

1 **Larger and denser: an optimal design for surface grids of EMG**
2 **electrodes to identify greater and more representative samples of**
3 **motor units**

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16

17 **Abstract**

18 The spinal motor neurons are the only neural cells whose individual activity can be non-invasively
19 identified. This is usually done using grids of surface electromyographic (EMG) electrodes and source
20 separation algorithms; an approach called EMG decomposition. In this study, we combined
21 computational and experimental analyses to assess how the design parameters of grids of electrodes
22 influence the number and the properties of the identified motor units. We first computed the percentage
23 of motor units that could be theoretically discriminated within a pool of 200 simulated motor units when
24 decomposing EMG signals recorded with grids of various sizes and interelectrode distances (IED).
25 Increasing the density, the number of electrodes, and the size of the grids, increased the number of motor
26 units that our decomposition algorithm could theoretically discriminate, i.e., up to 83.5% of the
27 simulated pool (range across conditions: 30.5-83.5%). We then identified motor units from experimental
28 EMG signals recorded in six participants with grids of various sizes (range: 2-36 cm²) and IED (range:
29 4-16 mm). The configuration with the largest number of electrodes and the shortest IED maximized the
30 number of identified motor units (56±14; range: 39-79) and the percentage of early recruited motor units
31 within these samples (29±14%). Finally, the number of identified motor units further increased with a
32 prototyped grid of 256 electrodes and an IED of 2 mm. Taken together, our results showed that larger
33 and denser surface grids of electrodes allow to identify a more representative pool of motor units than
34 currently reported in experimental studies.

35

36 **Significance statement**

37 The application of source separation methods to multi-channel EMG signals recorded with grids of
38 electrodes enables users to accurately identify the activity of individual motor units. However, the design
39 parameters of these grids have never been discussed. They are usually arbitrarily fixed, often based on
40 commercial availability. Here, we showed that using larger and denser grids of electrodes than
41 conventionally proposed drastically increases the number of identified motor units. The samples of
42 identified units are more balanced between early- and late-recruited motor units. Thus, these grids
43 provide a more representative sampling of the active motor unit population. Gathering large datasets of
44 motor units using large and dense grids will impact the study of motor control, neuromuscular
45 modelling, and human-machine interfacing.

46

47 **Introduction**

48 Decoding the neural control of natural behaviors relies on the identification of the discharge activity of
49 individual neural cells. Classically, arrays of electrodes are implanted close to the cells to record their
50 electrical activity. The application of algorithms that separate the simultaneous and overlapping activity
51 of these cells has enabled researchers to study neural processes in multiple areas of the brain (Stringer
52 et al., 2019), such as in the motor or the sensorimotor areas (Churchland and Shenoy, 2007; Gallego et
53 al., 2020). At the periphery of the nervous system, it is also possible to record the activity of individual
54 motor neurons innervating muscle fibers (Duchateau and Enoka, 2011; Heckman and Enoka, 2012;
55 Farina et al., 2016). The motor unit, i.e., a motor neuron and the muscle fibers it innervates, acts as an
56 amplifier of the neural activity, as one action potential propagating along a motor neuron's axon
57 generates an action potential in each of the innervated muscle fibers. The discharge activity of motor
58 units can be identified by decomposing surface electromyographic (EMG) signals into trains of motor
59 unit action potentials (MUAPs) using, e.g., blind-source separation algorithms (Holobar and Farina,
60 2014; Farina and Holobar, 2016). The multiple observations for source separation are obtained by
61 recording EMG signals with grids of electrodes. This approach usually allows for the reliable analysis
62 of 5 to 40 concurrently active motor units (Del Vecchio et al., 2017; Del Vecchio et al., 2020; Hug et
63 al., 2021a).

