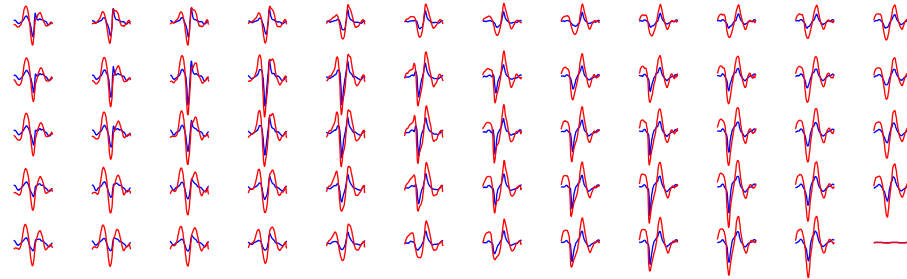
Fused MU twitch forces



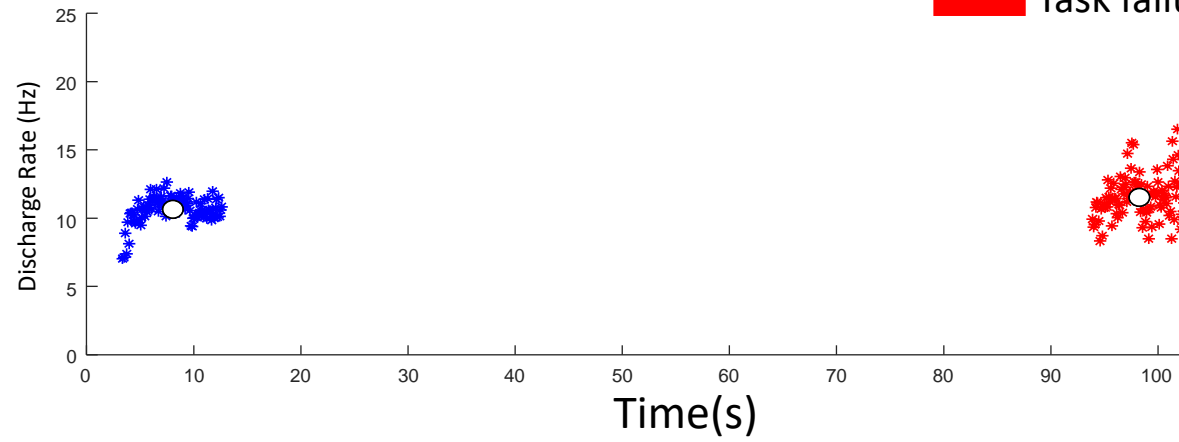
15 s

