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1 **Changes in motor unit behavior following isometric fatigue of the first dorsal interosseous**  
2 **muscle**

3 by

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8 Abbreviated Title: Changes in motor unit action potentials following fatigue

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22 X.H. and N.L.S. approved final version of manuscript.

23 **ABSTRACT** The neuro-muscular strategies employed to compensate for fatigue-induced muscle  
24 force deficits are not clearly understood. This study utilizes surface electromyography (sEMG)  
25 together with recordings of a population of individual motor unit action potentials (MUAPs) to  
26 investigate potential compensatory alterations in motor unit (MU) behavior immediately following a  
27 sustained fatiguing contraction, and after a recovery period.

28 EMG activity was recorded during abduction of the First Dorsal Interosseous in 12 subjects at 20%  
29 maximum voluntary contraction (MVC), before and directly after a 30% MVC fatiguing contraction  
30 to task failure, with additional 20% MVC contractions following 10 minutes rest. The amplitude,  
31 duration and mean firing rate (MFR) of MUAPs extracted with a sEMG decomposition system were  
32 analyzed, together with sEMG root-mean-square (RMS) amplitude and median frequency (MPF).

33 MUAP duration and amplitude increased immediately post-fatigue, and were correlated with changes  
34 to sEMG MPF and RMS respectively. After 10 minutes, MUAP duration and sEMG MPF recovered  
35 to pre-fatigue values but MUAP amplitude and sEMG RMS remained elevated. MU MFR and  
36 recruitment thresholds decreased post-fatigue and recovered following rest.

37 The increase in MUAP and sEMG amplitude likely reflects recruitment of larger MUs, while  
38 recruitment compression an additional compensatory strategy directly post-fatigue. Recovery of MU  
39 MFR in parallel with MUAP duration suggests a possible role for metabolically sensitive afferents in  
40 MFR depression post-fatigue.

41 This study provides insight into fatigue-induced neuromuscular changes by examining the properties  
42 of a large population of concurrently recorded single MUs, and outlines possible compensatory  
43 strategies involving alterations in MU recruitment and MFR.

44 **Key words:** Motor unit action potential, surface electromyography decomposition, isometric fatigue

## 45 INTRODUCTION

46 Adaptations in motor unit (MU) recruitment and firing rate modulation during contraction-induced  
47 fatigue have been proposed as compensatory mechanisms counteracting the decline in muscle force  
48 generating capacity. The progressive recruitment of new MUs during fatiguing contractions  
49 performed at a submaximal level has been demonstrated in a number of studies, employing both  
50 sustained (Garland et al. 1994; Jensen et al. 2000; Maton and Gamet 1989) and intermittent (Adam  
51 and De Luca 2003; Bigland-Ritchie et al. 1986b; Carpentier et al. 2001; Christova and Kossev 1998;  
52 Dorfman et al. 1990; Enoka et al. 1989) fatiguing protocols. This recruitment appears to compensate  
53 for fatigue-induced loss, and to aid in preserving force generation.

54 The role of alterations in MU firing rate and recruitment threshold in response to fatigue are less  
55 clearly defined. It has been suggested that these changes are not uniform across the motor unit pool  
56 (Carpentier et al. 2001; Garland et al. 1994) and that adjustments to individual MUs may depend on  
57 activation history (Farina et al. 2009). During sustained and intermittent voluntary contractions,  
58 motor units active from the beginning of the contraction typically exhibit a decline in discharge rate  
59 (Carpentier et al. 2001; Enoka et al. 1989; Garland et al. 1994), however newly recruited MUs have  
60 been shown to exhibit a range of behaviors. Later recruited MUs have been reported to fire at a  
61 constant rate or even to increase steadily (Garland et al. 1994), to exhibit no significant change in  
62 discharge rate (Adam and De Luca 2005), or to show varying rates with time (Carpentier et al. 2001).  
63 Although a decrease in the recruitment threshold of higher threshold MUs following fatigue is  
64 generally reported (Adam and De Luca 2003; Calder et al. 2008; Carpentier et al. 2001; Jensen et al.  
65 2000), some studies have observed increased recruitment thresholds for early recruited MUs  
66 (Carpentier et al. 2001; Farina et al. 2009). Others have reported a homogenous and monotonic  
67 decrease in the recruitment threshold of their sample motor unit population as fatigue progressed  
68 (Adam and De Luca 2003; Christova and Kossev 1998).

69 A key factor that may account for the variations observed could be the type of motor unit recordings  
70 that are utilized. The use of surface EMG is often preferred over intramuscular recordings as a more

71 stable recording during dynamic or fatiguing contractions, with surface EMG providing a summary  
72 representation of motor unit behavior. Yet, in order to gain a comprehensive view of compensatory  
73 recruitment and firing rate strategies, it is imperative to analyze changes occurring at the motor unit  
74 level. These in turn can provide context from which to interpret the adaptations in the sEMG signal.

75 Recent advances in surface EMG decomposition methods have provided a promising new method for  
76 analyzing the activity of individual motor units without the limitations associated with intramuscular  
77 EMG. A number of different surface EMG configurations have been employed to-date (Gazzoni et al.  
78 2004; Hogrel 2003; Holobar et al. 2009; Kleine et al. 2008; Nawab et al. 2010). Kallenberg and  
79 Hermens (2008) observed an increase in the number of MUAPs detected per second over the course  
80 of a sustained fatiguing contraction in the biceps brachii. Although the method collectively  
81 considered both MU recruitment and firing rate, the corresponding increase in the mean root mean-  
82 squared (RMS) amplitude of identified MUAPs suggests recruitment was the primary factor in  
83 maintaining force output. Calder et al. (2008) used a decomposition-based algorithm to identify firing  
84 times of individual MUs, and extracted corresponding MUAPs by spike trigger averaging the sEMG  
85 signal from the biceps brachii. They similarly reported that increases in mean MUAP amplitude and  
86 area were mirrored by increases in the sEMG signal amplitude as the contractions progressed. This  
87 was accompanied by a significant decrease in mean MU firing rates, despite a constant torque output.

88 A reduction in mean firing rates was also reported for MUAPs detected in the Vastus Lateralis, but  
89 not in the Vastus Medialis, in response to an intermittent fatiguing protocol (Stock et al. 2012).

90 It has not yet been evaluated whether a systematic approach to MU firing rate modulation and MU  
91 recruitment strategies is employed to compensate for force deficits in a fatigued muscle, due partly to  
92 the varied fatiguing protocols employed. In particular, it remains unclear whether firing rate  
93 adaptations due to fatigue are uniform across the motoneuron pool and whether there are simultaneous  
94 changes in MU recruitment strategies, such as compressed recruitment and/or recruitment of larger  
95 motor units. For technical reasons, compensatory mechanisms in a fatigued muscle have not yet been  
96 systematically assessed over a large population of concurrently recorded units.

97 To address these questions, the objective of our study was to compare MU behavior directly after  
98 isometric fatigue, and following partial recovery, to pre-fatigue conditions in the First Dorsal  
99 Interosseous (FDI) muscle. The aim of this approach is to comprehensively assess the relative  
100 contribution of MU firing rate and recruitment strategies in accommodating for muscle force  
101 impairment in a fatigued hand muscle. In addition, the ability to record a large sample MU population  
102 using a sEMG decomposition system (dEMG) allows us to determine the degree of uniformity of  
103 these adaptations across the MU pool.

104 This study characterizes the response of a population of motor units following isometric fatigue using  
105 a dEMG system that utilizes a novel surface sensor array recording electrode, coupled with advanced  
106 pattern recognition software to identify single MUAPs. The dEMG approach has been validated with  
107 a range of different techniques (De Luca and Nawab 2011; Hu et al. 2013a; b), using a two-source  
108 recording method (Hu et al. 2014), as well as several advanced simulation studies. It has been used to  
109 investigate the control of multiple motor units in voluntary contractions under in intact subjects (De  
110 Luca and Hostage 2010; Defreitas et al. 2014), during fatigue (Beck et al. 2012; Stock et al. 2012) and  
111 in stroke survivors (Hu et al. 2012). The dEMG system allows motor unit behavior in a fatigued  
112 muscle to be characterized using both the standard surface EMG signal and information from  
113 individual MUAPs. The relationship between the two recordings can also be examined to determine  
114 which MU properties predominate in mediating the changes in the sEMG signal.