64 While the design of intracortical (e.g., (Jun et al., 2017; Steinmetz et al., 2018)) and intramuscular (e.g.,
65 (Muceli et al., 2015; Muceli et al., 2022)) electrodes arrays has scaled up over the years to record larger
66 samples of neural cells, the configuration of grids of surface EMG electrodes has not systematically
67 evolved. Most researchers currently use grids with 64 electrodes arranged in 13×5 or 8×8 montages,
68 the interelectrode distance (IED) between adjacent electrodes (e.g., 4 mm, 8 mm, or 10 mm) being
69 dictated by the size of the muscle to cover. Yet, optimizing these parameters, i.e., grid size and IED,
70 may influence the performance of EMG decomposition. Currently, there are no recommendations on
71 optimal designs for grids of electrodes.

72 Source separation algorithms are based on the necessary condition that identifiable motor units have a
73 unique representation of their action potentials across the multi-channel EMG signals (Farina et al.,
74 2008; Holobar and Farina, 2014; Farina and Holobar, 2016). This implies that the three-dimensional
75 waveform of a MUAP (one time dimension and two spatial dimensions) is unique within the pool of
76 active motor units detected by the grid of electrodes. In practice, the identified motor units are those that
77 innervate larger numbers of muscle fibers, as their action potentials tend to have the largest energy.
78 Conversely, low-threshold motor units usually remain hidden since their energy is close to the baseline
79 noise. Increasing the density of electrodes would increase the spatial sampling of EMG signals (Farina
80 and Holobar, 2016), which in turn should improve the discrimination of MUAPs, allowing the
81 identification of a higher number of motor units. Additionally, increasing the density of electrodes may

82 reveal the hidden low-threshold motor units by sampling their action potentials across a higher number
83 of electrodes, leading to a better compensation of the additive noise in the mixture model of the EMG
84 signal (Farina and Holobar, 2016).

85 In this study, we combined computational and laboratory experiments to identify the optimal design
86 parameters of grids of surface electrodes with the aim to maximize the number of identified motor units.
87 We first simulated a pool of 200 motor units and the associated EMG signals recorded from grids of
88 electrodes of various sizes and densities. These simulations showed that the greater the size and the
89 density of the grid, the higher the percentage of theoretically identifiable motor units and the relative
90 ratio of theoretically identifiable deep units. We confirmed these theoretical results with experimental
91 signals recorded with a grid of 256 electrodes with a 4-mm IED that was down-sampled in the space
92 domain to obtain six grid configurations (surface range: 2-36 cm² and IED range: 4-16 mm). Finally,
93 we prototyped a new grid of 256 electrodes with a 2-mm IED and demonstrated that the number of
94 identified motor units further increased with 2-mm IED. The entire dataset (raw and processed data) and
95 codes are available at <https://figshare.com/s/f4a94d9bdf470bf10f8>.

96 **Methods**

97 **Computational study**

98 A pool of 200 motor units was simulated to test whether increasing the density and the size of surface
99 grids of electrodes would impact the number of theoretically identifiable motor units. The simulations
100 were based on an anatomical model entailing a cylindrical muscle volume with parallel fibers (Farina et
101 al., 2008; Konstantin et al., 2020), in which subcutaneous and skin layers separate the muscle from the
102 surface electrodes. Specifically, we set the radius of the muscle to 25.4 mm and the thicknesses of the
103 subcutaneous and skin layers to 5 mm and 1 mm, respectively. The centers of the motor units were
104 distributed within the cross section of the muscle using a farthest point sampling technique. The farthest
105 point sampling filled the cross-section by iteratively adding centers points that were maximally distant
106 from all the previously generated motor unit centers, resulting in a random and even distribution of the
107 motor unit territories within the muscle. The number of fibers innervated by each motor neuron followed
108 an exponential distribution, ranging from 15 to 1500. The fibers of the same motor unit were positioned
109 around the center of the motor unit within a radius of 0.2 to 9.8 mm, and a density of 20 fibers/mm².
110 Because motor unit territories were intermingled, the density of fibers in the muscle reached 200
111 fibers/mm². The MUAPs were detected by circular surface electrodes with a diameter of 1 mm. The
112 simulated grids were centered over the muscle in the transverse direction, with a size ranging from 14.4
113 to 36 cm², and an IED ranging from 2 to 36 mm.

