

Tutorial

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27 **Abstract**

28 Recent work demonstrated that it is possible to identify motor unit discharge times from high-density
29 surface EMG (HDEMG) decomposition. Since then, the number of studies that use HDEMG
30 decomposition for motor unit investigations has increased considerably. Although HDEMG
31 decomposition is a semi-automatic process, the analysis and interpretation of the motor unit pulse trains
32 requires a thorough inspection of the output of the decomposition result. Here, we report guidelines to
33 perform an accurate extraction of motor unit discharge times and interpretation of the signals. This
34 tutorial includes a discussion of the differences between the extraction of global EMG signal features
35 versus the identification of motor unit activity for physiological investigations followed by a
36 comprehensive guide on how to acquire, inspect, and decompose HDEMG signals, and robust
37 extraction of motor unit discharge characteristics.

38

39 **Introduction**

40 The generation of movement is accomplished by the transmission of synaptic inputs to motoneuron
41 pools. The transducer of synaptic input into forces is the motor unit, which comprises a group of muscle
42 fibres (muscle unit) and an alpha motor neuron. The neural information is transmitted by the motor unit
43 through axonal action potentials (neural drive to the muscle) that elicit action potentials in the innervated
44 muscle unit (motor unit action potentials, Figure 1). The summation and time-course of the motor unit
45 action potentials determine the characteristics of the surface electromyogram (EMG) recorded with
46 electrodes placed on the skin during motor tasks (Day and Hulliger, 2001; Fuglevand et al., 1992;
47 Heckman and Enoka, 2012; Milner-Brown et al., 1973). The shapes of the surface-recorded motor unit
48 action potentials are influenced by the properties of the volume conductor (Dimitrov and Dimitrova,
49 1974; Enoka and Duchateau, 2015; Farina et al., 2002b; Mañanas et al., 2016; Merletti et al., 2003;
50 Stegeman et al., 1997).

51 Due to the physiological safety factor at the neuromuscular junction, the identification of motor unit
52 action potentials from the interference EMG signals informs us about the discharge activity of individual
53 motoneurons (Desmedt and Godaux, 1977; Duchateau and Enoka, 2011; Enoka and Duchateau, 2015;
54 Gandevia et al., 1990; Henneman et al., 1965; Milner-Brown et al., 1973; Milner-Brown and Stein,
55 1975). Based on this approach, the motoneuron is the only nerve cell that can be noninvasively
56 recorded in humans. For these reasons, several surface EMG decomposition methods have been
57 proposed over the past three decades (Chen et al., 2018; Chen and Zhou, 2016; De Luca et al., 2006;
58 Farina et al., 2010; Gazzoni et al., 2004; Holobar et al., 2014; Holobar and Zazula, 2007; Kumar et al.,
59 2020; Nawab et al., 2010; Negro et al., 2016a). Of these methods, in this tutorial we focus exclusively
60 on those based on blind source separation (BSS) methods applied to high-density surface EMG.

61 Over the past two decades, non-invasive high-density surface EMG (HDEMG) electrodes have been
62 used to identify motor unit discharge times (Drost et al., 2001; Farina et al., 2002a; Gazzoni et al.,
63 2005; Masuda and De Luca, 1991; Merletti et al., 2008, 1999; Zwarts and Stegeman, 2003). These
64 recordings provide a spatial sampling of the motor unit action potentials at the skin surface (Holobar et

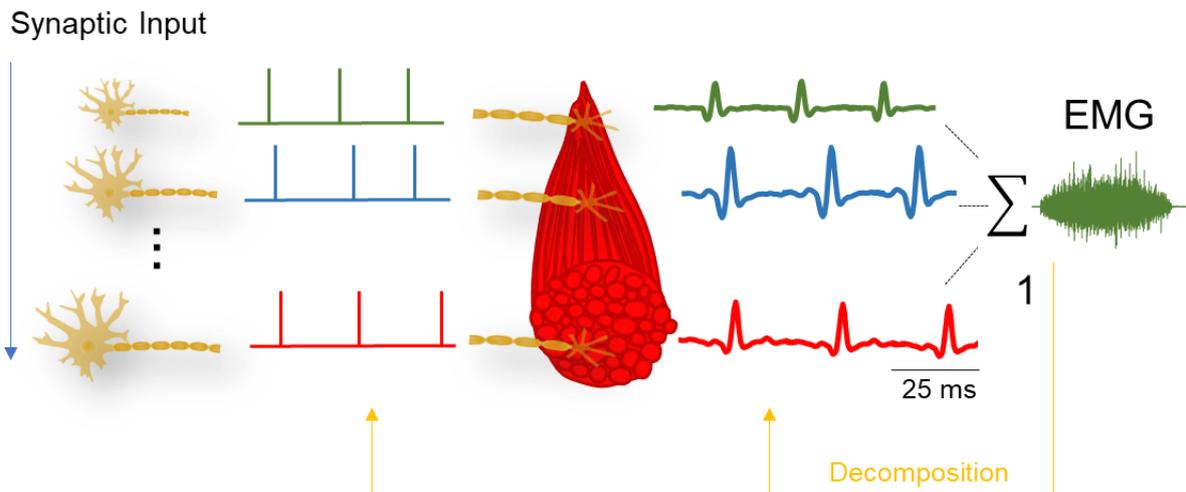
65 al., 2010; Merletti and Farina, 2016; Negro et al., 2016a; Zwarts and Stegeman, 2003). From these
 66 recordings, blind source separation (BSS) procedures can identify motor unit discharge times (Chen
 67 and Zhou, 2016; Holobar et al., 2010; Negro et al., 2016a) during a range of isometric tasks (A Del
 68 Vecchio et al., 2019c; Gallego et al., 2015; Martinez-Valdes et al., 2017). Although BSS decomposition
 69 procedures are performed in an automatic way, they require user-inspection of the identified motor unit
 70 spike trains (Enoka, 2019).

71 The aim of this tutorial article is to provide guidelines for the decomposition of HDEMG recordings.
 72 Moreover, we discuss the limits, the potential, and how to further validate the results obtained with
 73 HDEMG decomposition. The future advances needed in EMG decomposition are also discussed, with
 74 an emphasis on the computational challenges required to remove the subjectivity during visual editing
 75 of the motor unit spike trains.

76

77 **1 – Extracting neural information from high-density EMG signals: Global EMG estimates vs.**
 78 **decomposition**

79 Since the surface EMG signal is the algebraic summation of motor unit action potentials (Day and
 80 Hulliger, 2001), it is influenced by both the discharge times and the waveforms of the action potentials
 81 of the active motor units (Figure 1).