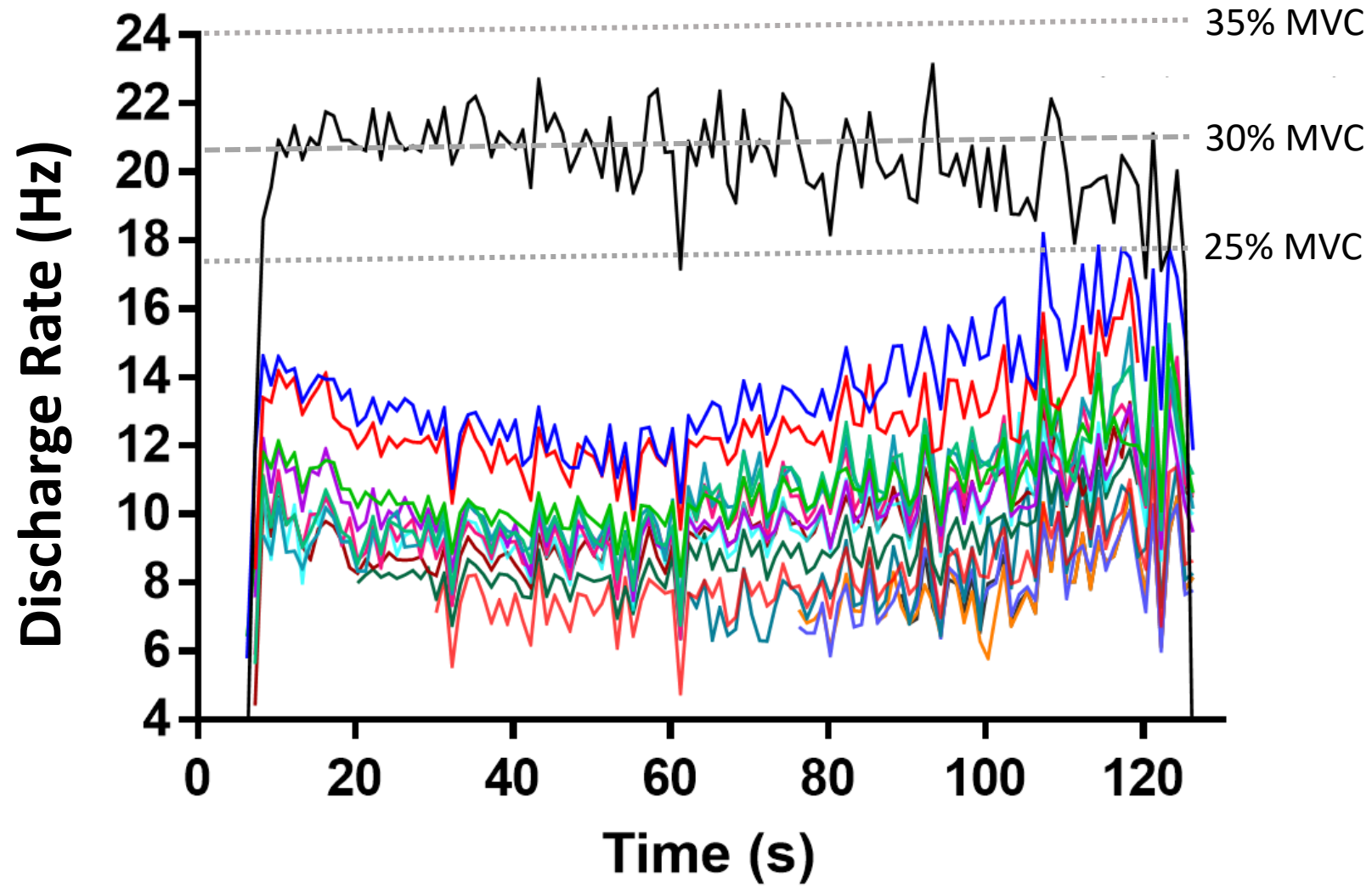


Cross-Correlation Coefficient = 0.87

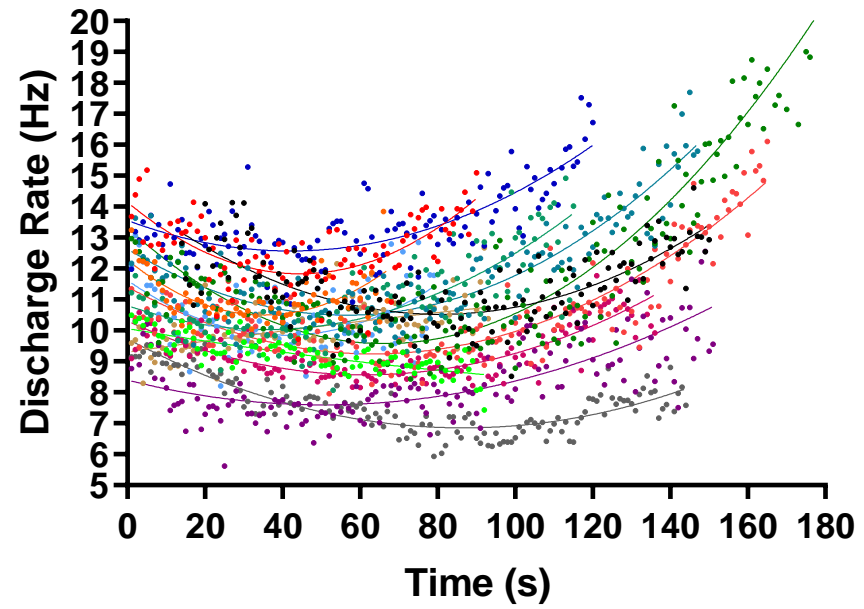