114

115 **Laboratory study**

116 Participants

117 Six healthy participants (all males; age: 26 ± 4 yr; height: 174 ± 7 cm; body weight: 66 ± 15 kg)
118 volunteered to participate in the first experimental session of the study. They had no history of lower
119 limb injury or pain during the months preceding the experiments. One of these individuals (age: 26 yr;
120 height: 168 cm; bodyweight: 51 kg) participated in a second experimental session to test the prototyped
121 grid with an IED of 2 mm. The Ethics Committee at Imperial College London reviewed and approved
122 all procedures and protocols (no. 18IC4685). All participants provided their written informed consent
123 before the beginning of the experiment.

124

125 Experimental tasks

126 The two experimental sessions consisted of a series of isometric ankle dorsiflexions performed at 30%
127 and 50% of the maximal voluntary torque (MVC) during which we recorded high density
128 electromyographic (HD-EMG) signals over the Tibialis Anterior muscle (TA). The participant sat on a
129 massage table with the hips flexed at 30°, 0° being the hip neutral position, and their knees fully
130 extended. We fixed the foot of the dominant leg (right in all participants) onto the pedal of a commercial

131 dynamometer (OT Bioelettronica, Turin, Italy) positioned at 30° in the plantarflexion direction, 0° being
132 the foot perpendicular to the shank. The thigh was fixed to the massage table with an inextensible 3-cm-
133 wide Velcro strap. The foot was fixed to the pedal with inextensible straps positioned around the
134 proximal phalanx, metatarsal and cuneiform. Force signals were recorded with a load cell (CCT
135 Transducer s.a.s, Turin, Italy) connected in-series to the pedal using the same acquisition system as for
136 the HD-EMG recordings (EMG-Quattrocento; OT Bioelettronica). The dynamometer was positioned
137 accordingly to the participant's lower limb length and secured to the massage table to avoid any motion
138 during the contractions.

139 All experiments began with a warm-up, consisting of brief and sustained ankle dorsiflexion performed
140 at 50% to 80% of the participant's subjective MVC. During the warm-up, all participants learnt to
141 produce isometric ankle dorsiflexion without co-contracting the other muscles crossing the hip and knee
142 joints. At the same time, we iteratively adjusted the tightening and the position of the straps to maximize
143 the comfort of the participant. Then, each participant performed two 3-to-5 s MVC with 120 s of rest in
144 between. The peak force value was calculated using a 250-ms moving average window, and then used
145 to set the target level during the submaximal contractions. After 120 s of rest, each participant performed
146 two trapezoidal contractions at 30% and 50% MVC with 120 s of rest in between, consisting of linear
147 ramps up and down performed at 5%/s and a plateau maintained for 20 s and 15 s at 30% and 50%
148 MVC, respectively. The order of the contractions was randomized. One participant (S2) did not perform
149 the contractions at 50% MVC.

150

151 High-density electromyography

152 In the first experimental session, four adhesive grids of 64 electrodes (13 x 5 with a missing electrode
153 in a corner; gold coated; 1 mm diameter; 4 mm IED; OT Bioelettronica) were placed over the belly of
154 the TA. The grids were carefully positioned side-to-side with a 4-mm-distance between the electrodes
155 at the edges of adjacent grids (Figure 1A). The 256 electrodes were centered to the muscle belly and
156 laid within the muscle perimeter identified through palpation. The skin was shaved, abraded and cleansed
157 with 70% ethyl alcohol. Electrode-to-skin contact was maintained with a bi-adhesive perforated foam
158 filled with conductive paste. The grids were wrapped with tape and elastic bands to secure the contact
159 with the skin. The four pre-amplifiers were connected in-series with stackable cables to a wet reference
160 band placed above the medial malleolus of the same leg. HD-EMG signals were recorded in monopolar
161 derivation with a sampling frequency of 2,048 Hz, amplified (x150), band-pass filtered (10–500 Hz),
162 and digitized using a 400 channels acquisition system with a 16-bit resolution (EMG-Quattrocento; OT
163 Bioelettronica).