115 Our results show an increase in sEMG and MUAP amplitude post-fatigue and following a recovery  
116 period, indicating the recruitment of larger motor units to compensate for the decline in the force  
117 generating capacity of the fatigued muscle. MUAP duration increased directly post-fatigue, but  
118 recovered after the rest period, suggesting the restoration of the ionic and metabolic changes to the  
119 muscle that slow muscle fiber conduction velocity (MFCV). This was accompanied by a parallel  
120 decrease and subsequent increase in MU firing rates, consistent across the MU pool. The reduction in  
121 firing rates coupled with continued recruitment may suggest a selective inhibition of early recruited  
122 motoneurons, mediated through increased activity of mechanically and metabolically sensitive  
123 afferents. The results indicate that a combination of supplementary MU activation and lower MU

124 recruitment threshold is favored over rate-coding to maintain the force after fatigue while motor units  
125 are available.

## 126 **METHODS**

### 127 *Participants*

128 Twelve right-dominant neurologically intact individuals (6 male, 6 female) volunteered to participate  
129 in this study. The force and EMG activity of the first dorsal interosseous muscle were examined  
130 during isometric abduction of the right index finger about the 2nd metacarpo-phalangeal (MCP) joint.  
131 All participants gave informed consent via protocols approved by the Institutional Review Board  
132 under the Office for the Protection of Human Subjects at Northwestern University.

### 133 *Experimental Setup*

134 Participants were seated upright in a Biodex experimental chair (Biodex Medical systems, Shirley,  
135 NY) with their upper arm comfortably resting on a plastic support. To standardize hand position and  
136 to minimize contributions of unrecorded muscles, the forearm was cast and placed in a ring mount  
137 interface attached to an elbow rest at the wrist. The elbow rest was securely mounted with magnetic  
138 stands to a heavy steel table. The forearm was placed in full pronation and the wrist was held neutral  
139 with respect to flexion/extension. The little, ring and middle fingers were separated from the index  
140 finger and strapped to the support surface. The thumb was secured at an approximately 60-degree  
141 angle to the index finger. The index finger was placed in line with the 2nd metacarpal and the long  
142 axis of the forearm creating a 0 degree or neutral (abduction/adduction) MCP joint angle. The  
143 proximal phalanx of the index finger was fixed to a ring-mount interface attached to a six degrees-of-  
144 freedom load cell (ATI, Inc., 3226). Recorded forces from the x (abduction/adduction) and y  
145 (extension/flexion) directions were low pass filtered (cut-off =200 Hz) and digitized at a sampling  
146 frequency of 1 kHz. The subjects were instructed to produce required abduction forces while  
147 minimizing the off-axis forces. There is evidence that both motor unit recruitment threshold (Enoka  
148 et al. 1989) and patterns of recruitment (Suresh et al. 2002) are directionally dependent (i.e. finger  
149 flexion vs. abduction) in the FDI. In order to prevent variations in rank order of MU recruitment with

150 different directions of contraction (Thomas et al. 2006) off-axis forces in the flexion direction were  
151 tightly controlled. The subject received visual feedback of the force in the x and y direction  
152 (flexion/extension), to minimize off axis forces and maximize the force exerted in the desired x  
153 direction.

154 The subject's skin was prepared using adhesive tape and alcohol pads. Surface EMG was recorded  
155 from the FDI using a surface sensor array (Delsys, Inc.) that consisted of 5 cylindrical probes (0.5 mm  
156 diameter) located at the corners and at the center of a  $5 \times 5$  mm square (Nawab et al. 2010). Pairwise  
157 differentiation of the 5 electrodes yielded 4 channels of sEMG signals (Figure 1 (a)). The sEMG  
158 sensor and a reference electrode were connected to 4 channels of a Bagnoli sEMG system (Delsys,  
159 Inc.). The signals were amplified and filtered between 20 Hz and 2 kHz. The signals were sampled at  
160 20 kHz and stored on a computer for further processing.

#### 161 *Protocol*

162 Subjects were asked to perform a series of three maximal voluntary contractions (MVCs) for 3 s, with  
163 1 min rest between trials, and the largest value was designated as 100 % MVC. The subjects were  
164 then asked to perform a series of six isometric voluntary contractions in which they followed a  
165 trapezoidal force trajectory as depicted in Figure 1 (b), in order to provide a pre-fatigue baseline.  
166 Feedback on the force direction and magnitude was presented to the subject in two-dimensional  
167 display on a computer screen as a visual aid. The dimensions of the target on screen were kept  
168 constant across subjects, therefore visual gain was maintained over the experiment for a single subject  
169 but varied between subjects according to subject MVC. Previous studies have indicated that  
170 alterations to visual gain can influence force fluctuations (Sosnoff and Newell, 2009) and  
171 motoneuronal excitability (Laine et al., 2014). However, in this study inter-subject differences in  
172 visual feedback were relatively small and were unlikely to have had significant effects on force  
173 variability (Baweja et al., 2009). The trapezoid trajectory contained 5 segments: a 3 s quiescent  
174 period for baseline noise calculation, an up-ramp increasing at a rate of 10% MVC/s, a constant force  
175 of 20% MVC for 10 s, a down-ramp decreasing at 10% MVC/s, and a final 3 s quiescent period. To



176 ensure that the subjects could trace the trapezoid trajectory closely, they were given practice trials  
177 before the main experiment. During this section of the experiment, the subject was given a 1-minute  
178 rest period between repetitions to minimize fatigue. After the six pre-fatigue trials, a sustained  
179 isometric contraction was performed at 30% MVC to task failure in order to induce fatigue. The  
180 subjects were given visual bar feedback of their force output and the time of task failure was defined  
181 as the time when the subject's force dropped 10% below the required output for a period of 5 or more  
182 seconds. A single MVC was performed directly following task failure, and six post-fatigue  
183 trapezoidal force trajectories at 20% MVC were subsequently performed, with no rest period given  
184 between trials to minimize recovery. The subjects were then allowed a 10-minute recovery period  
185 before a series of four more trapezoidal trajectories (20% MVC) was performed. A final maximum  
186 voluntary contraction was recorded in 10 of the subjects following the recovery trials.

## 187 ***Figure 1***

### 188 *Data Analysis*

189 To be selected for further analysis, surface EMG signals were required to have a peak to peak (P-P)  
190 baseline noise  $< 20 \mu\text{V}$  and signal to noise ratio  $> 5$  with no sudden change (i.e., larger than 20%  
191 MVC/s) in the up-ramp force. The analysis was confined to the surface EMG signals recorded before  
192 and directly after the fatiguing contraction, and following the recovery period, in order to adhere to  
193 specific experimental conditions for which the dEMG system has been previously validated, i.e. short  
194 duration isometric contractions. Next, discriminable MUs were extracted using the dEMG  
195 decomposition system (version 1.0.0.28). For each identified MU, the output of the algorithm  
196 consisted of MU firing times and 4 MUAP waveforms (for the 4 recorded channels). Detailed  
197 information for the decomposition algorithms is described in Nawab et al. (2010) and De Luca and  
198 Hostage (2010).

199 Spike triggered averaging (STA) of the sEMG was performed to characterize the MU waveform  
200 recorded from the surface electrodes. A STA was performed on each of the 4 channels of the sEMG  
201 signals, using the identified firing times for each MU as triggers, resulting in 4 representative STA

202 MUAP estimates for each MU. The time interval used to derive the template estimate was 10 ms  
203 prior to and after the firing time. The peak-to-peak amplitude was calculated as the voltage difference  
204 between the maximum peak and the adjacent minimum peak within the time window. The time  
205 between the nearest zero crossings to the maximum and minimum peaks was calculated to provide the  
206 MUAP duration.

207 Two separate reliability tests were performed to determine which decomposed MUAP estimates  
208 would be retained for further analysis, using the procedure outlined in Hu et al. (2013b). To quantify  
209 the variation of the STA MUAP over time, the coefficient of variation (CV) was calculated for the  
210 peak-to-peak (P-P) amplitude of the MUAP templates. This coefficient was implemented as a  
211 measure of the stability of the waveform average over the duration of the contraction and the accuracy  
212 of the firing time estimation. The maximum linear correlation coefficient between the STA estimate  
213 (calculated over the entire trial duration) and the decomposition-estimated templates was computed as  
214 a second measure of the reliability of the STA estimates of the MUAP. The MUs with a correlation  
215 coefficient (between the STA MUAP estimate and the decomposition MU template)  $>0.7$  and CV of  
216 P-P  $<0.3$  across all four channels were selected for later analysis. The average correlation coefficient  
217 between the STA MUAP and MU template, as well as the average CV of P-P amplitude derived from  
218 each of the four channels, was used to qualify each MU for use in further analysis. For each identified  
219 MU, the combined results from all four channels was used only for the MU selection process,  
220 subsequent analysis of the selected MUs was simplified by using the channel of maximum median  
221 amplitude for each subject.