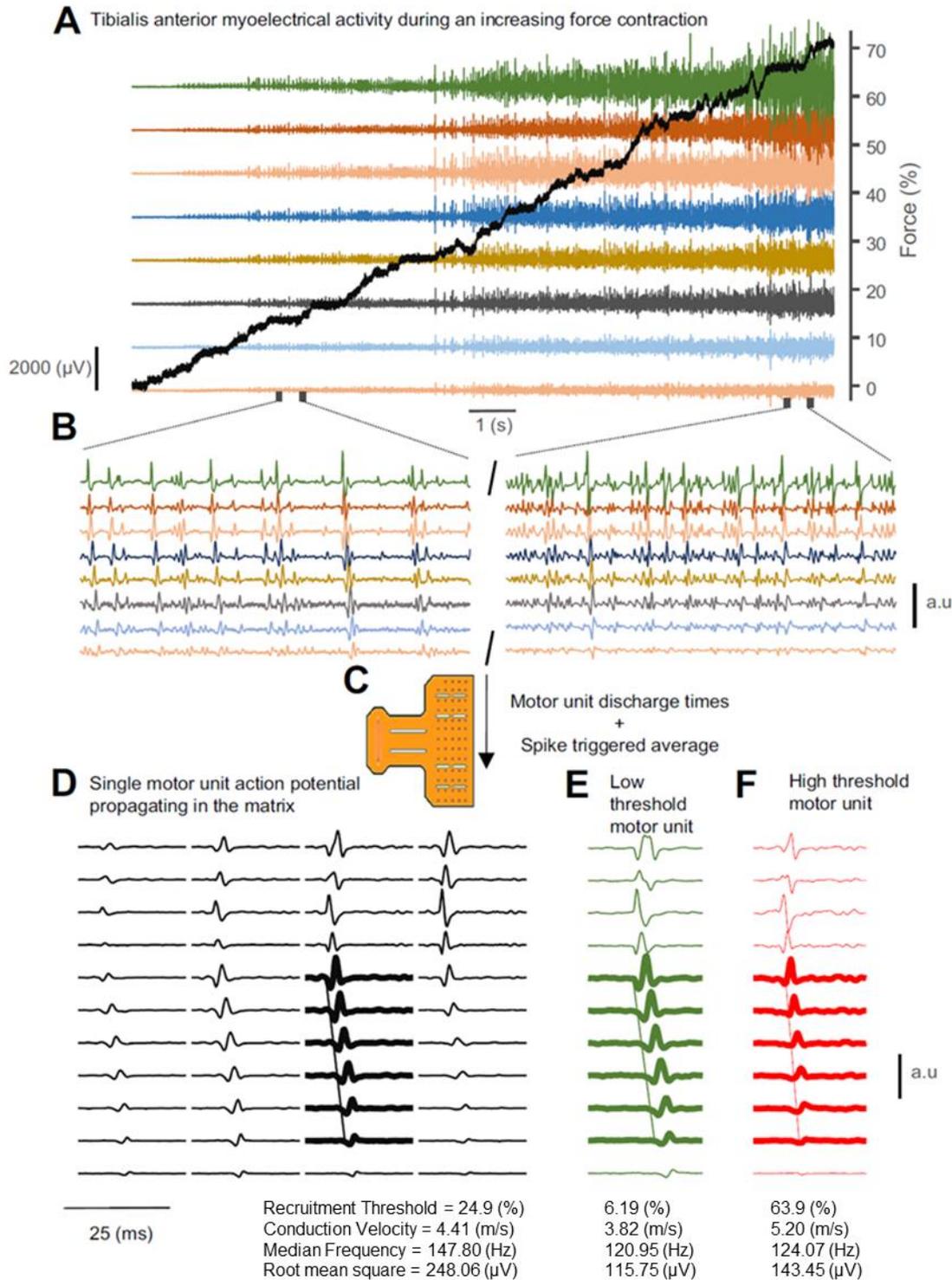
82

83 **Figure 1** The one-to-one correspondence between axonal action potentials and motor unit action
 84 potentials. A pool of motoneurons discharges a series of action potentials (left) that are transformed by
 85 the muscle unit in a time series of motor unit action potentials (right). The motor unit action potential
 86 vary in amplitude and these differences are not always associated with the size of the motor unit, due
 87 to the influence of the volume conductor. The summation of the motor unit action potentials corresponds
 88 to the recorded EMG signals. Due to these effects, the association between the strength of the neural
 89 drive to the muscle and EMG amplitude is not always linear. Rather, the neural drive to the muscles
 90 can only be estimated from the motor unit discharge times, such as by decomposition of high-density
 91 surface EMG recordings (line 1 in orange). Conversely, conventional EMG analyses often estimate the
 92 neural drive to the muscle by extracting global features of the signal, such as amplitude or spectral
 93 moments. The decomposition of the EMG signal identifies the series of action potentials for individual
 94 motor units (red spikes). Due to several limitations with the global EMG, however, it is not always correct
 95 to infer the motoneuron population activity from global EMG signals, for example, because of the effects
 96 of amplitude cancellation and the non-linear relation between action potential sizes and recruitment
 97 thresholds. *Note that the innervation zones of the motoneurons are shown in largely different positions

98 of the muscle only to improve figure clarity while often the innervation zones are clustered in relatively
99 small muscle portion.

100 The characteristics of the motor unit action potentials depend on many factors; for example, action
101 potential amplitude and conduction velocity, which scale with the diameter of the muscle fibre
102 (Håkansson, 1956; Plonsey and Barr, 1988). The amplitude of the motor unit action potentials also
103 depends on the number of innervated muscle fibres, which is associated to the motor unit recruitment
104 threshold (the voluntary force level corresponding to the first discharge of a motor unit) (Milner-Brown
105 and Stein, 1975). However, this association is confounded by the influence of the volume conductor
106 and, therefore, by the distance between the muscle fibres and the recording electrodes (Besomi et al.,
107 2019) Consequently, the association between recruitment threshold and motor unit action potential
108 amplitude is usually weak (Del Vecchio et al., 2017; Keenan et al., 2006), which influences the
109 associations between EMG amplitude and the strength of the neural drive to the muscle and between
110 EMG amplitude and force (Del Vecchio et al., 2017; Dideriksen et al., 2011; Fuglevand et al., 1993;
111 Keenan et al., 2006; Komi and Viitasalo, 1976). It also makes it challenging to compare EMG amplitude
112 across subjects, muscles, and time (Besomi et al., 2019).

113 Experimental results on the association between the amplitude of motor unit action potentials and motor
114 unit size, which are consistent with simulation results of EMG generation (Farina et al., 2014), indicate
115 that the amplitude of the EMG is only a crude indicator of the neural strategies used to control muscle
116 force (Enoka, 2019; Enoka and Duchateau, 2015). Figure 2, for example, shows that the amplitude of
117 the action potential waveforms for three motor units can be unrelated to the recruitment thresholds (Del
118 Vecchio et al., 2017).



119

Figure 2 Association between motor unit action potential properties and recruitment threshold. **A** Eight double-differential EMG signals of the tibialis anterior muscle during an isometric ankle-dorsiflexion contraction at up to 70% of maximal voluntary force at a rate of 5% MVC/s (thick black trace). **B** 500 ms of EMG activity for the 8 channels. **C** Motor unit action potentials were identified by EMG decomposition and spike-triggered averaging. **D-E-F** Three representative motor unit action potentials with recruitment thresholds 24.9, 6.2, and 63.9 % of maximal force. The estimated conduction velocity, root mean square amplitude, and mean power spectral frequency are also shown for each motor unit action potential. (a.u = arbitrary units, scaled amplitude of the EMG). Reproduced with permission from Del Vecchio et al. (2017).

120 Contrary to surface action potential amplitude, the estimated conduction velocity of the motor unit action
121 potentials has been shown to be associated with motor unit recruitment threshold across subjects and
122 muscles, and to be influenced by different types of training interventions (Andreassen and Arendt-
123 Nielsen, 1987; Casolo et al., 2019; Del Vecchio et al., 2017; Gazzoni et al., 2005; Martinez-Valdes et
124 al., 2018; Masuda et al., 1996; Masuda and De Luca, 1991; Zwarts and Arendt-Nielsen, 1988). The
125 conduction velocity estimated from the global EMG signal is the weighted average of the motor unit
126 conduction velocities.

127 Due to the challenges associated with interpreting the features extracted from the surface EMG (Del
128 Vecchio et al., 2017; Farina et al., 2014, 2004), intramuscular (LeFever et al., 1982; LeFever and De
129 Luca, 1982; McGill et al., 2005; Stashuk and de Bruin, 1988) and surface EMG decomposition methods
130 have been proposed (Chen et al., 2018; Chen and Zhou, 2016; De Luca et al., 2006; Farina et al., 2010;
131 Gazzoni et al., 2004; Holobar et al., 2014; Holobar and Zazula, 2007; Nawab et al., 2010; Negro et al.,
132 2016a). These methods identify individual motor unit action potentials during voluntary contractions
133 and, therefore, allow the comparison of motor unit properties across subjects and time. Moreover, the
134 same motor unit can be tracked over time (Del Vecchio and Farina, 2019; Martinez-Valdes et al., 2017)
135 and compared across sessions including before and after training interventions (A Del Vecchio et al.,
136 2019a; Martinez-Valdes et al., 2018). In contrast to global EMG analysis, the identification of the
137 discharge times of individual motor units provides a direct estimate of the neural drive to muscle.

138 As an example of the information that can be obtained when decomposing EMG signals with respect
139 to global analysis, we recently showed that the activity of motoneurons identified by EMG decomposition
140 is predictive of the maximal rate of force development (A Del Vecchio et al., 2019c). Similarly, the
141 detrimental influence of aging on force steadiness was shown to be associated with the variability in the
142 common synaptic input to motoneurons, as estimated by EMG decomposition (Feeney et al., 2018).

143 Researchers now have a new tool to observe the neural code for movement in humans directly with a
144 non-invasive approach that can be used in a variety of conditions. Nonetheless, surface EMG
145 decomposition must be used carefully and requires expertise in signal acquisition, interpretation of
146 results, and manual assessment of decomposition quality. After testing the validity of HDEMG
147 decomposition algorithms in several methodological studies (e.g., Holobar *et al.*, 2010, 2014; Marateb
148 *et al.*, 2011; Negro *et al.*, 2016a; Del Vecchio & Farina, 2019a), here we now share guidelines on how
149 to perform HDEMG decomposition by BSS accurately and how to identify motor unit properties reliably.

150

151 **2 - High-density surface EMG signals: acquisition**

152 Prior to applying the high-density electrode grids (Figure 2C), the skin should be shaved, lightly
153 abraded, and cleansed with an alcoholic solution and with abrasive paste (Piervigili et al., 2014).
154 Source separation is based on the assumption that action potential waveforms of motor units are unique
155 when recorded by the grid. Therefore, the EMG electrodes should be placed in a location that assures
156 maximal variations in shape of the action potentials of different motor units. For example, when
157 recording from fusiform muscles, it is preferable to position the EMG array with its centre approximately

158 above a primary innervation zone. In other types of muscles (e.g., pennate muscles) the BSS is less
159 sensitive to the position of the electrode array, although the electrodes will still need to be placed over
160 the muscle belly. Interestingly, these requirements for decomposition are opposite to those often
161 discussed for extracting global features from the EMG (Barbero et al., 2012).