164 In the second experimental session, one ultra-dense prototyped grid of 256 electrodes (Figure 1H; 26 x
165 10 with a missing electrode in each corner; gold coated; 1 mm diameter; 9 cm² area; 2-mm IED; custom-

166 manufactured for this study by OT Bioelettronica) was placed over the belly of the TA and the HD-
167 EMG signals were recorded using the same procedure as previously described.

168

169 Grid configurations

170 During the first experimental session, we recorded EMG signals from the TA with a total of 256
171 electrodes covering an area of 36 cm² over the muscle (10 cm x 3.6 cm, 4-mm IED, Figure 1A). To
172 investigate the effect of electrode density, we down-sampled the grid of 256 electrodes by successively
173 discarding rows and columns of electrodes and artificially generating three new grids covering the same
174 area with IEDs of 8 mm, 12 mm, and 16 mm, that involved 256, 64, 35, and 20 electrodes, respectively
175 (Figure 1B-D). It is noteworthy that the 8-mm and 16-mm grids covered a surface of 32 cm² because
176 they included an odd number of rows and columns. To investigate the effect of the size of the grid, we
177 discarded the peripheral electrodes to generate grids of 63, 34 and 19 electrodes with a 4-mm IED,
178 covering areas of 7.7, 3.8 and 2 cm² over the muscle (Figure 1E-G). We chose these sizes to match the
179 number of electrodes used in the density analysis, thus comparing grids with similar number of
180 electrodes, but different densities and sizes (in Figure 1, B versus E, and C versus F).

181 During the second experimental session, we recorded EMG signals from the TA with an ultra-dense grid
182 of 256 electrodes covering an area of 9 cm² over the muscle (5 cm x 1.8 cm, 2-mm IED, Figure 1H).
183 Using the same procedure as above, we generated two artificial grids of 64 and 32 electrodes with an
184 IED of 4 mm and 8 mm, respectively.

185

186 HD-EMG decomposition

187 We decomposed the signals recorded in all the conditions using the same algorithm, parameters, and
188 procedure. First, the monopolar EMG signals were band-pass filtered between 20 and 500 Hz with a
189 second-order Butterworth filter. The channels with low signal-to-noise ratio or artifacts were discarded
190 after visual inspection. The HD-EMG signals were then decomposed into individual motor unit pulse
191 trains using convolutive blind-source separation, as previously described (Negro et al., 2016). In short,
192 the EMG signals were first extended by adding delayed versions of each channel. We kept the same
193 extension factor for all the conditions to reach 1000 extended channels, as previously suggested (Negro
194 et al., 2016). The extended signals were spatially whitened to make them uncorrelated and of equal
195 power. Thereafter, a fixed-point algorithm was applied to identify the sources embedded in the EMG
196 signals, i.e., the motor unit pulse trains, or series of delta functions centered at the motor unit discharge
197 times. In this algorithm, the contrast function $g(x) = \log(\cosh(x))$ was iteratively applied to the EMG
198 signals to skew the distribution of the values of the motor unit pulse trains toward 0, and thus maximize
199 the level of sparsity of the motor unit pulse train. The high level of sparsity matches the physiological