222 The recruitment threshold was defined as the threshold force at recruitment, calculated as the  
223 averaged force over the interval -50 to +100 ms relative to the first firing event. The mean firing rate  
224 was calculated with a non-overlapping window of 0.5 s length from a 4-s averaging window in the  
225 middle of the steady state hold phase of the contraction. The relationship between the MUAP  
226 amplitude/duration and the recruitment threshold force of the MU was examined by fitting a linear  
227 regression line to the data and calculating the slope and r squared values of the fit.

228 To assess the relationship between spectral properties of the global signal and characteristics of the  
229 decomposed MUAPs, the mean RMS and the median frequency of the power spectrum (MPF) value  
230 of the sEMG signal during the trapezoidal force trajectories were calculated for each subject, for each  
231 of the three conditions (pre-, post-fatigue, and recovery). The average values were obtained using a 1  
232 second moving average window and step size of 0.5 s over 2 seconds of the steady state hold phase  
233 (7-9 s) using the first trial at 20% MVC.

234 The RMS value of the EMG signal was calculated across a 1 second time window (5-6 s) during the  
235 30% MVC fatiguing contraction for each subject. In order to control for between-subject variations in  
236 sEMG amplitude, this RMS value was used to normalize the RMS amplitude of the global signal  
237 detected for each condition. The mean and standard deviation of the decomposed MUAP amplitudes  
238 were calculated for the MUs detected in the pre-fatigue condition for each subject and used to  
239 standardize the pre-fatigue MUAP amplitude distribution to have a zero mean and unit variance. The  
240 pre-fatigue mean and standard deviation were then used to standardize the post-fatigue and recovery  
241 MUAP distributions within the same subject. Standardized distributions were used to examine the  
242 changes in MUAP amplitude with condition across subjects and reduce subject specific variability in  
243 the mean and variance of the distribution.

#### 244 *Statistics*

245 For each subject, the median value of the MPF and RMS of the global sEMG signal and the amplitude  
246 and duration of the surface decomposed MUAPs were calculated for the pre-fatigue, post-fatigue and  
247 recovery states. The probability distributions of the decomposed MUAP amplitudes and durations  
248 were analyzed per condition for each subject. A one-way within-subjects (or repeated measure)  
249 analysis of variance (ANOVA) was conducted to compare the effect of condition on each parameter  
250 across subjects, with a statistic test for pre-fatigue, post-fatigue and recovery states. Mauchly's Test  
251 of Sphericity was implemented to check the assumption of sphericity, and if violated, a Greenhouse-  
252 Geisser correction was applied to the data. Post hoc tests to examine pairwise differences between  
253 conditions were conducted using the Fisher's Least Significant Difference (LSD) test. A regression

254 analysis using a generalized linear model was performed to examine the change of MPF and RMS of  
255 sEMG during the fatigue protocol.

256

## 257 **RESULTS**

258 The properties of the sEMG signal and the characteristics of multiple discriminated MU spike trains,  
259 including mean firing rates and the estimated threshold force, were examined before and after a  
260 fatiguing contraction. The alterations in median MUAP amplitude and duration were related to the  
261 changes observed in the RMS amplitude and mean frequency of the surface signal. Changes in  
262 MUAP amplitude and duration and MU firing rate were examined as a function of MU recruitment  
263 threshold to assess the uniformity of the adaptations across the MU pool.

264

### 265 *Force Properties*

266 Changes in subject MVC across the conditions (for n=10 subjects) were analyzed with an ANOVA  
267 before and after the sustained isometric 30% MVC fatiguing contraction to task failure ( $196.8 \pm 55$   
268 seconds). The results indicate a significant difference in subject MVC between pre- and post-fatigue  
269 conditions ( $F(2, 18) = 33.895, p < .0001$ ). Post hoc tests revealed a significant reduction ( $p < .001$ ) in  
270 MVC following fatigue ( $54.15 \pm 11.53$  N to  $30.43 \pm 13.36$  N, respectively). The median subject  
271 MVC failed to recover after the period of rest and remained significantly depressed ( $42.75 \pm 16.75$  N,  
272  $p < .001$ ) compared to initial pre-fatigue values, though higher than that recorded directly post-fatigue  
273 ( $p < 0.005$ ).

### 274 *Figure 2*

275

### 276 *Surface EMG signal*

277 The results of an ANOVA, with Greenhouse-Geisser correction show that there was a significant  
278 effect of condition (pre-fatigue, post-fatigue and recovery) on the EMG MPF, ( $F(1.18, 12.99) =$   
279  $20.123, p < .0001$ ) (Figure 2 (a)). Post hoc tests revealed that the sustained, fatiguing contraction  
280 resulted in a significant decrease in global signal MPF from pre-fatigue to post-fatigue conditions  
281 ( $166.9 \pm 52.09$  Hz to  $100.38 \pm 31.42$  Hz, respectively,  $p < .001$ , standardized values  $0 \pm 1$  to  $-1.27 \pm$   
282  $.6$ ). However, after the recovery period the MPF increased significantly ( $170.87 \pm 79.04$  Hz,  $p = .002$ ,

283 standardized value  $0.08 \pm 1.5$ ), and was found not to be statistically different from MPF values before  
284 fatigue ( $p = .709$ ).

285 ANOVA on the mean scores for normalized RMS amplitudes also confirmed a significant effect of  
286 condition ( $F(2, 22) = 7.08, p = .004$ ) (Figure 2 (b)). Median normalized RMS amplitude increased  
287 significantly ( $p = .023$ ) from pre-fatigue to post-fatigue conditions ( $1.01 \pm 0.075$  to  $1.36 \pm 0.47$  and  
288  $0.19 \pm 0.18$  to  $0.26 \pm 0.14$ , normalized and non-normalized values, respectively). After the period of  
289 10 minutes recovery mean RMS amplitude ( $1.49 \pm 0.56$ , normalized,  $0.29 \pm 0.13$ , non-normalized)  
290 remained elevated with respect to the initial RMS mean ( $p = .012$ ), however, the values were not  
291 significantly different to post-fatigue values ( $p = .198$ ).

292 **Figure 3**

293 **Figure 4**

294 *Decomposed MUAPs*

295 Within each condition pre-fatigue, post-fatigue and after the recovery period, 78.6% (1164 of 1480  
296 MUs), 76% (1052 of 1384 MUs) and 78% (784 of 1001 MUs) were accepted respectively. Unless  
297 otherwise stated the values reported are based on the analysis of accepted units. Over all conditions,  
298 77% of decomposed motor units met the criteria to be accepted for further analysis. The probability  
299 density distribution in Figure 3 displays the probability of occurrence of an accepted MU of a  
300 particular amplitude plotted against increasing amplitude values in a single representative subject, and  
301 across all subjects for pre-fatigue, post-fatigue and after 10 minutes recovery. Boxplots display the  
302 mean (line), median ('+'), standard deviation and outliers of the distribution for the indicated MU  
303 population. Figure 4 shows the corresponding probability distribution of the MU durations in a single  
304 representative subject and across all subjects.

305 **Figure 5**

306 Repeated measures ANOVAs were then employed to examine the intrinsic signal properties of the  
307 decomposed MUAPs. The change in MVC (Figure 5 (a)) was accompanied by a significant change in

308 the duration of the decomposed MUAPs with condition ( $F(2, 22) = 79.97, p < .0001$ ) (Figure 5 (b)).  
309 Median MU duration increased significantly ( $p < .0001$ ) from pre-fatigue to post-fatigue conditions  
310 ( $7.05 \pm 1.29$  ms vs.  $10.21 \pm 2.03$  ms, respectively). However, after the recovery period median MU  
311 durations then decreased significantly ( $7.26 \pm 1.74$  ms,  $p < .0001$ ), and were found not to be  
312 statistically different from durations observed before fatigue ( $p = 0.329$ ).