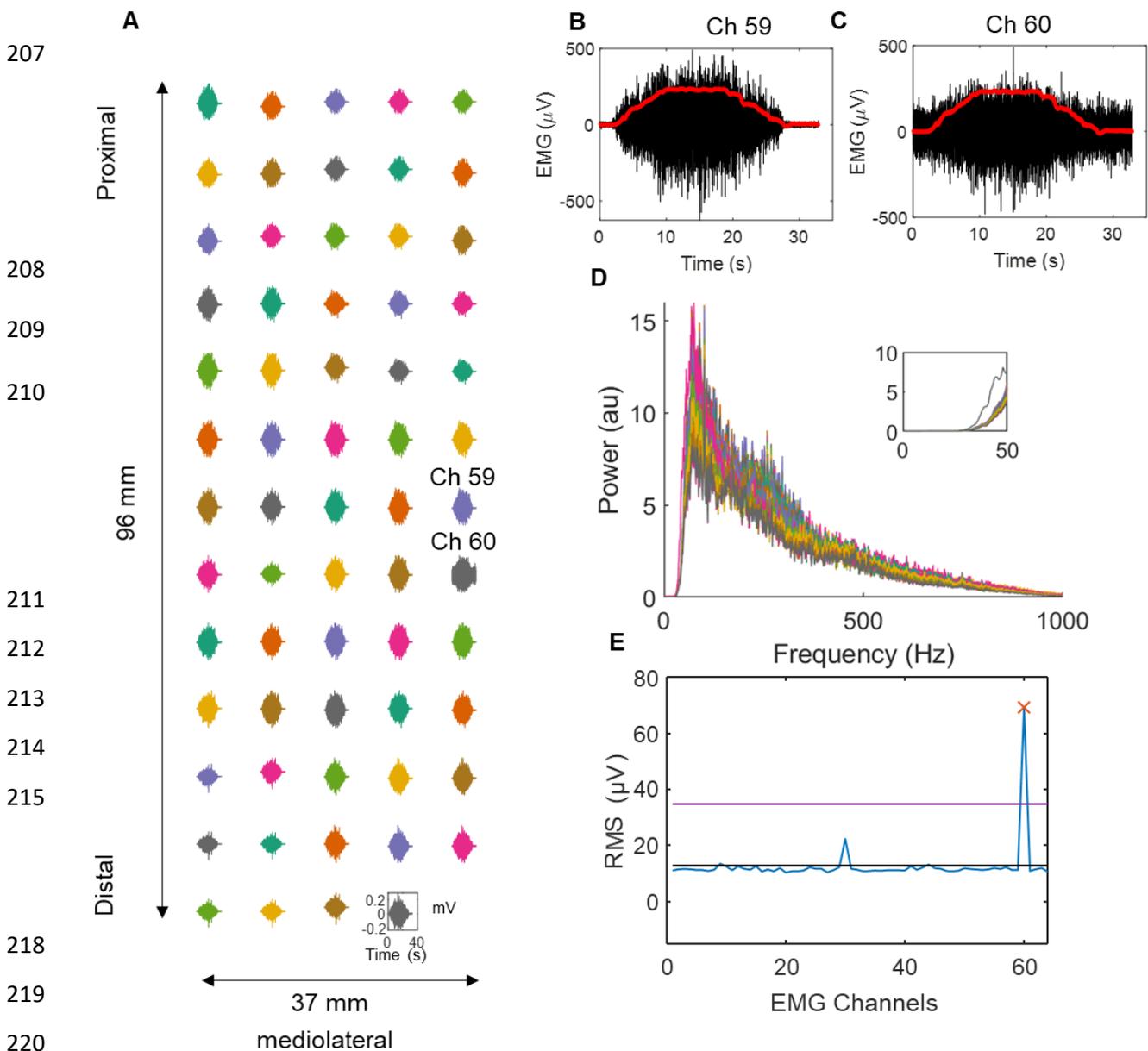
162 The interelectrode distances used for HDEMG usually range from 3-4 mm to 10 mm (Drost et al., 2001;
163 Merletti and Muceli, 2019; Zwarts and Stegeman, 2003; Del Vecchio et al., 2018; Farina et al., 2010;
164 Feeney et al., 2018; Gazzoni et al., 2005; Holobar et al., 2010; Negro et al., 2016a). It should be noted
165 that the electrode array does not need to satisfy the requirement for spatial Nyquist sampling frequency
166 for successful BSS. Whether or not the spatial Nyquist criterion needs to be met depends on how the
167 decomposition results will be used; for example, high spatial sampling may be necessary when
168 analysing the spatial distribution of the identified motor unit action potentials (Merletti and Muceli, 2019).
169 Therefore, the choice of the interelectrode distance is usually dictated by practical criteria, such as the
170 size of the muscle.

171 After the electrode grids are applied, the signals should be assessed for quality. This should preferably
172 be done by displaying the signals as monopolar recordings, as these signals are the most sensitive to
173 interference. The visual inspection of monopolar signals allows the operator to find and remove the
174 sources contaminating the recordings. The monopolar derivation is usually the most sensitive to signal
175 interferences and therefore poses the highest constraints on signal quality, whereas the bipolar
176 derivation better reveals the short-circuited EMG channels and also their spatial diversity. When the
177 main sources of EMG signals are located at greater distances, it is not uncommon to observe EMG
178 signals with high amplitudes in monopolar derivation but small amplitudes in bipolar derivation, because
179 of the filtering of common spatial signal components by the bipolar system. In such cases, the spatial
180 variation across different EMG channels is substantially reduced, effectively decreasing the number of
181 useful EMG channels and, thus, the yield of BSS techniques. Accepted baseline noise levels for
182 HDEMG signals are in the order of 10 – 40 μV RMS, but this requirement may vary with contraction
183 intensity. From empirical experience, at low EMG amplitudes signal noise should be no more than one
184 half of the power of the signal to ensure reliable decomposition (Del Vecchio et al., 2017; A Del Vecchio
185 et al., 2019a). Aside from the electrode-skin and electronic-amplification noise (signal noise), EMG
186 decomposition can only identify relatively few active motor units. The activity of the unidentified motor
187 units is an additional, and often the main, source of noise for the decomposition process.

188 The EMG signals are usually band-pass filtered between 10-20 Hz at the low end and 400-500 Hz at
189 the high end. This range keeps most of the EMG signal power while filtering out the contributions of
190 signal noise. The decomposition process will be influenced by the choice of filter settings as this may
191 alter the action potential waveforms. In general, the smaller the bandwidth, the greater the similarity of
192 action potentials for different motor units. However, a smaller bandwidth does decrease the level of
193 noise. The use of zero-phase filters, when possible, is recommended to avoid variable delays
194 introduced for action potentials of different motor units and to keep the energy of motor unit action
195 potentials concentrated in short intervals of time. Nonlinear filtering techniques change the EMG mixing
196 model and should be avoided.

197 Noise may differ across channels and it may be necessary to remove some channels from the analysis.
 198 Among the methods that can be used to identify channels with low signal-to-noise ratio, one approach
 199 is to check the quality of the signal by estimating the power spectral density for each electrode in the
 200 grid and comparing it with the baseline. Figure 3 shows an example of 63 (from a total of 64) signals
 201 with high signal-to-noise ratio and shows how channels with poor signal quality can be identified. After
 202 having identified the electrodes showing high signal-to-noise ratio, potential power line interferences
 203 can be removed with filtering techniques (e.g., notch filters). Similar considerations apply for notch filters
 204 as for the choice of the bandpass filters discussed above.

205 After the EMG signal quality check, visual confirmation, and filtering of the EMG signals, the BSS
 206 decomposition can be initiated.



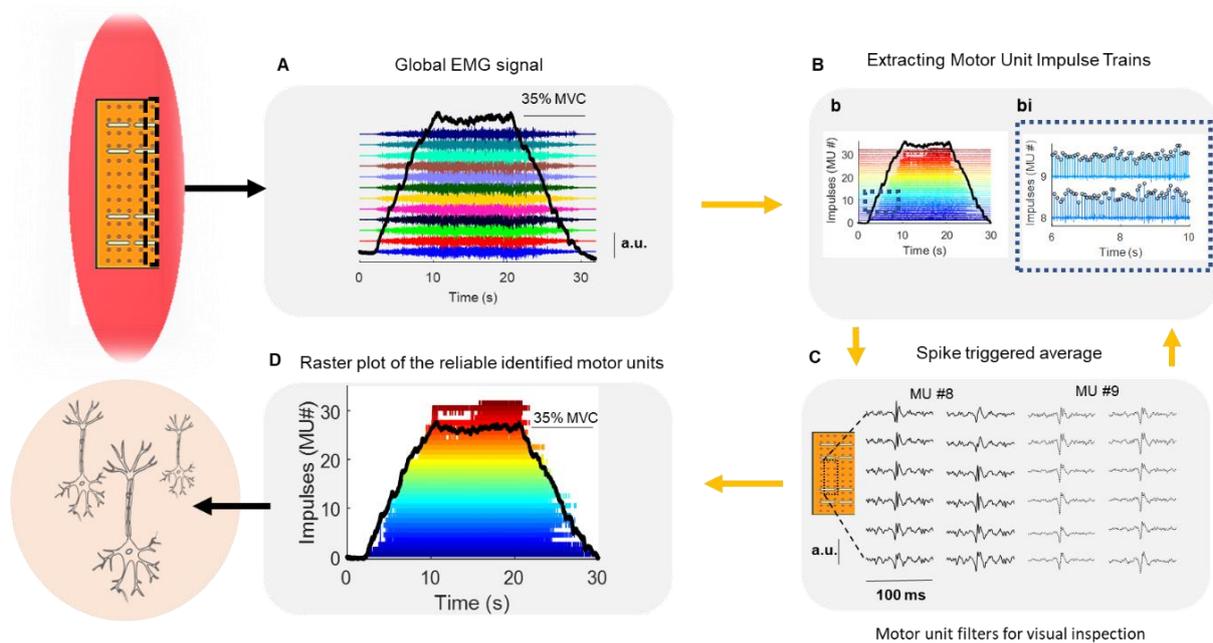
222 **Figure 3** Example of detection and visual display of channels with poor signal-to-noise ratio. **A** Sixty-
 223 four monopolar EMG signals from the tibialis anterior muscle during a contraction at 35% of maximal

224 force. Two signals (channel 59 and 60) are highlighted and displayed in **B**. The force trace is indicated
 225 by the red lines. The 60th channel shown in **C** has a high level of noise at baseline, as it can be seen
 226 from its power spectral density (**D**) and from the baseline amplitude of the EMG. **D**. The power spectral
 227 density of each of the 64 channels, as computed from the full contraction duration (~24 s). Note that
 228 one channel (in the inset, grey line) shows higher power at lower frequencies than all the others. This
 229 indicates poor signal-to-noise ratio (channel 60, au for auxiliary units) **E**. Three standard deviations from
 230 the EMG root mean square (RMS) baseline across the grid shows the outlier channel.

231 **3 - High-density surface EMG signals: decomposition**

232 High-density EMG signals are decomposed into individual motor unit action potentials with methods
 233 that have limited a-priori information. Figure 4 shows an overview of the decomposition process:
 234 acquisition of HDEMg recordings, separation of sources (motor units) via BSS, visual inspection, and
 235 raster plot of the reliably identified motor units. BSS procedures usually estimate one motor unit spike
 236 train at a time by iteratively optimizing the motor unit separation filter and applying it to the recorded
 237 EMG signals. Importantly, optimization of the motor unit filter builds on a measure of sparseness for the
 238 motor unit spike train based on a predefined time interval. Different measures of spike-train sparseness
 239 have been proposed (Chen and Zhou, 2016; Holobar and Zazula, 2007; Negro et al., 2016a), but they
 240 all require relatively long EMG recordings for the spike train to be estimated reliably. Consequently,
 241 current BSS algorithms should be applied to EMG signals that last at least 5 s.