200 properties of motor units, with a relatively small number of discharges per second (< 50 discharge
201 times/s during submaximal isometric contractions). The convergence was reached once the level of
202 sparsity did not substantially vary (with a tolerance fixed at 10^{-4}) when compared to the previous
203 iteration (Negro et al., 2016). At this stage, the motor unit pulse train contained high peaks (i.e., the delta
204 functions from the identified motor unit) and lower values due to the activities of other motor units and
205 noise. High peaks were separated from lower values using peak detection and K-mean classification
206 with two classes. The peaks from the class with the highest centroid were considered as the discharge
207 times of the identified motor unit. A second algorithm refined the estimation of the discharge times by
208 iteratively recalculating the motor unit filter and repeating the steps with peak detection and K-mean
209 classification until the coefficient of variation of the inter-spike intervals was minimized. This
210 decomposition procedure has been previously validated using experimental and simulated signals
211 (Negro et al., 2016). After the automatic identification of the motor units, duplicates were automatically
212 removed. For this purpose, the pulse trains identified from pairs of motor units were first aligned using
213 a cross-correlation function to account for a potential delay due to the propagation time of action
214 potentials along the fibers. Then, two discharge times were considered as common when they occurred
215 within a time interval of 0.5 ms, and two or more motor units were considered as duplicates when they
216 had at least 30% of their identified discharge times in common (Holobar et al., 2010). In principle, the
217 limited level of synchronization between individual motor units results in a few simultaneous discharges
218 between pairs of motor units. A threshold of 30% is therefore highly conservative to ensure the removal
219 of all motor units with a level of synchronization well above physiological values. It is worth noting that
220 most of the motor units identified as duplicates after the automatic decomposition had almost 100% of
221 their discharge times in common. In that case, the motor unit with the lowest coefficient of variation of
222 the inter-spike intervals was retained for the analyses. At the end of these automatic steps, all the motor
223 unit pulse trains, i.e., the output of the decomposition resulting from the projection of EMG signals onto
224 individual motor unit filters, were visually inspected, and manual editing was performed to correct the
225 false identification of artifacts or the missed discharge times (Del Vecchio et al., 2020; Hug et al., 2021b;
226 Avrillon et al., 2023). The update of the motor unit filters with the corrected discharge times and the
227 recalculation of the motor unit pulse trains always improved the distance between the discharge times
228 and the noise, quantified with the pulse-to-noise ratio (PNR) (Holobar et al., 2014). Note that this manual
229 step is highly reliable across operators, as previously demonstrated by Hug et al. (2021b). Duplicates
230 were checked a second time after manual editing, with very rare cases of removal as most of the
231 duplicates were automatically identified after the automatic decomposition. Only the motor unit pulse
232 trains which exhibited a $\text{PNR} > 28$ dB after manual editing were retained for further analysis.

233

234 We further tested whether decomposing subsets of electrodes within a highly populated grid of 256
235 electrodes increased the number of identified motor units. Indeed, the lower ratio of large motor units

236 sampled by each independent subset of 64 electrodes could allow the algorithm to converge to smaller
237 motor units that contribute to the signal. For a similar number of iterations, it is likely that these motor
238 units would have otherwise contributed to the noise component of the mixture model of the EMG signal
239 (Farina and Holobar, 2016). Thus, we decomposed the grids of 256 electrodes (4-mm and 2-mm IED,
240 Figure 1A, H) as four separated grids of 64 electrodes before removing the motor units duplicated
241 between grids.

242

243

244 **Analyses**

245 Computational study

246 We first estimated the theoretical percentage of identifiable motor units for each of the simulated
247 conditions. To do so, the simulated MUAPs detected over the entire set of electrodes were compared
248 with each other. The comparisons were done pairwise by first aligning the MUAPs in time using the
249 cross-correlation function, and then computing the normalized mean square difference between the
250 aligned action potentials. Pairs of action potentials with a mean square difference below 5% were
251 considered not discriminable. The 5% criterion was based on the variability of motor unit action
252 potential shapes observed experimentally for individual motor units (Farina et al., 2008). After
253 computing all pair-wise comparisons, we then computed the percentage of action potentials that could
254 be discriminated from all others, i.e., the theoretical percentage of identifiable motor units. This metric
255 is independent from the algorithm used for decomposition and establishes a theoretical upper bound in
256 the number of motor units that can be identified by any decomposition algorithm. For each theoretically
257 identifiable motor unit, we also computed the distance between the center of the territory of the
258 corresponding muscle fibers and the skin surface.

259

260 Laboratory study – number of identified motor units

261 We reported the absolute number of motor units (PNR > 28 dB) identified with all the grid
262 configurations. For each participant, the number of identified motor units was then normalized to the
263 maximal number of motor units found across all conditions, yielding normalized numbers of identified
264 motor units \bar{N} expressed in percentage. For each condition, we calculated the mean and standard
265 deviation of the \bar{N} values across participants. To investigate the effects of density and size of the grid,
266 we fitted logarithmic trendlines to the relationships between the averaged \bar{N} values and IED or grid size.
267 We also fitted a logarithmic trendline to the average \bar{N} values and their corresponding number of
268 electrodes, in which case the conditions involving the same number of electrodes, but different grid size

269 and density, were given a weight of 0.5 in the minimization function. We reported the r^2 and p-value for
270 each regression trendline. To maintain consistency with the computational study investigating the
271 number of theoretically identifiable motor units across grid designs, the trendlines were fitted on the
272 results obtained when the complete grids of 256 electrodes were decomposed as independent subsets of
273 64 electrodes, which systematically returned the highest number of identified motor units. The trendlines
274 fitted on the results obtained with the decomposition of the 256 electrodes as a whole are reported in
275 Figure 4-1.