313 The results of an RM ANOVA, with Greenhouse-Geisser correction, on the median standardized  
314 amplitudes of decomposed MUs reveal that the median MUAP amplitude was significantly affected  
315 by fatigue ( $F(1.3, 14.3) = 7.57, p = .01$ ) (Figure 5 (c)). An increase in median standardized MU  
316 amplitude was observed from pre-fatigue to post-fatigue conditions ( $-0.2 \pm 0.058$  vs.  $0.46 \pm .97$ ,  
317 respectively), which was statistically significant ( $p < .05$ ). However, after the period of 10 minutes  
318 recovery MUAP amplitudes ( $0.7 \pm 0.99$ ) remained significantly higher than pre-fatigue values ( $p <$   
319  $.01$ ). No statistical difference was observed between the medians of the two sets of MUAP  
320 amplitudes recorded after the fatiguing contraction, whether recorded directly after or following the  
321 recovery period ( $p = .068$ ).

322 In order to investigate whether there was any systematic bias in the method employed to retain MUs  
323 for further analysis, the ANOVAs were repeated using all of the original decomposed MUs. There  
324 was no difference in the direction of any of changes in MU amplitude or duration for each condition.

## 325 **Figure 6**

### 326 *MU firing rate changes*

327 Repeated measures ANOVAs were then employed to examine the mean firing rates over each  
328 condition. The results show that there was a significant effect of condition on the firing rates of the  
329 decomposed MUAPs ( $F(2, 22) = 10.04, p < .001$ ), Figure 6 (b). Median MU mean firing rate  
330 decreased significantly ( $p < .015$ ) from pre-fatigue to post-fatigue conditions ( $10.9 \pm 1.26$  Hz vs.  
331  $10.15 \pm 1.47$  Hz, respectively). However, after the recovery period median MU firing rates then  
332 increased significantly ( $11.23 \pm 1.27$  Hz,  $p < .001$ ), and were found not to be statistically different from  
333 discharge rates observed before fatigue ( $p = .218$ ).

334 The average firing rate of MUs was calculated within a bin width equal to 1% MVC for each subject  
335 over all conditions and the mean result for all subjects is displayed in Figure 6 (b). A significant  
336 negative correlation was observed between threshold of recruitment and average firing rates per bin  
337 for pre-fatigue, post-fatigue and recovery conditions ( $r(101) = -.7, p < .001, r(85) = -.6, p < .001$  and  
338  $r(103) = -.73, p < .001$  respectively).

### 339 **Figure 7**

#### 340 *MU Recruitment Threshold*

341 Figure 7 presents the probability distribution of the recruitment threshold for the MUAP pool. The  
342 results of a repeated measures ANOVA show a significant effect of condition on the median threshold  
343 of recruitment for decomposed MUAPs ( $F(2, 22) = 3.497, p < .05$ ) (Figure 7). Median MU threshold  
344 of recruitment was significantly lower directly post fatigue ( $5.75 \pm 2.06$  % MVC) than both pre-  
345 fatigue and recovery conditions ( $7.83 \pm 2.96$  % MVC,  $p < .05$ , and  $7.89 \pm 3.09$  % MVC,  $p < .05$ ,  
346 respectively).

347 The normalized MUAP amplitude for each subject was binned with respect to its threshold of  
348 recruitment with a bin width of the normalized force equal to 1% MVC. The average MUAP  
349 amplitude in the post-fatigue and recovery conditions was greater than the corresponding pre-fatigue  
350 average at each threshold bin; however there was no consistent trend to suggest that lower or higher  
351 threshold MUs were affected disproportionately. Similarly, the increase in the average duration of  
352 MUAPs detected at each 1% MVC force interval after fatigue did not appear to be influenced by the  
353 initial MU recruitment threshold.

#### 354 *Effect of recruitment threshold on measured parameters*

355 The uniformity of MUAP property changes across the MU pool were investigated by examining the  
356 relationship between the decomposed MUAP amplitude/duration and the recruitment threshold force  
357 of the MU. No correlation was observed between MUAP duration and recruitment threshold force,  
358 the linear regression slope did not differ significantly from zero for 96% of the trials. We found no



359 systematic change with condition for either the slope or the r-squared value of the linear regression fit  
360 to the MUAP amplitude versus threshold plot when analyzed with a RM ANOVA ( $F(2, 22) = 2.62$ ,  $p$   
361  $= .095$  and  $F(2, 22) = .076$ ,  $p = .927$  respectively). This may indicate compensatory strategies post-  
362 fatigue are subject specific, with a combination of recruitment compression (increased slopes) and  
363 supplementary MU recruitment (no change in slope values) employed. A significant increase in  
364 amplitude with threshold was observed in only 47% of trials, which may occur as a result of the low  
365 force of the contraction. Although no consistent change in the slope of the MUAP amplitude versus  
366 threshold plot was observed, higher mean slopes were reported directly post-fatigue ( $0.057 \pm 0.07$ )  
367 and after recovery ( $0.039 \pm 0.035$ ), than the slopes observed pre-fatigue ( $0.029 \pm 0.036$ ). In contrast,  
368 similar mean r-squared values were reported for all three conditions ( $0.32 \pm 0.26$ ,  $0.33 \pm 0.2$  and  $0.32$   
369  $\pm 0.2$ ; pre-fatigue, post-fatigue and after recovery respectively).

#### 370 *Co-variation of global surface EMG measures and MU parameters*

371 An approximately linear relationship was observed between the median amplitude of decomposed  
372 MUAPs and the RMS amplitude of the global sEMG signal. The scatterplot in Figure 8 (a) presents  
373 the relationship between MUAP amplitude and sEMG amplitude for all of the three conditions, along  
374 with the Pearson product-moment correlation coefficient. A strong, statistically significant positive  
375 correlation was observed between MUAP amplitude and the amplitude of the sEMG signal,  $r(36) =$   
376  $.86$ ,  $p < .0001$ . A Spearman's rank-order non-parametric correlation was performed to determine the  
377 non-linear but monotonic relationship between the median duration of decomposed MUAPs and the  
378 MPF of the global sEMG signal across each of the three conditions. The scatterplot in Figure 8  
379 summarizes the results, a strong, statistically significant negative correlation was observed between  
380 MUAP duration and the median frequency of the global signal,  $r(36) = -.95$ ,  $p < .0001$ .

381 The square of the Pearson product-moment correlation coefficient was computed to assess the  
382 approximately linear relationship between the median amplitude and firing rate of decomposed  
383 MUAPs and the percentage change between conditions for the median duration and firing rate of  
384 decomposed MUAPs. A strong, statistically significant negative correlation was observed between

385 MUAP amplitude and firing rate,  $r(36) = -.4$ ,  $p < .05$  and percentage change between conditions for  
386 MUAP duration and discharge rate,  $r(36) = -.726$ ,  $p < .0001$ .

387 *Figure 8*

## 388 **DISCUSSION**

389 In this study, surface EMG and motor unit action potential properties, including motor unit  
390 recruitment and firing rates, were examined collectively to provide insight into changes due to  
391 isometric muscle fatigue and in the following recovery period. While many of these techniques are  
392 well established, the capacity to decompose the surface EMG signal into constituent motor unit action  
393 potentials offers a unique overview of the distribution of MUAP waveform characteristics across a  
394 large population of MUs, an approach that has been difficult to implement using traditional surface or  
395 intramuscular EMG methods to-date. The sEMG decomposition technique also allows a  
396 comprehensive, simultaneous analysis of firing rate and recruitment properties across the motor  
397 neuron pool, in a manner not previously attainable.