242



243

244 **Figure 4** Example of high-density surface EMG decomposition with blind-source separation and visual
 245 inspection of the signals. **A**. Tibialis anterior monopolar EMG activity during an isometric contraction.
 246 The rate of force development was 5% MVC/s with a plateau phase of 10 s. One column of the high-
 247 density EMG grid (64 electrodes in total, with 8 mm of interelectrode distance, au arbitrary units) is
 248 shown color-coded. Specifically, the channels highlighted by the dotted black trace (over the muscle,
 249 left side of the figure) are shown in **A**. In this example, the signal-to-noise ratio is similar for all 64
 250 electrodes in the matrix. **B**. Extraction of motor unit pulse trains by blind-source separation. The 64
 251 channels are decomposed blindly, and the output of the algorithm are impulse trains with heights
 252 corresponding to the weights of the motor unit action potential shapes in the matrix obtained by the

253 independent component analysis process. The two insets in B (b and bi) show the motor unit impulses
254 extracted by blind-source separation for each motor unit. The next iteration is to check each motor unit
255 action potential visually, as shown in **bi**, and reiterate the source separation manually by triggering the
256 motor unit action potential in a fixed time window, usually in the order of 3-5 s, as shown in **bi** and **C**.
257 After visual inspection of all the motor unit spike trains, it is possible to observe the raster plot of all
258 identified motor units (**D**). The motor unit waveforms in **C** represents the motor unit waveforms
259 corresponding to 12 electrodes after spike-triggered averaging.

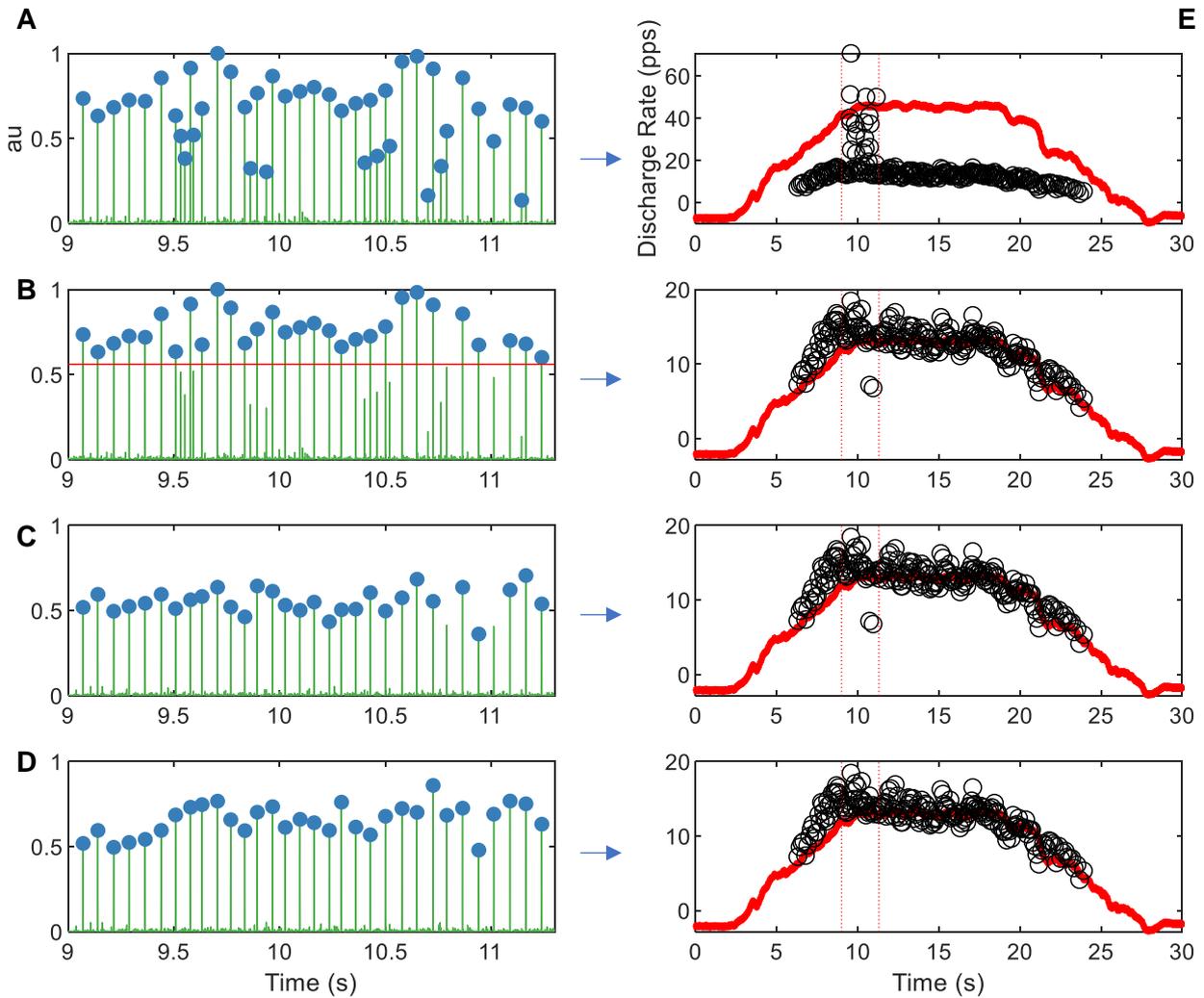
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261 **4 - High-density surface EMG signals: visual inspection of decomposition results**

262 Due to the sparseness of the motor unit spike train, BSS calculates the motor unit separation filter from
263 those time instants in the EMG recording when the motor unit was likely to be active. Once the motor
264 unit spike train is identified, the motor unit filter can be re-calculated based only on the identified motor
265 unit spikes, in an iterative way. This can be accomplished by inspecting the results of the BSS algorithm,
266 so that the operator can manually identify and remove from the calculation of the separation filter the
267 spikes of lower quality. Note that this partly manual selection is for the calculation of the separation filter
268 only and not for the output of the decomposition (see also below). This selection can often improve the
269 motor unit separation filter estimates beyond the level achieved by the BSS algorithm used fully
270 automatically. For example, when decomposing EMG signals that contain artefacts, the BSS algorithm
271 will try to optimize the motor unit filter on all the motor unit spikes, including those occurring concurrently
272 with artefacts. It is exactly this noise and the residual activity of the other motor units that is measured
273 by some signal-based metrics of accuracy, such as the pulse-to-noise ratio (Holobar et al., 2014).

274 Under assumption of nonstationary noise and artefacts, following the initial automatic decomposition it
275 is always possible to identify the portions of a spike train with low pulse-to-noise ratio and exclude those
276 portions from the motor unit filter calculation. It is not a simple matter to implement the exclusion of the
277 low-quality portions of the motor unit spike train automatically in a BSS algorithm. Indeed, the pulse-to-
278 noise ratio (and therefore the quality of spike train portions) may change due to many factors such as
279 the contraction level (increase of contraction level increases the contributions of other motor units),
280 changes of skin-electrode contact noise, instrument noise, and signal artefacts. The human operator
281 builds on the knowledge of the experimental protocol and currently can decide which signal intervals to
282 exclude from the motor unit filter optimization better than a BSS algorithm, which has no knowledge on
283 the experimental conditions.

284 After exclusion of spike-train intervals with poor signal quality, the motor unit filter should be re-
 285 calculated and re-applied to the entire EMG signal in order to re-estimate (objectively, without any
 286 manual intervention) the entire motor unit spike train. An example of this procedure is shown in Figure
 287 5.



288

Figure 5. Visual reiterations of the motor unit discharge times identified by blind source separation (A-D). The blind source separation automatically identified the discharge times of a motor unit. In A, the left plot depicts the identified spike train with many spikes below the average spike height. Automatically identified motor unit firings are depicted by blue circles. The discharge times of the motor unit (right plot) show a strong mismatch with the average motor unit discharge rate and force trace in red. In these instances, a time window of ~3 s is centred in the location of interest (red dashed lines in E). Within this location, the motor unit filter is reconstructed after removing the firings below a certain threshold, as depicted in B. The motor unit filter is then reapplied to the HDEMg signals, yielding a new spike train estimate that is depicted in C. Afterwards, two more spikes are recognized as motor unit firings and manually added in D. In this way, the motor unit filter that was identified by blind source separation is visually edited and yields a robust estimate of the motor unit firings.