■ Beginning
■ Task failure

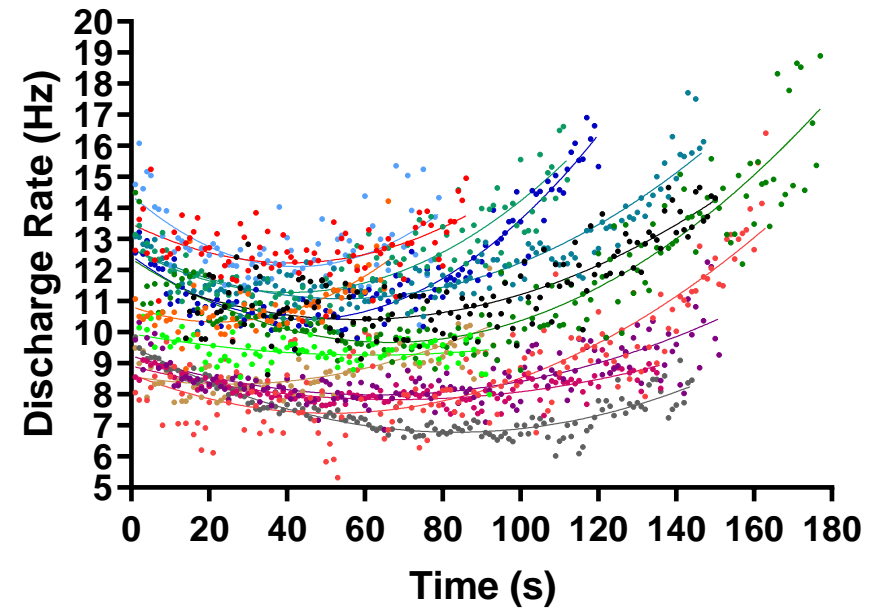


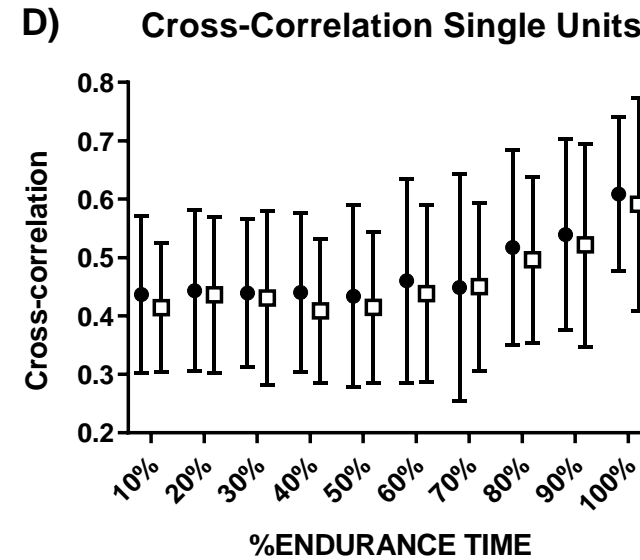