### 398 *Changes to the Global EMG signal*

399 For each subject, a sustained, fatiguing contraction induced a reduction in the force generating  
400 capacity of the FDI muscle immediately following the contraction. The reduction in maximal force  
401 persisted beyond the 10 minute recovery period (Figure 5 (a)). This incomplete recovery of force  
402 following a rest period has also been reported previously in the biceps brachii and the FDI muscles,  
403 using a similar sustained, submaximal fatiguing protocol (Esposito et al. 1998; Fuglevand et al.  
404 1993b; Post et al. 2008). The presence of muscle fatigue is supported by a significant progressive  
405 decrease in the median frequency of the EMG signal during the sustained fatiguing contraction, and  
406 immediately post-fatigue (Figure 2 (a)). This is consistent with the well-established decline in muscle  
407 fiber conduction velocity as fatigue progresses. The sEMG spectral properties recorded immediately  
408 after the fatiguing contraction suggest a change in the metabolic and ionic state of the muscle,  
409 resulting in a depression of muscle fiber conduction velocity, and subsequently the MPF (Figure 2  
410 (a)). After the rest period of 10 minutes, the MPF had recovered to pre-fatigue values. The frequency

411 compression of the power spectrum is primarily governed by shape alterations in the MUAP  
412 waveforms that comprise the signal, with minimal influence from motor unit firing rates (Hermens et  
413 al. 1992). The recovery of the MPF thus suggests that the MFCV and hence duration of the MUAP  
414 have also been restored to pre-fatigue values.

415 The decline in MPF post-fatigue was accompanied by a simultaneous rise in surface EMG amplitude  
416 (Figure 2 (b)). Although the complexity of the surface signal limits the utility of EMG amplitude as  
417 an index of muscle activation, an increase during and immediately following sustained submaximal  
418 contractions is usually considered to indicate an enhancement of the central drive (Bigland-Ritchie et  
419 al. 1986b; Fuglevand et al. 1993a), though it can be influenced by surges in  $Na^+ - K^+$  pump activity,  
420 MU synchronization and rate-coding, and prolonged duration of the underlying MUAP shape due to  
421 decreased conduction velocity (Farina et al. 2004; Hicks and McComas 1989; Lowery and O'Malley  
422 2003). This increase in the intensity of the central drive increases the number of motor units recruited  
423 and/or modulates the firing rates of previously active MUs (Fuglevand et al. 1993b).

424 In the present study, a reduction in mean firing rates was observed post-fatigue which suggests that  
425 MU rate coding was not a contributing factor to the rise in surface EMG amplitude (Gabriel and  
426 Kamen 2009). However, the contribution of MU synchronization, which has been proposed to  
427 increase following fatigue (Holtermann et al. 2009), in augmenting surface EMG amplitude cannot be  
428 excluded. After a period of rest, surface EMG amplitude remained elevated, disparate from the  
429 observed recovery of the signal frequency content. The decoupling of spectral and amplitude  
430 properties provides evidence that changes in the muscle fiber action potential waveforms and  
431 reduction in conduction velocity are not the primary factor responsible for the enlargement of the  
432 EMG signal amplitude.

#### 433 *Changes in MUAP Amplitude and Duration*

434 Properties of decomposed MUAP waveforms mirrored the changes observed in the global EMG  
435 signal (Figure 8), and a strong correlation was observed between MUAP amplitude and RMS sEMG  
436 (Figure 8 (a)) and MUAP duration and sEMG MPF (Figure 8 (b)). The inverse relationship between

437 MUAP duration and MPF has been previously demonstrated in simulation studies (Hermens et al.  
438 1992; Lowery and O'Malley 2003), and investigated using dEMG techniques (Calder et al. 2008).  
439 However, the large sample population in this study enabled a significant correlation between the two  
440 parameters to be demonstrated experimentally for the first time. MUAP duration increased  
441 significantly immediately post-fatigue and returned to initial values after 10 minutes recovery (Figure  
442 5 (b)). However, MUAP amplitude increased directly following the fatiguing contraction and  
443 remained elevated after the rest period (Figure 5 (c)), despite the median MU duration returning to  
444 pre-fatigue levels (Figure 5 (b)). This suggests that the increase in MUAP amplitude observed post-  
445 recovery is unlikely to be due to the ionic disturbances that alter MUAP waveforms during fatigue. A  
446 more plausible interpretation is that the increase in amplitude is predominantly due to the recruitment  
447 of additional large MUs to compensate for the reduction in the force generating capacity of the  
448 muscle, (Figure 6 (a)) (Bigland-Ritchie et al. 1986b; Carpentier et al. 2001; Enoka et al. 1989; Maton  
449 and Gamet 1989).

450 A uniform increase in the median and standard deviation of the MUAP durations was observed post-  
451 fatigue in Figure 4 (b). If larger MUs, associated with higher conduction velocities and shorter  
452 MUAP durations, are recruited to compensate for force loss, a greater range in the population  
453 durations may be expected. However, the results of this study support the findings of Gazzoni et al.  
454 (2005), who determined that changes in membrane properties due to the activity of recruited muscle  
455 fibers influence the conduction velocity of quiescent fibers and newly recruited motor units. Thus an  
456 increase in the duration of newly recruited MUAPs with respect to their durations in an unfatigued  
457 state would be expected, and is substantiated by the absence of lower range durations post-fatigue in  
458 this study, despite their presence before fatigue and after recovery.

459 Lastly, surface-detected action potentials from motor units located at greater depths within the muscle  
460 will tend to have larger durations due to the spatial low pass filtering effect of the tissue. The volume  
461 conductor effect means that MUAPs of deeper motor units will have longer durations and attenuated  
462 amplitudes with respect to more superficial units (Dimitrova and Dimitrov 2003; Lowery et al. 2002).  
463 The recruitment of motor units according to spatial distribution, however, is unlikely to bias the

464 overall result of the study, as the FDI muscle has been reported to have a uniform distribution of large  
465 and small motor units, with the muscle fibers that comprise each unit widely dispersed (Milner-Brown  
466 and Stein 1975).

467 While the changes in motor unit duration during fatigue are well-established, the variations in MUAP  
468 amplitude are less clear. Evidence for peripheral factors inducing changes in MUAP amplitude  
469 during fatigue can be found in the literature. A decline in M-wave amplitude has been reported  
470 directly after fatiguing contractions when there remained a strong influence of ion channel activity  
471 and concentration gradients on the intracellular action potential (Carpentier et al. 2001; Fuglevand et  
472 al. 1993a). A full recovery of the M-wave has been demonstrated after a 10 minute rest period  
473 (Fuglevand and Keen 2003; Post et al. 2008), indicating that the restoration of membrane excitability  
474 follows a similar time course. The recovery of MPF (Figure 2 (a)), and action potential duration  
475 (Figure 6 (b)), after the rest period in the present study suggest the ionic and metabolic changes are  
476 unlikely to have substantially influenced the increased sEMG and MUAP amplitudes following  
477 recovery.

#### 478 *Response to the Impairment of Muscle Force Generating Capacity*

479 It is not possible to infer from the sEMG and MUAPs parameters whether one single mechanism or a  
480 combination of mechanisms is responsible for the decrease of the mechanical muscle force (Allen et  
481 al. 2008; Enoka and Duchateau 2008). Prolonged low-frequency force depression and reduced MVC  
482 and twitch force following sustained contractions have been previously attributed to excitation-  
483 contraction coupling failure in several studies (Hill et al. 2001; Westerblad et al. 1998). The  
484 prolonged reduction in the force generating capacity of the muscle is compensated by the recruitment  
485 of larger motor units, as reflected in the increase of the amplitude of the sEMG signal (Figure 2 (c)).  
486 A reduction in the threshold of MU activation for previously active MUs may be an additional  
487 compensatory strategy employed directly post-fatigue, where there is evidence of recruitment  
488 compression (Figure 7). The lowering of MU recruitment threshold may be peripherally mediated by  
489 changes in the mechanical and metabolic properties of the muscle, and could potentially counteract

490 the decline in force attributable to the reduction in MU firing rates. Despite the absence of  
491 recruitment compression post-recovery, the percentage increase in the mean amplitude of the MUs  
492 recruited at each threshold, averaged over all subjects, remained elevated, particularly at the latter  
493 stages of recruitment. This suggests that fatigue-induced lowering of the recruitment threshold of  
494 larger MUs was also employed post-recovery to compensate for the continued force impairment of  
495 those MUs already activated (Adam and De Luca 2003; Calder et al. 2008; Carpentier et al. 2001;  
496 Farina et al. 2009). The slope of the regression fit to the MUAP amplitude and threshold relationship  
497 exhibited an overall tendency to increase directly post-fatigue, which corresponds with the reported  
498 recruitment compression. However, across all subjects there was no systematic change observed in  
499 the slope of the regression fit after the recovery period, which implies that the compensatory strategies  
500 employed to cope with a force deficit may be specific to the individual. Supplementary MU  
501 activation and lower MU recruitment thresholds could both be present in varying combinations after  
502 fatigue.