276

277 Laboratory study – properties of identified motor units

278 To investigate the effects of electrode density and grid size on the properties of the identified motor unit,
279 we used a typical frequency distribution of the motor unit force recruitment thresholds in the human TA
280 (Caillet et al., 2022b), where $F^{th}(j)$ is the force recruitment threshold of the j^{th} motor unit in the
281 normalized motor unit pool ranked in ascending order of F^{th} .

$$282 \quad F^{th}(j) = 0.50 \cdot (58.12 \cdot j + 120^{j^{1.83}}), j \in [0; 1]$$

283 The identified motor units were then classified according to this relationship and their measured force
284 recruitment threshold, the first half of the active pool being ‘early recruited’, and the second half ‘late
285 recruited’ (Henneman and Mendell, 1981; Caillet et al., 2022a). For each condition, we reported the
286 percentage of identified motor units that were ‘early recruited’. We did not report this metric when five
287 or fewer motor units were identified in one condition for three or more participants.

288

289 Laboratory study – correlation between observations

290 We assessed how the density of electrodes impacted the information redundancy in EMG signals
291 recorded by adjacent electrodes. To this end, MUAP shapes were identified over the 256 electrodes with
292 the spike-triggered averaging technique. To do so, the discharge times were used as a trigger to segment
293 and average the HD-EMG signals over a window of 50 ms. For each motor unit, we identified the
294 electrode with the highest action potential peak-to-peak amplitude and calculated the average correlation
295 coefficient ρ between this action potential and those recorded by the four adjacent electrodes with an
296 IED of 4 mm, 8 mm, 12 mm, and 16 mm. We also repeated this correlation analysis for the ultra-dense
297 grid of 256 electrodes using an IED of 2 mm, 4 mm, and 8 mm.

298 **Results**

299 All the datasets (raw and processed data) and codes used to process the data are available at
300 <https://figshare.com/s/f4a94d9bdf470bf10f8>.

301

302 **Computational study**

303 We simulated the discharge activity of 200 motor units recorded by 84 configurations of grids of
304 electrodes (Figure 2; surface range: 14.4 to 36 cm², IED range: 2 to 36 mm). The number of theoretically
305 identifiable motor units increased with the size of the grid, from $46.7 \pm 7.7\%$ of the motor units
306 theoretically identifiable with a grid of 14.4 cm² to $77.8 \pm 5.5\%$ of the motor units theoretically
307 identifiable with a grid of 36 cm². The number of theoretically identifiable motor units also increased
308 with shorter interelectrode distances. For example, with a grid of 36 cm², the number of theoretically
309 identifiable motor units increased from 63.5% to 83.5% of the motor units with an IED of 36 and 2 mm,
310 respectively (Figure 2B). Increasing the surface size and the density of the grid of electrodes revealed
311 deeper motor units. The averaged distance of theoretically identifiable motor units from the skin
312 increased with the size of the grid (Figure 2C; 14.3 ± 0.1 mm vs. 16.5 ± 0.2 mm with grids of 14.4 and
313 36 mm², respectively), but not with the IED of the grid (Figure 2D; 15.6 ± 1.1 mm vs. 15.5 ± 0.9 mm
314 with an IED of 36 and 2 mm, respectively).