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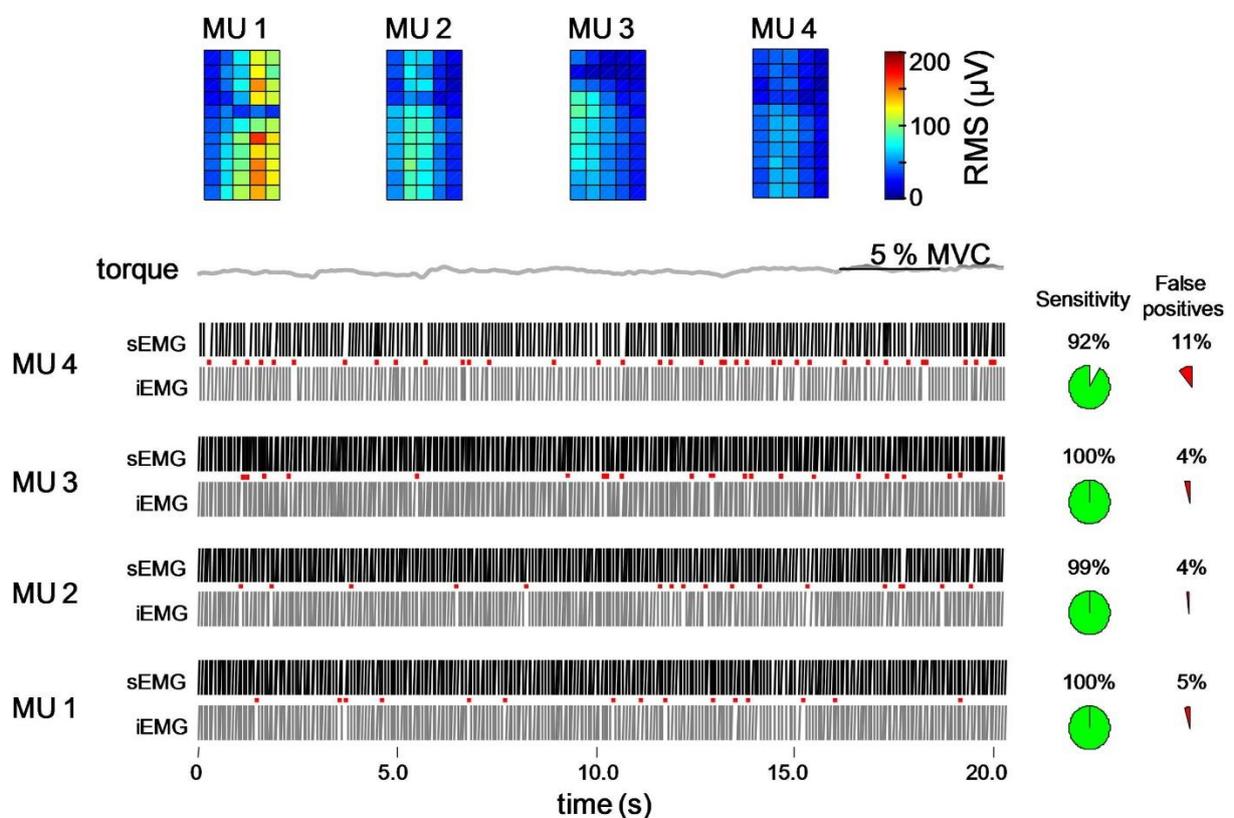
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291 Manual exclusion of spike-train intervals in manual optimization of the motor unit filter may or may not
 292 rely on the human knowledge of motor unit firing regularity. Although this additional information may be
 293 beneficial, it may also bias the selection of motor unit spikes that are taken into consideration when
 294 manually re-calculating motor unit filters. Importantly, manual spike selection should only be used for
 295 motor unit filter optimization. Afterwards, manually optimized motor unit filters should be applied to the
 296 entire EMG signal and objective spike segmentation procedures need to be followed to discriminate
 297 spikes from baseline noise in the identified motor unit spike train. Subjective selection of motor unit
 298 spikes in the final motor unit spike train (final decomposition result) should be avoided as it may lead to
 299 biasing the decomposition results.

300

301 **5 - High-density surface EMG signals: decomposition accuracy**

302 The extraction of motor unit action potentials from high-density EMG signals has been extensively
 303 validated, but mainly during isometric contractions. The current accepted approach for the validation of
 304 surface EMG decomposition is a variant of the two-source method previously introduced by Mambrito
 305 & De Luca (1984) for intramuscular EMG decomposition. With this method, intramuscular and HDEMG
 306 signals are concurrently recorded and the results of their decomposition compared (Holobar et al., 2014,
 307 2010; Hu et al., 2014; Marateb et al., 2011). Figure 6 shows a raster plot of motor units concurrently
 308 identified from surface and intramuscular signals, with the respective accuracies.



309

310 **Figure 6** Two-source method to assess accuracy. The intramuscular electromyogram (iEMG) is
 311 recorded concurrently with high-density surface EMG (sEMG) from the abductor digiti minimi muscle at
 312 5% of the maximal voluntary contraction. The sensitivity and false positive rate for discharge time

313 identification are computed by comparing the output of intramuscular and surface EMG decomposition.
314 In this example, the bottom raster plot shows the motor unit discharge times that were identified from
315 the intramuscular EMG signal decomposition and, at the top, those identified by blind-source separation
316 of the HDEMG signals. The top plot in each pair shows the distribution of amplitude of the motor unit
317 action potential waveforms over the high-density EMG grid. The sensitivity of the high-density EMG
318 (right) represents the number of discharge times that are concurrently identified by the surface and
319 intramuscular EMG decomposition divided by the total number of discharges identified from the
320 intramuscular EMG. The percent of false positives corresponds to the number of discharges identified
321 by the surface but not by the intramuscular EMG decomposition, divided by the total number of
322 discharges identified from the intramuscular EMG. MU, motor unit. Reproduced with permission from
323 Farina et al. 2010.

324 Indirect methods of validating surface EMG decomposition use shape analysis of two-dimensional
325 motor unit action potentials identified by BSS (Del Vecchio and Farina, 2019; Hu et al., 2015, 2013a;
326 Thompson et al., 2018) and simulation approaches (Farina et al., 2010; Holobar and Zazula, 2007). For
327 example, accuracy measures, such as pulse-to-noise ratio (Holobar et al., 2014), the silhouette
328 measure (Negro et al., 2016a), or the motor unit action potential similarity after spike-triggered
329 averaging (see below) across the contractions with or without injection of gaussian noise (Del Vecchio
330 and Farina, 2019; Thompson et al., 2018), can be used to infer the accuracy of motor unit spike
331 identification. All of these measures are asymptotic and increase their precision with the number of
332 identified spikes in the spike train. Therefore, they should not be used to assess the accuracy of spike
333 trains with less than 30 spikes (Holobar et al., 2014) or to assess the accuracy of each individual spike
334 in a spike train.

335 Some information about accuracy can be obtained from the spike-triggered averaging of EMG signals
336 (Del Vecchio and Farina, 2019; Hu et al., 2015, 2013b; Thompson et al., 2018). With this approach, the
337 discharge times of identified motor units are used as triggers for an average that is accumulated over
338 time intervals of 25 to 100 ms. Due to the possibility that motor unit action potential shapes change
339 during an isometric contraction, a relatively small number of motor unit discharge times should be used
340 in the spike-triggered average. We empirically observed that 3 s to 5 s (~30-100 spikes) are sufficient
341 to robustly extract motor action potential waveforms during sustained and fast isometric contractions (A
342 Del Vecchio et al., 2019c). Also, the reliability of an identified motor unit pool can be examined by
343 identifying the same motor units across days (see *Motor unit Tracking*).

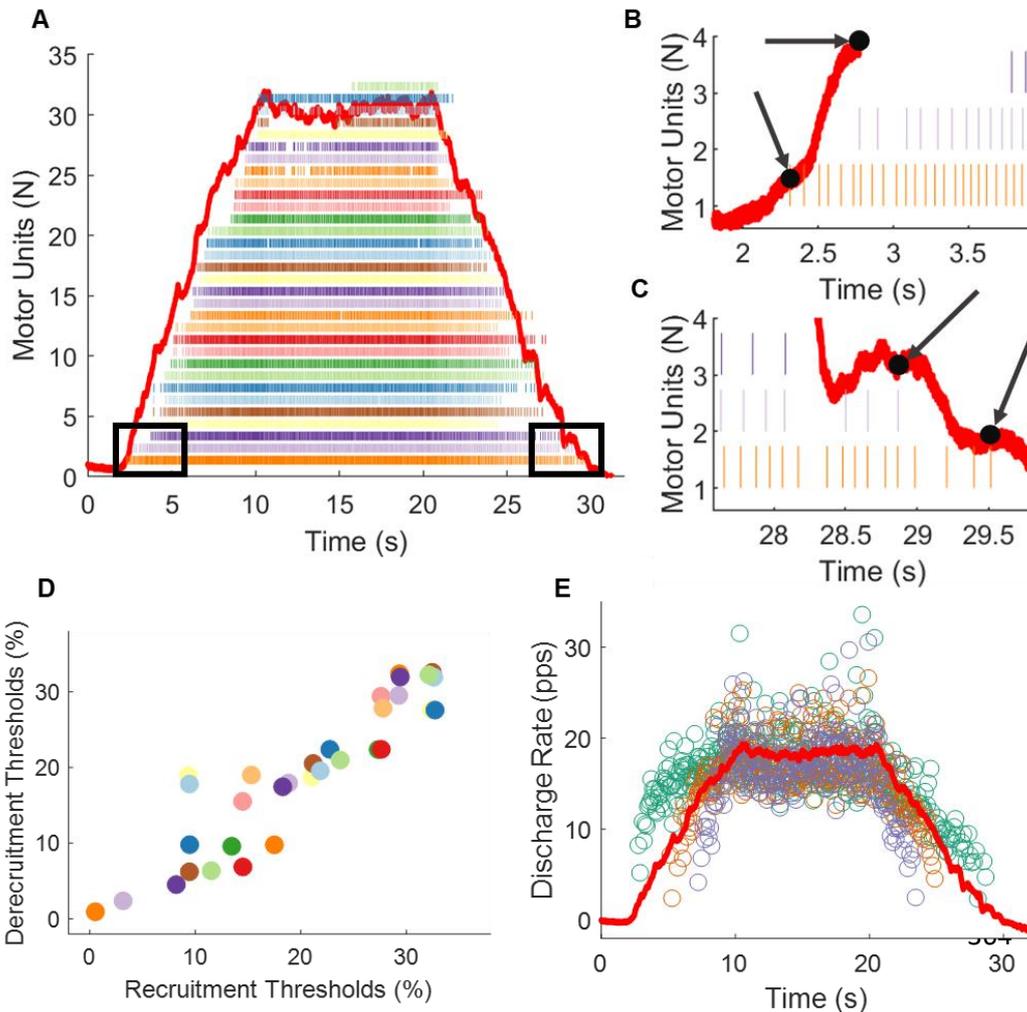
344 **6 – Assessment of motor unit properties**

345 From the discharge times of identified motor units, the characteristics of the engaged motor units can
346 be identified. One key characteristic is the recruitment threshold, which corresponds to the force when
347 the first motor unit action potential occurs. The ensuing force that is produced by the muscle fibres
348 innervated by the motoneuron (the muscle unit) occurs with a delay that depends on the axonal
349 conduction velocity and on the properties (active and passive) of the muscle fibres. To obtain reliable
350 estimates of recruitment and derecruitment thresholds, subjects must practice performing slow linear
351 ramp contractions.