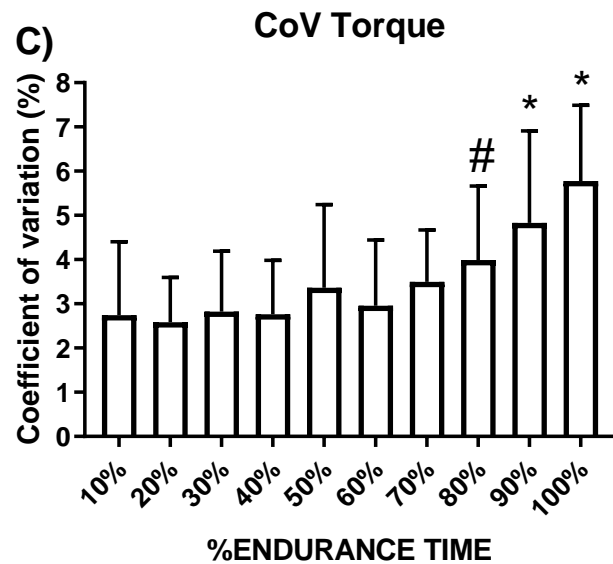
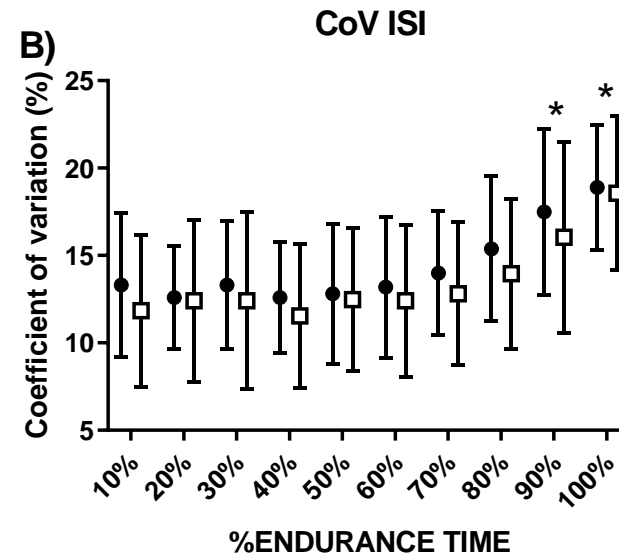
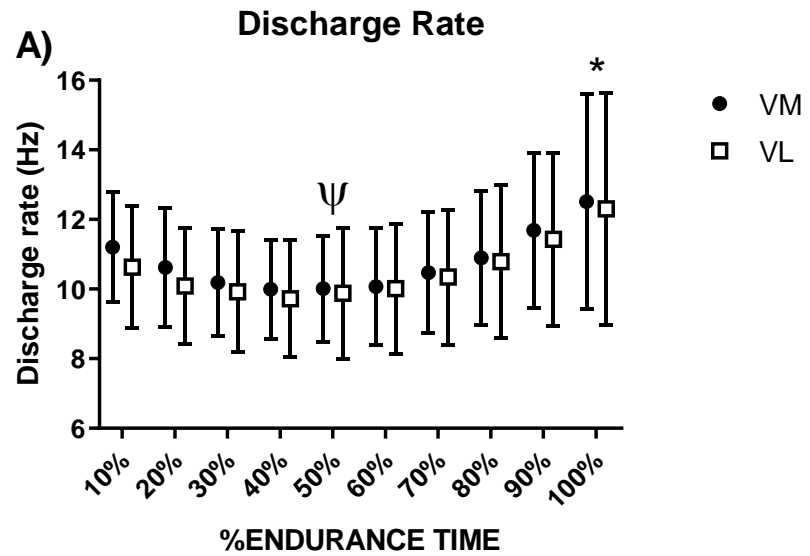