315

316 **Laboratory study - grids of 256 electrodes with an IED of 4-mm**

317 Number of identified motor units

318 The motor unit pulse trains automatically identified across all conditions, intensities, and participants
319 were visually inspected and carefully edited when a missing discharge time or a falsely identified artifact
320 were observed. On average, $9 \pm 4\%$ and $22 \pm 9\%$ of the motor units automatically identified at 30%
321 and 50% MVC, respectively, were removed after visual inspection and manual editing. Furthermore,
322 when the four grids of 64 electrodes were separately decomposed, $30 \pm 5\%$ and $24 \pm 6\%$ of the
323 automatically identified motor units were removed because they were identified in more than one grid
324 (only one pulse train was retained in case of duplicates). The highest number of identified motor units
325 was systematically reached with the separate decomposition of the four grids of 64 electrodes with an
326 IED of 4 mm, with 56 ± 14 motor units (PNR = 34.2 ± 1.1) and 45 ± 10 motor units (PNR = 34.0 ± 0.9)
327 at 30% and 50% MVC, respectively (Figure 3). At least 82% of the motor units identified in one
328 condition were also identified in the conditions involving a higher number of electrodes. Similarly, 91%
329 to 100% of the motor units identified in one condition were also identified with the 256-electrode
330 configuration (4-mm IED, 36-cm² size, Figure 1A) with the four grids decomposed separately.

331

332 When considering the effect of electrode density (grid size fixed at 32-36 cm², Figure 1A-D), we found
333 the lowest number N of identified motor units with the 16-mm IED, with 3 ± 1 motor units and 2 ± 1
334 motor units at 30% and 50% MVC, respectively (Figure 4A, C). Additional motor units were gradually
335 identified with greater electrode densities. The highest number of identified motor units was observed
336 with the highest density (4-mm IED), with 56 ± 14 and 45 ± 10 motor units at 30% and 50% MVC,
337 respectively, with the 4×64-electrode decomposition procedure (Figure 4A, C). With the 256- electrode
338 decomposition procedure, 43 ± 11 and 25 ± 6 motor units were identified at 30% and 50% MVC,
339 respectively (Figure 4A, C). Finally, we found a decreasing logarithmic relationship between the
340 normalized number \bar{N} of motor units, averaged for each participant, and the IED, with $r^2 = 1.0$ ($p =$
341 $2.5 \cdot 10^{-5}$) and $r^2 = 0.99$ ($p = 0.001$) at 30% and 50% MVC, respectively (Figure 4B, D).

342 When considering the effect of the size of the grid (IED fixed at 4 mm, Figure 1A, E-G), we found the
343 lowest number N of motor units with a grid of 2 cm², with 4 ± 2 motor units and 4 ± 2 motor units at
344 30% and 50% MVC, respectively (Figure 6A, C). Additional motor units were then gradually identified
345 with larger grid sizes. The highest number of motor units was observed with a grid of 36 cm², with 56
346 ± 14 and 45 ± 10 motor units at 30% and 50% MVC, respectively, with the 4×64-electrode
347 decomposition procedure (Figure 4A, C). With the 256- electrode decomposition procedure, 43 ± 11
348 and 25 ± 6 motor units were identified at 30% and 50% MVC, respectively (Figure 4A, C). Finally, we
349 found an increasing logarithmic relationship between the normalized number of motor units \bar{N} , averaged
350 for each participant, and the size of the grid, with $r^2 = 0.99$ ($p = 3.0 \cdot 10^{-4}$) and $r^2 = 0.98$ ($p = 0.001$) at
351 30% and 50% MVC, respectively (Figure 6B, D). It is noteworthy that the parameters of the fits were
352 very similar at 30% and 50% MVC in both analyses.

353 As both the density and the size of the grid determine the number of electrodes, we finally fitted the
354 relationship between the normalized number of motor units \bar{N} and the number of electrodes. As observed
355 previously, more motor units were identified with a larger number of electrodes, following a logarithmic
356 tendency with $r^2 = 0.98$ ($p = 0.018$) and $r^2 = 0.95$ ($p = 0.016$) at 30% and 50% MVC, respectively (Figure
357 6). A plateau should theoretically be reached with grids of 1024 and 4096 electrodes (36-cm² grids with
358 2-mm and 1-mm IED, respectively), with a prediction of 50% and 90% more motor units.