352 A common approach used to estimate recruitment threshold and to measure the discharge
353 characteristics of motor units is the performance of trapezoidal force trajectories with controlled rates
354 of increase and decrease in force (5-20% MVC/s) to a moderate plateau force (35-70% of maximal

355 force). Given the current limitations in HDEMG decomposition analysis in uniformly sampling motor
 356 units across recruitment thresholds, it is best practice to use a range of target forces (30 to 70-90% of
 357 maximum force) depending on the test muscle and type of contraction.

358



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365

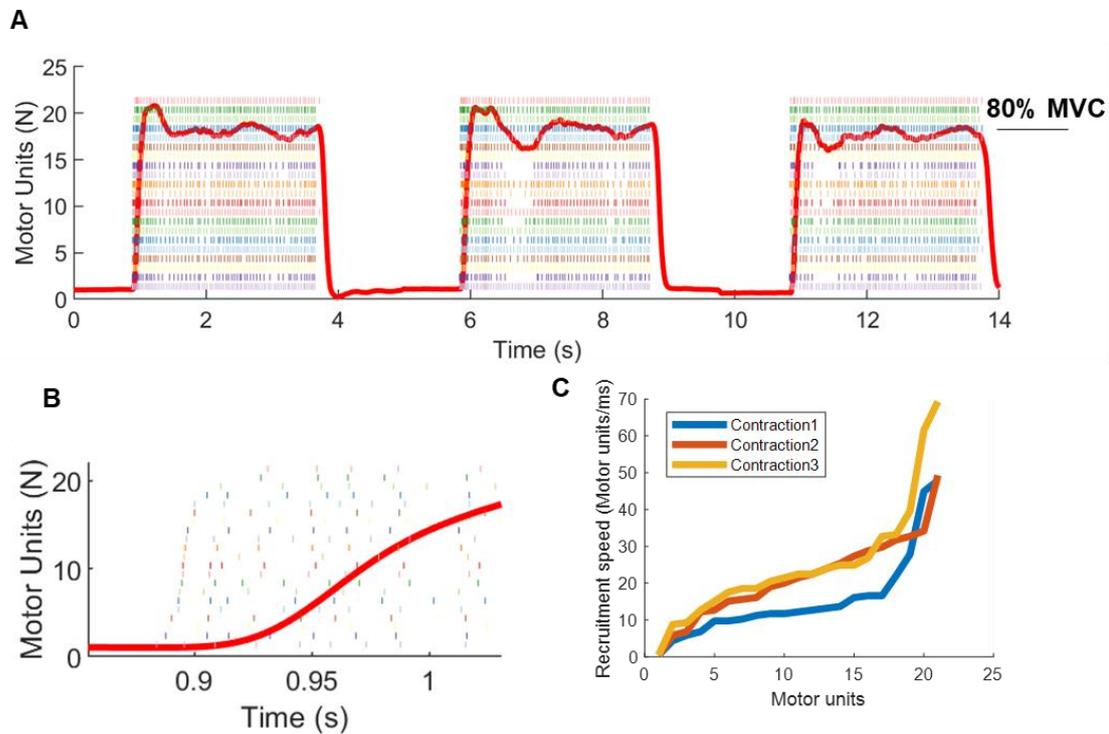
Figure 7. Motor unit properties: recruitment thresholds and discharge rates during an isometric trapezoidal contraction (plateau 35% of maximum). **A.** Raster plot of 32 identified motor units during an isometric contraction of the tibialis anterior muscle. The black boxes highlight the recruitment (**B**) and derecruitment (**C**) phases for three motor units with the specific force indicated with a black arrow. **D.** The association between recruitment threshold and derecruitment thresholds. **E.** The instantaneous discharge rate (the inverse of the interspike interval) as a function of time for the three representative motor units. The force trace is in red colour.

366 Figure 7 shows the raster plot of discharge times of 32 motor units during a trapezoidal contraction up
 367 to 35% of the maximum force of the tibialis anterior muscle. The recruitment and derecruitment
 368 thresholds are highlighted in Figure 7A-C. Once the interspike intervals are known, the motor unit
 369 discharge rates can be determined during the recruitment, plateau, and derecruitment phases, as
 370 shown in Figure 7E for three representative motor units.

371

372 Estimates of motor unit recruitment threshold during fast contractions can provide a measure of the
373 speed of recruitment (Fig. 8).

374



375

Figure 8 Motor unit recruitment during fast contractions. **A.** Three rapid isometric contractions of the tibialis anterior muscle. The plateau of the force is ~80% of maximum (red-trace). **B.** One representative contraction during the first 100 ms. The discharge times of identified motor units are shown as tick marks. **C.** Motor unit recruitment speed represents the time interval between the first discharge times of consecutive motor units (B). This value is calculated by taking the average of the derivative of the first discharge times of the motor unit pool (sorted by recruitment order). The x-axis label in C is sorted with respect to the motor units showing the smallest time interval. In this example, all the identified motor units were recruited in a small time window (<50 motor units/ms).

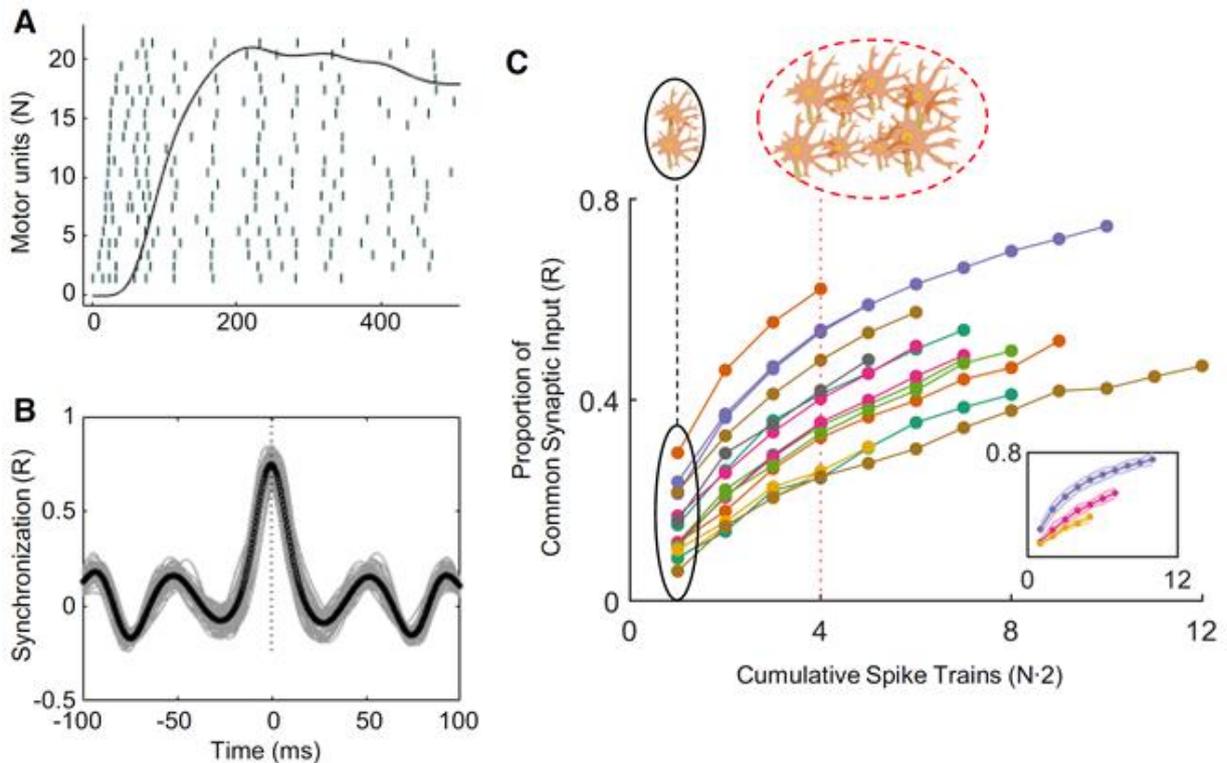
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377

378 From the discharge times of the motor units, it is possible to extract characteristics of the common
379 synaptic input to the motoneuron pool. These measures can be obtained in both the time and frequency
380 domain. One time domain approach is to compute the cross-correlogram between motor unit discharges
381 (Nordstrom et al., 1992). This method, originally proposed for pairs of motor units, can be extended to
382 populations of motoneurons by summing the motor unit spike trains (binary signal) to generate the
383 cumulative spike trains (CST). The cross-correlogram is then performed between the CSTs of randomly
384 permuted groups of motor units (Figure 9). The rate of increase in correlation between CSTs when the
385 number of motor units used for the CST calculation increases is associated to the relative proportion of
386 common input with respect to independent input. This proportion can also be quantified by non-linear
387 fitting of the peak correlation values in the frequency domain (Negro et al., 2016b), or in the time domain.
388 These estimates provide information on a bandwidth of motor neuron input that depends on the filtering

389 of the CSTs. For example, by using a Hanning window of 25-ms (A Del Vecchio et al., 2019b), the
390 analysed bandwidth is approximately 40 Hz.

391



392

393 **Figure 9.** Calculation of the proportion of common input from the cross-correlogram. **A** Raster plot of
394 21 motor units during a fast contraction. **B** The cross-correlogram was obtained in 100-ms time windows
395 with a 5-ms overlap. Each shaded grey line corresponds to a time window. For each calculation, the
396 motor unit spike trains were divided in two equally sized groups and convolved with a 25-ms Hanning
397 window. **C** Individual subject data (color-coded) for the strength of correlation between CSTs as a
398 function of the number of motor units used for each CST. The inset in C shows three representative
399 subjects with standard deviation across three rapid contractions (shaded colour). Modified from Del
400 Vecchio et al. 2019 with permission.