Vastus Medialis

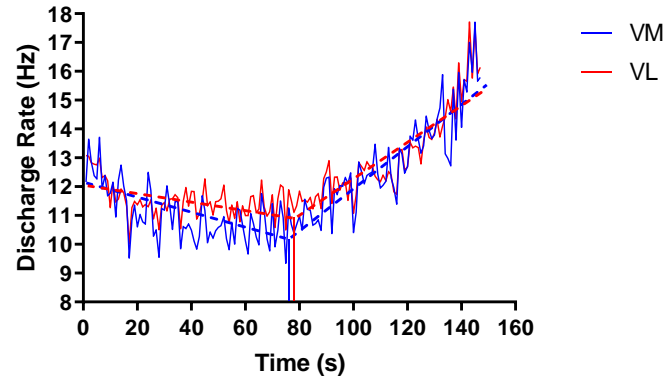


Vastus Lateralis

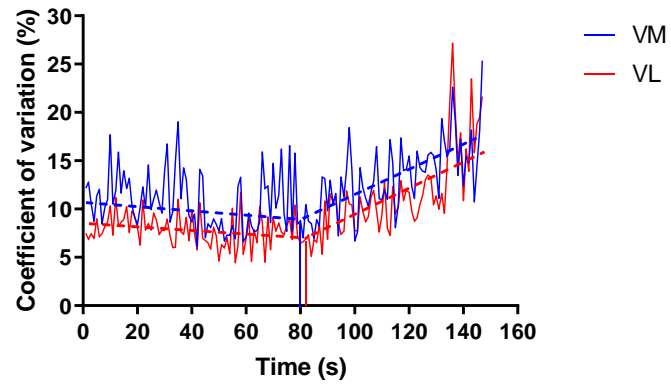




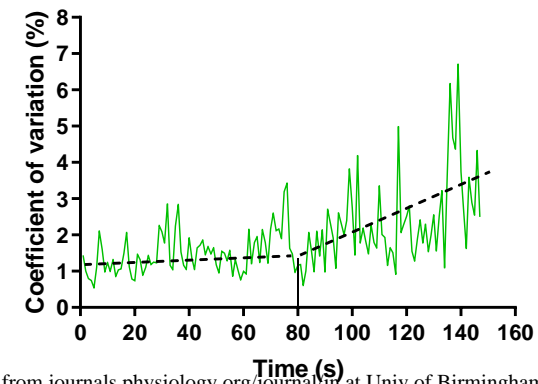
A) Discharge rate breakpoint



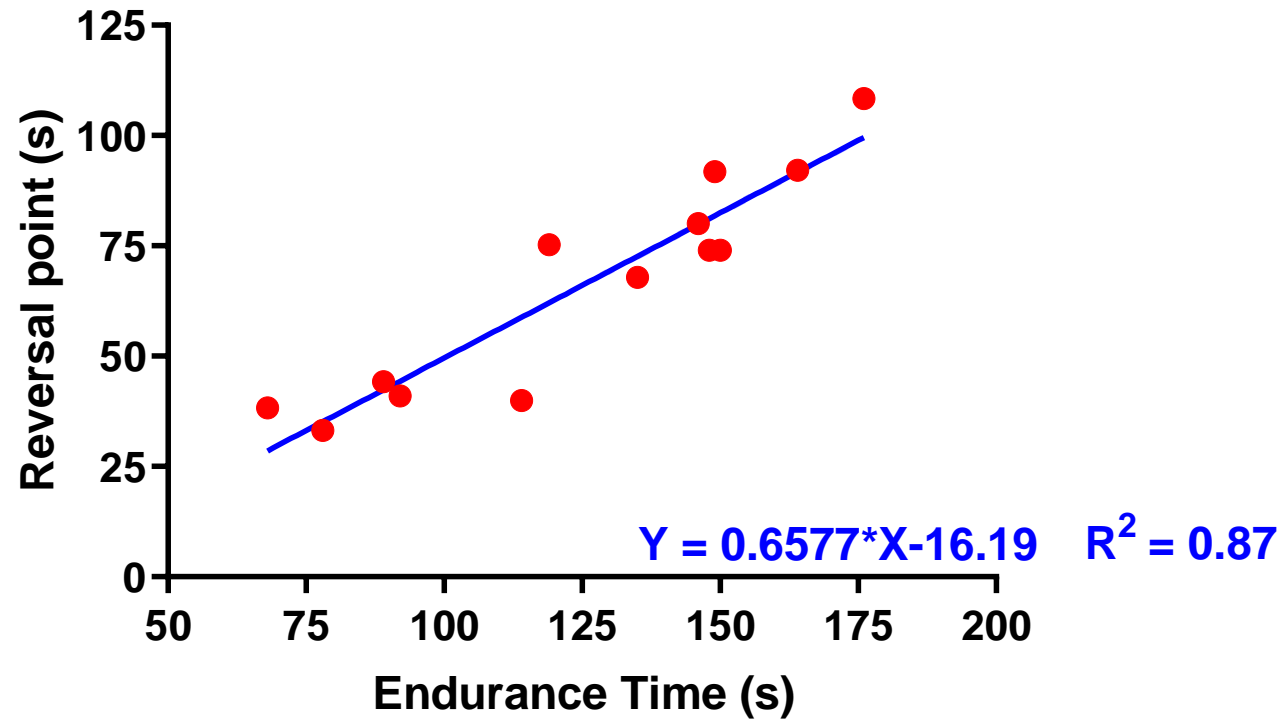
B) CoVisi breakpoint



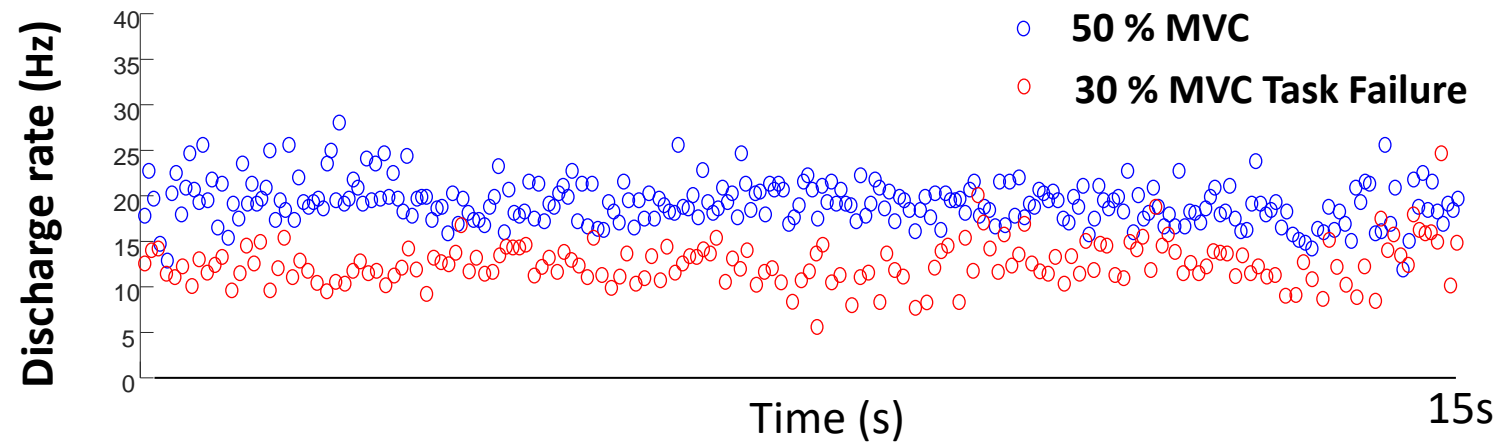