### 503 *Firing Rate and Uniformity of Changes*

504 The current study offers a more comprehensive insight into firing rate alterations after muscle fatigue  
505 than previous reports on pooled single motor unit observations, by simultaneously examining the  
506 firing patterns of hundreds of motor units in each condition, across all subjects. In addition, the  
507 recording configuration allows the uniformity of motor unit adaptations to be investigated across a  
508 sample population with different thresholds of recruitment. The reduction in the mean MU firing  
509 rates observed following fatigue mirrors previous studies reporting an overall decline in MU  
510 discharge rates with the development of fatigue (Calder et al. 2008; Christova and Kossev 1998;  
511 Stock et al. 2012). Furthermore, average MU firing rates were consistently lower over the range of  
512 MU recruitment thresholds (Figure 6 (b)), which suggests that the reported decline was not a simply a  
513 consequence of the lower discharge rates of newly recruited MUs. Although previous studies have  
514 shown that newly recruited MUs can increase their discharge rate as fatigue progresses (Adam and De  
515 Luca 2005; Garland et al. 1994), the results of this study show that over the MU population discharge  
516 rates display a uniform reduction in response to fatigue (Figure 6 (b)). The reduction in discharge

517 frequency may occur as a result of a decline in the intrinsic excitability of MUs with previous activity  
518 (Kernell and Monster 1982), or alternatively, firing rate may be modulated by inhibitory afferent  
519 signals from receptors sensitive either to changes in the muscle contractile properties or to the  
520 metabolic state of the muscle (Bigland-Ritchie et al. 1986a). In this study, alterations to MU  
521 discharge rates were negatively correlated with changes in MUAP duration, which may imply a role  
522 for metabolically activated inhibitory inputs in regulating MU firing. Proprioceptive feedback from  
523 muscle spindles (De Luca and Kline 2012) and Golgi tendon organs (Kirsch and Rymer 1987) may  
524 also contribute to the control of MU activity, although simultaneous recovery of firing rate and  
525 MUAP duration was observed after rest with only partial restoration of force.

526 There were no consistent trends in the alterations to MU discharge rate or MUAP amplitude or  
527 duration to suggest that higher threshold MUs were systematically more affected by fatigue, and  
528 results varied by subject. This finding may be due to the low target force of the 20% MVC test  
529 contractions sampling a relatively homogeneous group of fatigue-resistant MUs. Alternatively, the  
530 longer hold phase of the fatigue task may have resulted in all detected MUs being active for similar  
531 durations, thus the activity dependent adjustments would be comparable across the MU pool. Lastly,  
532 it is likely that the intensity of the fatigue task induced widespread changes in the conduction velocity  
533 of all muscle fibers and was not confined to distinct motor unit territories.

## 534 **CONCLUSION**

535 This study is the first to simultaneously examine global sEMG and the properties of a large population  
536 of individual MUAPs, complete with recruitment threshold and firing rate information, before and  
537 immediately after a sustained isometric fatiguing contraction, and following a recovery period.  
538 Changes in the properties of the recruited motor unit population were found to be consistent with  
539 alterations observed in the global EMG signal and displayed well-established manifestations of  
540 fatigue over a large MU population. The observed increase in sEMG and MUAP amplitude post-  
541 fatigue is consistent with previous studies that have demonstrated MU recruitment during fatiguing  
542 contractions using intramuscular, conventional surface EMG and surface decomposition techniques.

543 MUAP duration and MPF were restored to initial pre-fatigue values following the rest period,  
544 suggesting that the fatigue-induced ionic and metabolic alterations that affect MFCV have also  
545 recovered. Despite the increase in central drive post-fatigue, motor unit firing rates were reduced,  
546 implying that to maintain force during fatigue, recruitment is favored over rate-coding while motor  
547 units are available. The presence of recruitment compression post fatigue may imply a peripherally  
548 mediated lowering of the MU recruitment threshold as an additional short-term compensatory  
549 mechanism to cope with large fatigue-induced force deficits. Though recruitment compression was  
550 absent after the recovery period, sEMG and MUAP amplitude remained elevated, and additional  
551 recruitment was still required to compensate for the continued impairment to the force. Motor unit  
552 discharge rates returned to initial values after rest, mirroring the recovery of MFCV. This provides  
553 evidence that the changes in MU firing are modulated by inhibitory afferents sensitive to the  
554 metabolic state of the muscle. In conclusion, this study employs sEMG decomposition techniques to  
555 examine fatigue-induced changes in the properties of a motor unit population and outlines possible  
556 recruitment strategies that may be employed to compensate for force deficits due to both short-term  
557 alterations in the metabolic state of the muscle and long-term variations in muscle contractile  
558 properties.

559



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687

688

689 **FIGURE CAPTIONS**

690 Figure 1. (a) Four channels of surface EMG recorded from the FDI using the Precision Decomposition System  
691 during a 20 second 20% MVC trapezoidal force trajectory (b).

692 Figure 2. Median values for (a) median power frequency and (b) root mean squared amplitude of the global  
693 surface EMG signal across all subjects for pre-fatigue, post-fatigue and recovery conditions. Median values  
694 were calculated from data recorded during the steady state hold (7-9 seconds) of the trapezoidal force trajectory.  
695 All values were standardized to the mean and standard deviation of pre-fatigue values. Significant differences at  
696 the  $p < .05$  and  $p < .005$  level are indicated with a single asterisk and a double asterisk respectively.

697 Figure 3. Probability distribution of MU amplitudes (a) for a single subject and (b) across all subjects. Boxplots  
698 display the mean (line), median ('+'), standard deviation and outliers of the distribution. MU amplitudes for  
699 each subject have been standardized to his or her pre-fatigue values for comparison. The asterisk indicates that  
700 for the post-fatigue and recovery conditions 6 and 7 MUAP amplitudes, respectively, were larger than 9.26.

701 Figure 4. Probability distribution of MU durations (a) for a single subject and (b) across all subjects. Boxplots  
702 display the mean (line), median ('+'), standard deviation and outliers of the distribution.

703 Figure 5. (a) Maximum voluntary contraction, (b) median MUAP duration and (c) median standardized MUAP  
704 amplitude for the decomposed motor unit action potentials across all subjects for pre-fatigue, post-fatigue and  
705 recovery conditions. Significant differences at the  $p < .05$  and  $p < .001$  level are indicated with a single asterisk  
706 and a double asterisk respectively.

707 Figure 6. (a) Distribution of mean MU firing rates across all subjects for pre-fatigue, post-fatigue and recovery  
708 conditions and (b) average firing rate of MUs binned with respect to the MU threshold of recruitment for all  
709 subjects. Significant differences at the  $p < .05$  and  $p < .001$  level are indicated with a single asterisk and a  
710 double asterisk respectively.