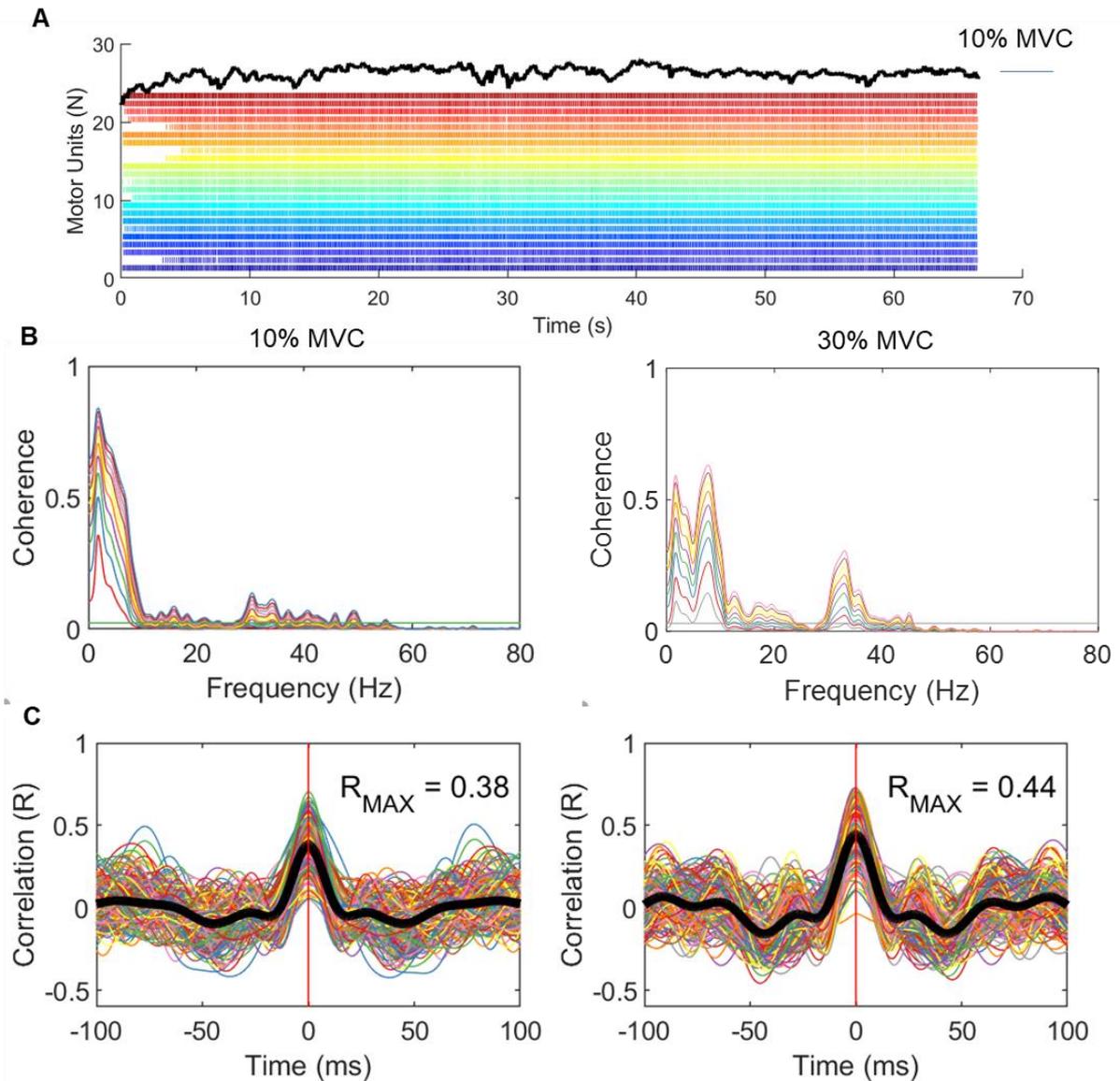
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402 It is further possible to estimate the frequency bands of the input shared by motoneurons (in the
403 assumption of an approximate linear input-output relation for the motoneuron population) during steady
404 contractions that last at least 20-30 s with the use of coherence functions. The coherence function
405 provides a cross-correlation analysis in the frequency domain. Figure 10 shows the procedure for this
406 calculation. Only motor unit spike trains without silent periods (>500 ms) should be included in this
407 analysis. The coherence function can be also applied to study the shared synaptic inputs within the
408 discharge timings of the populations of motoneurons. For this purpose, the coherence function is
409 applied to groups of motor units that belong to different muscles, as described previously (Del Vecchio
410 et al., 2019; Laine et al., 2015).

411 Another information that can be extracted from the motor unit discharge times is an estimate of the
412 strength of persistent inward currents (PICs) to motoneurons from the discharge rates at recruitment

413 and derecruitment (Gorassini et al., 2002; Heckman et al., 2005). This measure reflects
414 neuromodulatory input received by motoneurons and has been recently performed from HDEMG
415 signal decomposition (Hassan et al., 2020).

416 From the shape of the motor unit action potential waveform it is also possible to extract other
417 physiological information. This information includes analysis of the motor unit waveform, such as
418 amplitude and conduction velocity (see paragraph 1-2 and Figure 2). The analysis of the motor unit
419 discharge times and action potential waveforms enables the analysis of neural and peripheral properties
420 concurrently. For example, the strong association between motor unit recruitment thresholds and motor
421 unit conduction velocities that have been reported for different muscles (Andreassen and Arendt-
422 Nielsen, 1987; Del Vecchio et al., 2018; Hogrel, 2003; Masuda and De Luca, 1991) is consistent with
423 the size principle. Although in some cases this information has been used to infer the type of recruited
424 (fast-twitch or slow twitch) muscle fibres, in-vivo studies show that there is no clustering of conduction
425 velocity values but rather a continuous distribution of conduction velocities and estimated muscle fibre
426 diameters (Del Vecchio et al., 2018; Troni et al., 1983), which agrees with basic physiological studies
427 (see Enoka et al., 2015 for review).



428

429 **Figure 10 A.** Raster plot of motor unit discharge times from the tibialis anterior muscle during a steady contraction
 430 at 10% of maximal voluntary force. **B.** The coherence function was calculated between increasing numbers of
 431 motor units (color coded) for a contraction at 10 and one at 30% of the maximum. Note that the increase in the
 432 number of motor units corresponds to an increase in the frequency coupling in all frequency bands above
 433 significance (the significant level was computed as the maximal value of coherence above 100 Hz). **C.** The
 434 correlation in the time domain obtained by the cross-correlogram in 100-ms windows.

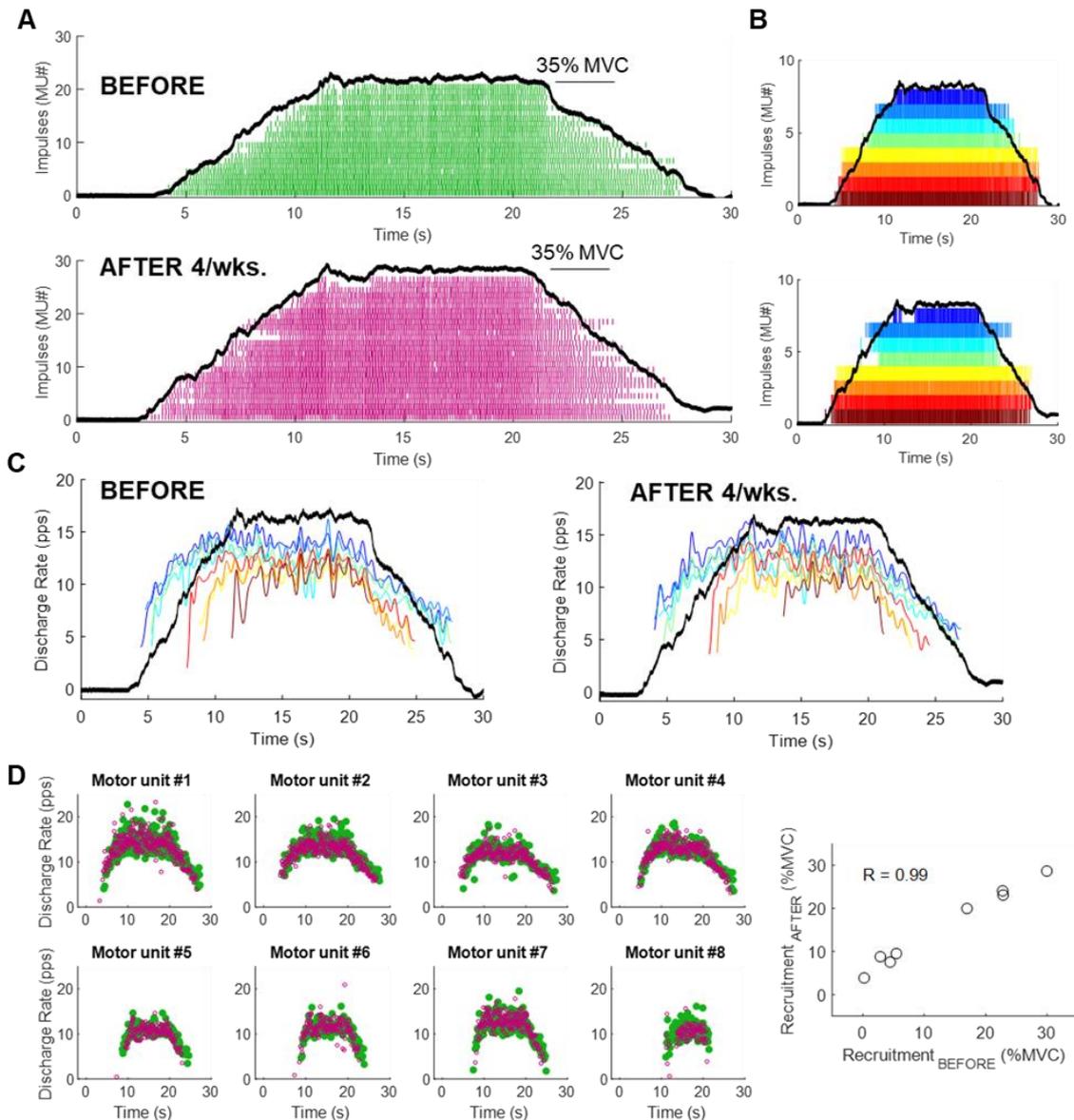
435 7 - Motor unit tracking

436 The comparison of motor unit properties during longitudinal studies, such as after a rehabilitation
 437 intervention, is only possible if the same motor unit can be identified before and after the intervention.
 438 One advantage of HDEMG recordings is that they usually provide high spatial resolution of the motor
 439 unit action potentials. There is a small likelihood that two motor units would show exactly the same
 440 action potential waveforms in all channels for a large electrode grid (Farina et al., 2008), which means
 441 that motor units can be tracked over multiple sessions when the grid is placed in a similar location in
 442 each session (Del Vecchio and Farina, 2019; Martinez-Valdes et al., 2017).