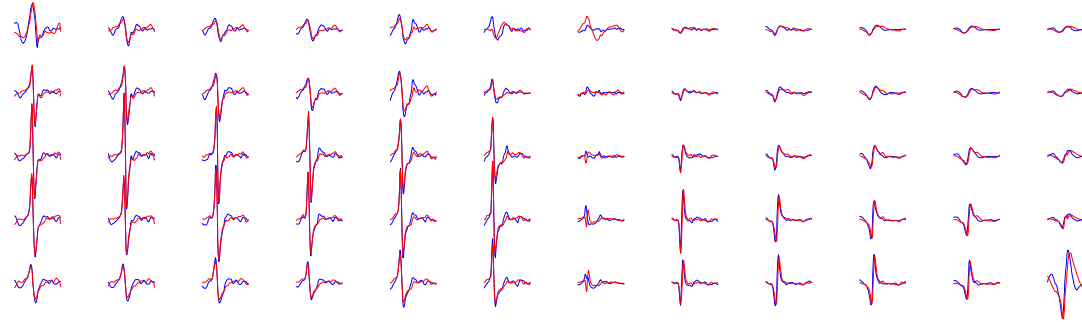
C) CoV torque breakpoint



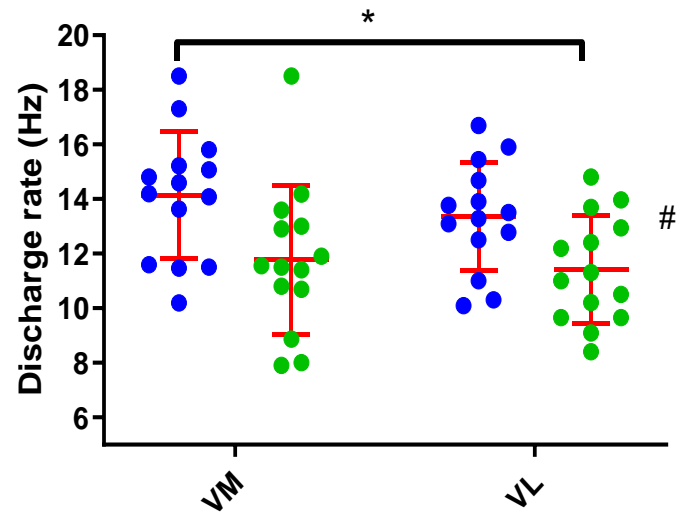
Endurance time vs Reversal point



Cross-Correlation Coefficient = 0.91



A) Early recruited units (50% vs 30% TF)



B) Late recruited units (50% vs 30% TF)