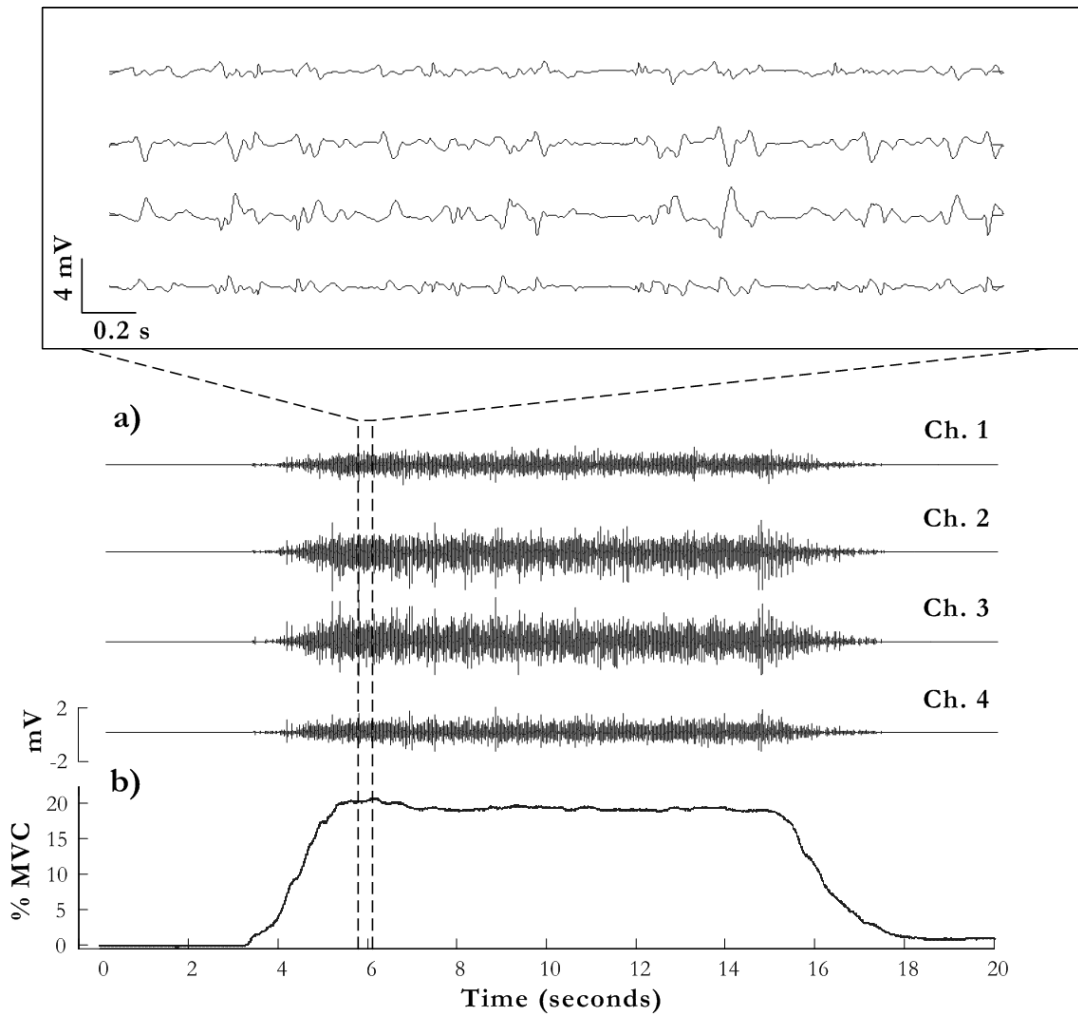
711 Figure 7. Probability distribution of MU recruitment thresholds (a) for a single subject and (b) across all  
712 subjects. Boxplots display the mean (line), median ('+'), standard deviation and outliers of the distribution.

713 Figure 8. Scatterplot of (a) the median decomposed MUAP amplitude and the RMS amplitude of the global  
714 sEMG signal for all subjects for each of the three conditions  $r = .86$  and (b) the median decomposed MUAP  
715 duration and the MPF of the global sEMG signal for all subjects for each of the three conditions  $r = -.95$ .

716 **FIGURES**

717 Figure 1

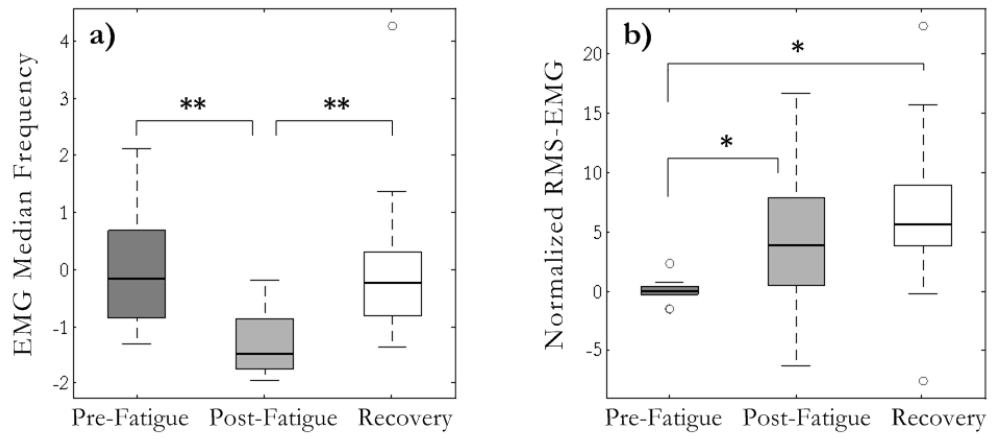
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721 Figure 2



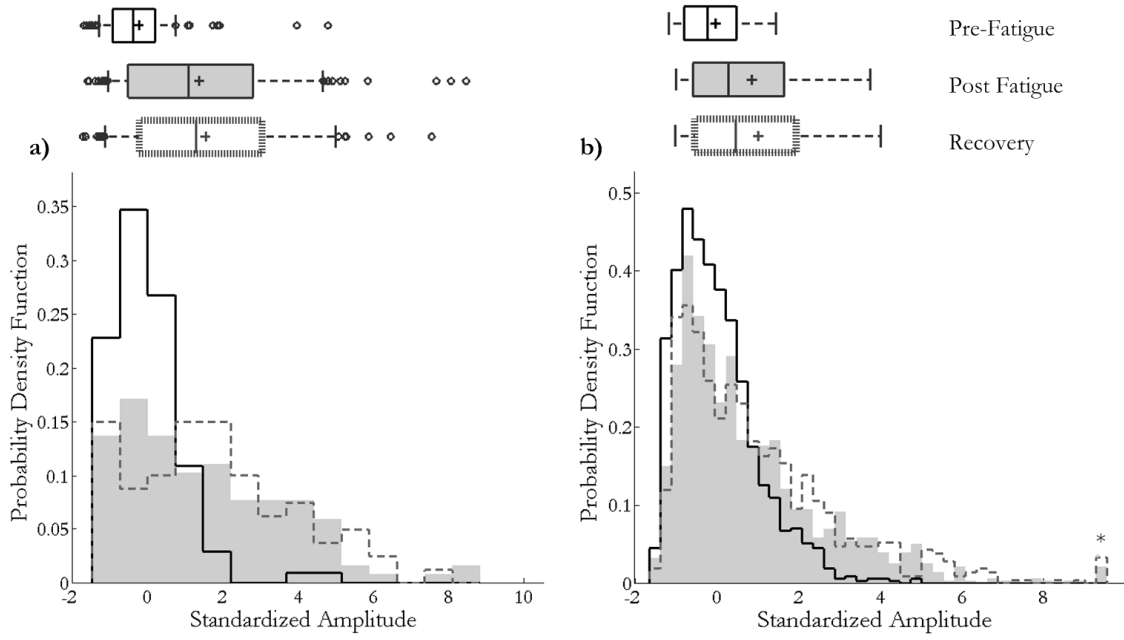
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724 Figure 3

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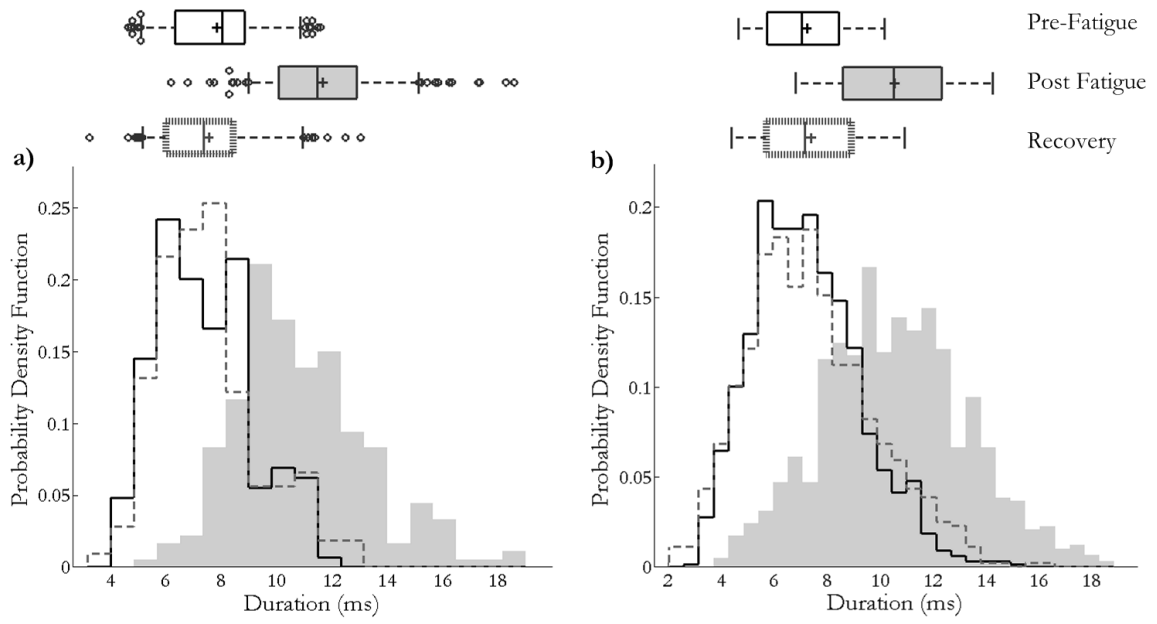