443 Figure 11 shows an example of motor unit tracking during an isometric contraction with the ankle
444 dorsiflexors. In this example, only some of the identified motor units could be tracked across
445 experimental sessions. In our experience, approximately 30% of the identified units can be tracked over
446 weeks in the tibialis anterior muscle. Motor unit tracking requires consistent placement of the high-
447 density grid and the establishment of a threshold in cross-correlation between motor unit action
448 potentials. When multiple motor units have a high cross-correlation between each other, which happens
449 occasionally, these motor units should be removed from the tracking (see Figure 3 in (A Del Vecchio et
450 al., 2019a)).

451 The motor unit tracking technique can also be used to test decomposition accuracy. Figure 11 shows
452 two pools of motor units identified during two experimental sessions four weeks apart during isometric
453 trapezoidal contractions of the tibialis anterior muscle. The action potential waveforms of these motor
454 units were used to track the motor units over time (Fig 11B). Once the motor units are tracked, it is
455 possible to test the accuracy and reliability of the discharge characteristics of the motor units, such as
456 discharge rate and recruitment thresholds. Figure 11C-D shows that the tracked motor units exhibited
457 strong reliability in discharge rate and recruitment threshold. It is important to note that the tracking
458 technique uses the 2D action potential waveforms, therefore the physiological properties of the motor
459 units are not taken into account during tracking. It is unlikely that a pool of motor units shows the same
460 discharge characteristics across days (as demonstrated by comparing random motor units across
461 sessions; Martinez-Valdes *et al.*, 2017) if the motor unit tracking and the initial decomposition were not
462 performed correctly (Figure 11).

463



464

465 **Figure 11. Motor unit tracking.** One method that can be used to assess decomposition accuracy is to
 466 track the same motor unit across time. **A.** Two isometric contractions were performed by the same
 467 subject with 4 weeks between contractions. The number of identified motor units (green vs purple)
 468 differs in the two contractions. **B.** The same motor unit is tracked across time by matching the action
 469 potential waveforms. Eight motor units that were successfully tracked in the two contractions. Note the
 470 similar smoothed discharge rate (**C**), the instantaneous discharge rate (**D**), and the recruitment
 471 thresholds (the tracked motor units are color-coded). The scatter plot in **D** shows a strong correlation
 472 ($P < 0.0001$) for recruitment thresholds before and after four weeks, thereby underscoring the accuracy
 473 in decomposition.

474

475 **8 – Influencing factors in motor unit decomposition: the influence of muscle, volume conductor,**
 476 **and target force**

477 There are three major limitations that limit the applicability of surface EMG decomposition in some
 478 experimental conditions. The output of the decomposition is sensitive to the muscles investigated, the
 479 volume conductor properties of the specific subject, and the contraction intensity. These limitations are
 480 due to anatomical constraints (the volume conductor between the recording electrodes and the muscle

481 units) and superimposition of the muscle fibre action potentials. With increasing contraction forces, the
482 number of motor units that can be identified by decomposition usually decreases. For example, in the
483 tibialis anterior muscle, which is a reliable muscle for decomposition (Del Vecchio and Farina, 2019;
484 Negro et al., 2016a), we observed a 30% reduction in the number of motor units that can be identified
485 when the target force increases from 35% to 70% of maximum force. Similarly, there is a trend for a
486 lower number of identified motor units for subjects with a thicker subcutaneous layer. These trends are
487 due to the decrease in discriminative information in the action potential waveforms of different motor
488 units when the signal bandwidth is reduced by the volume conductor (Farina et al., 2008). There are
489 still not sufficient data to reach a conclusion on the number of identified motor units between sexes.

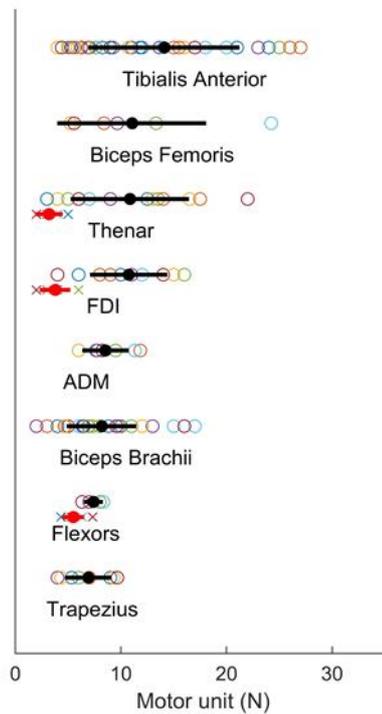
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491 Figure 12 shows the number of identified motor unit across muscles, sex, and contraction intensity for
492 a relatively large dataset of decomposed signals collected in the laboratories of the Authors. Some
493 muscles yield higher numbers of motor units irrespective of the contraction intensity (such as tibialis
494 anterior, see Fig 12). We have noted that muscles with fibres that are not all parallel to each other
495 usually yield a greater number of identified motor units by decomposition. This is likely due to the larger
496 discriminative information between motor unit action potentials of different units in muscles with varying
497 anatomy.

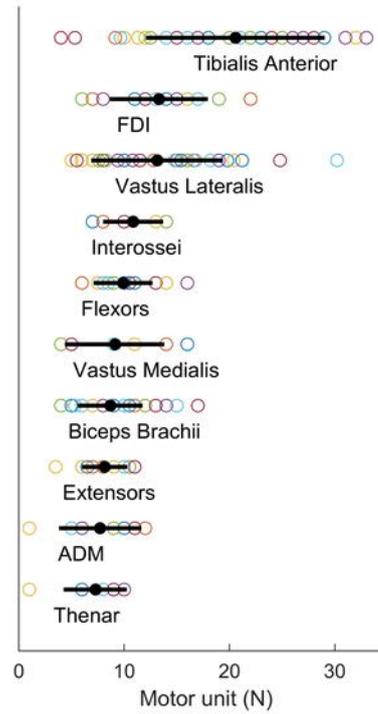
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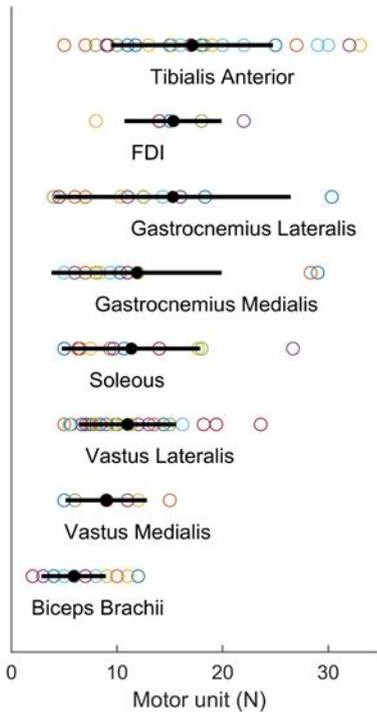
Contraction intensity $\leq 20\%$ MVC



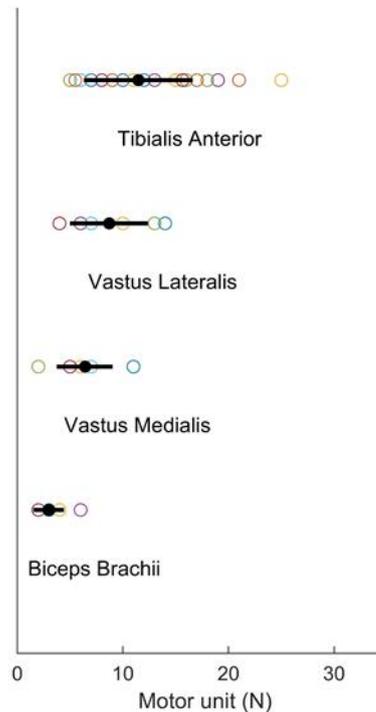
$> 20\% \leq 40\%$ MVC



$> 40\% \leq 70\%$ MVC



$> 70\%$ MVC



500

501 **Figure 12** Number of identified motor units across muscles and contraction intensities. The average
 502 number (black dots) and standard deviation (black line) across subjects (color-coded), muscles, and
 503 contraction intensities. The red line and x dots for three muscles at $\leq 20\%$ MVC indicate data for women.
 504 Note that some muscles yield a greater number of identified motor units irrespective of the contraction
 505 intensity. The data shown here are from recordings from the laboratories of the Authors. All the motor

506 units reported in this graph were decomposed with a pulse-to-noise ratio >30dB and were visually
507 inspected as described in this tutorial.

508

509 **Conclusions**

510 In this tutorial we present guidelines for the extraction of motor unit discharge characteristics from
511 HDEMG signals. This article provides an overview of the rationale for decomposition of EMG signals
512 and then describes the step-to-step guidelines on how to perform an accurate decomposition,
513 interpretation, and analysis of motor unit discharge times. Although the advances in software and
514 hardware technology obtained in the last two decades potentially allows any experimenter to record
515 motor units, there are many challenges that need to be overcome and many limitations that need to be
516 solved through thorough experimental testing and the development of additional software and hardware. We
517 emphasise that the output of decomposition must be inspected carefully. Moreover, progress is still
518 needed to improve surface EMG decomposition to reduce the limitations associated with variability of
519 performance due to muscle and subject anatomy.

520 **Acknowledgement**

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524

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