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729 Figure 4

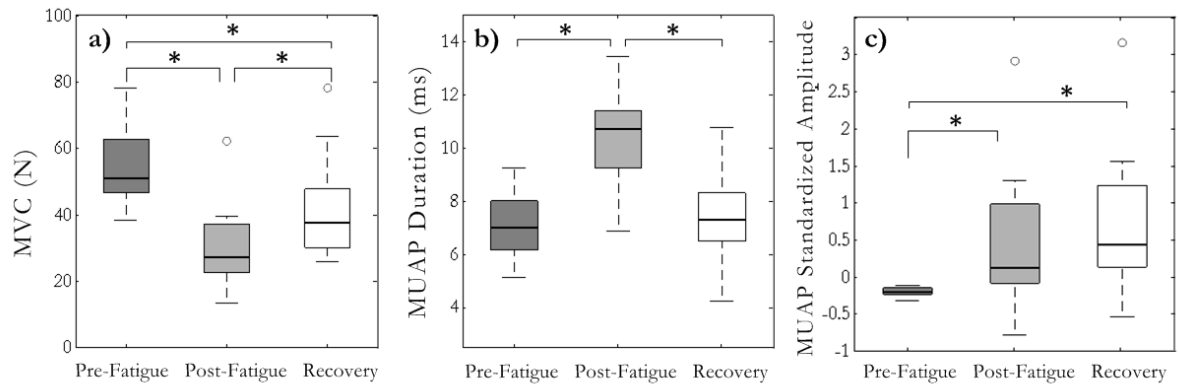


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732 Figure 5

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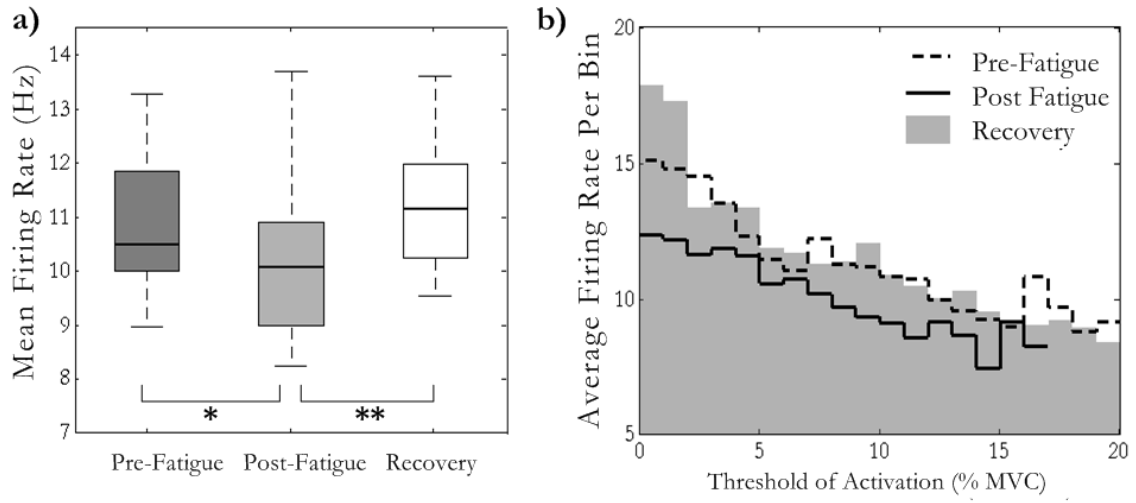
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736 Figure 6

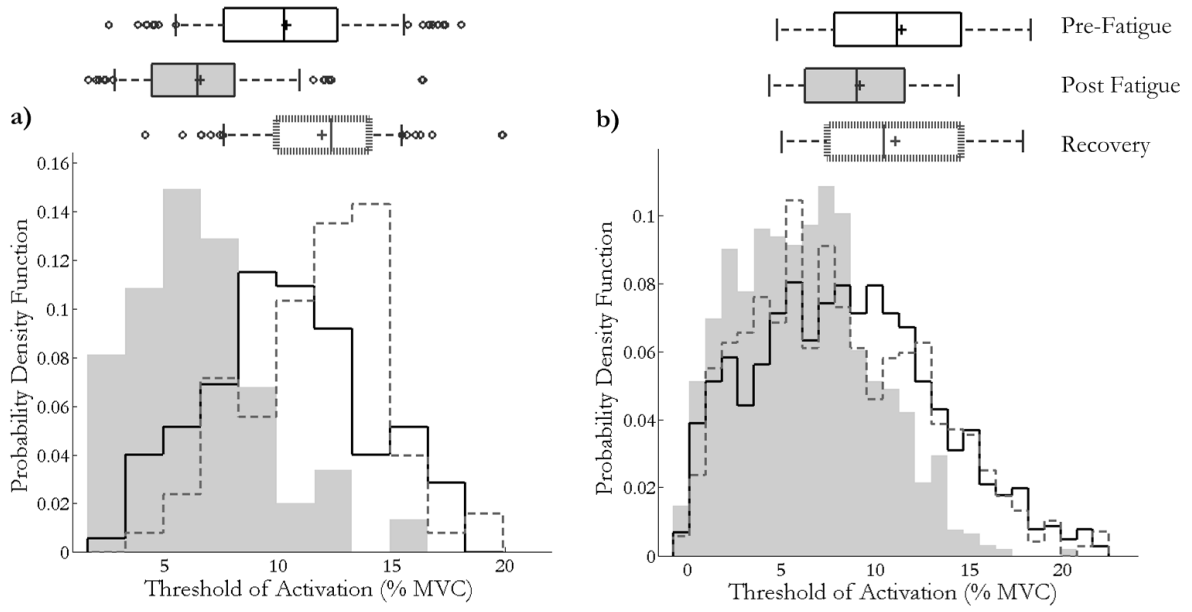
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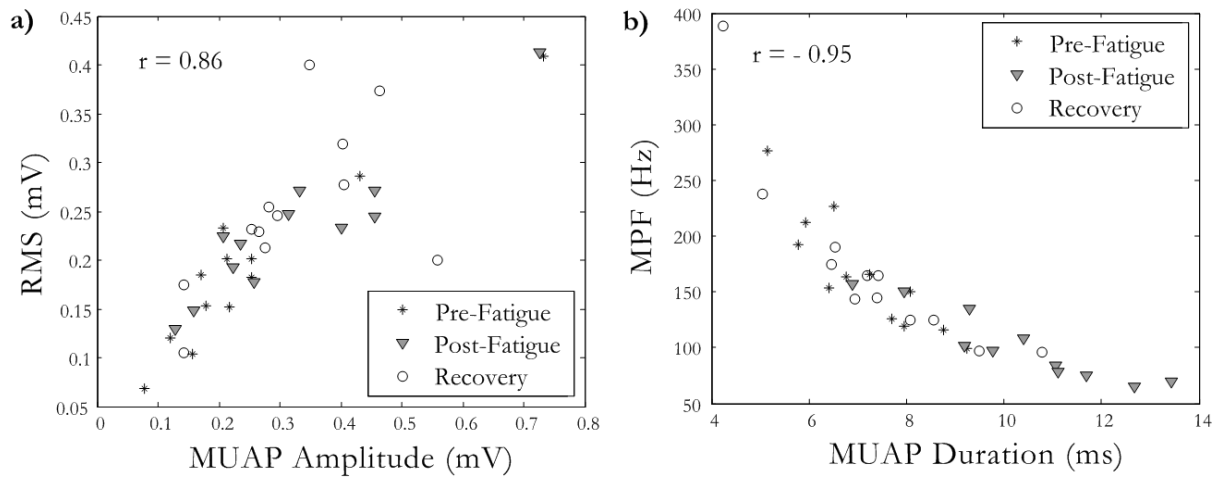


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744 Figure 8

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