359 For a fixed number of electrodes, it is noteworthy that the size and the density, although linked, may
360 have different impact on the number of identified motor units (black crosses in Figure 6). For example,
361 1.25 times more motor units were obtained with the 64-electrode condition (32 cm², 8-mm IED, Figure
362 1B) than with the 63-electrode condition (7.7 cm², 4-mm IED, Figure 1E) for the group of participants
363 at 30% MVC.

364

365 Characteristics of identified motor units

366 We found an increasing logarithmic relationship between the percentage of early recruited motor units
367 for each participant and the density of the grid, with $r^2 = 0.91$ ($p = 2.8 \cdot 10^{-3}$) at 30% MVC (Figure 7F).
368 Contrary to the density, the size of the grid did not impact the percentage of early recruited motor units,
369 with the percentage ranging from 20 to 29% across all sizes, and the logarithmic trendline returning a
370 negligible slope and a low $r^2 = 0.28$ (Figure 7C, G). Such differences were also not observed at 50%
371 MVC, where the percentage of early recruited motor units remained below 10% for all conditions.

372 To support the above observations made at 30% MVC, grids with the same number of electrodes, but
373 different densities and sizes, were directly compared. 62% of the motor units identified with the grids
374 of 64 electrodes (32 cm², IED 8 mm) and 63 electrodes (7.7 cm², IED 4 mm) were identified in both
375 conditions at 30% MVC. $28 \pm 9\%$ of the motor units specific to the 8-mm IED grid were early recruited,
376 while $44 \pm 11\%$ of the motor units specific to the 4-mm IED condition were early recruited. Similar
377 results were obtained with the grids of 35 (36 cm², 12-mm IED) and 34 electrodes (3.6 cm², 4-mm IED),
378 where a higher number of early recruited motor units were specifically identified with denser rather than
379 larger grids.

380

381 Correlation between MUAPs from adjacent electrodes

382 Figure 8 reports the effect of the density of electrodes on the level of correlation ρ between the profiles
383 of action potentials recorded by adjacent electrodes. The lowest average correlation coefficient ρ was
384 observed with an IED of 16 mm ($\rho = 0.87 \pm 0.03$ and $\rho = 0.88 \pm 0.04$ at 30% and 50% MVC,
385 respectively). The level of correlation increased with a shorter IED, with $\rho = 0.96 \pm 0.04$ and $\rho = 0.95$
386 ± 0.05 between the profiles of action potentials recorded by adjacent electrodes with a 4-mm IED at
387 30% and 50% MVC, respectively (Figure 8B, C).

388

389 **Laboratory study with an ultra-dense prototyped grid of 256 electrodes with 2-mm IED**

390 31 and 26 motor units (PNR > 28 dB) were identified for one participant with the ultra-dense grid of
391 256 electrodes (2-mm IED, 9 cm², Figure 1H) at 30% and 50% MVC, respectively (Figure 9B). Note
392 that the signals from four independent subsets of 64 electrodes were decomposed separately. For that
393 participant, more motor units were identified with the ultra-dense grid of 256 electrodes than with the
394 grid of 64 electrodes covering the same area (Figure 5A, C). Indeed, 31 and 26 motor units were
395 respectively identified at 30% and 50% MVC with the grid of 256 electrodes (Figure 9C), while 25 (24
396 ± 5 for the group) and 19 (18 ± 4 for the group) motor units were identified with the grid of 64 electrodes

397 (Figure 5A, C). Moreover, fewer motor units were identified when the electrode density of the ultra-
398 dense grid was decreased (Figure 9C), with 22 and 13 motor units identified with a 4- and 8-mm IED at
399 30% MVC, respectively, and 21 and 9 motor units identified with a 4- and 8-mm IED at 50% MVC,
400 respectively. At 30% MVC, the rate of increase of N between 4- and 2-mm IED followed the prediction
401 computed in Figure 4B and illustrated by the dash line in Figure 9C. At 50% MVC, the rate of increase
402 of N (dotted line in Figure 9C) was lower than the prediction. As previously observed, the correlation
403 between adjacent MUAPs increased from $\rho = 0.92$ with an 8-mm IED to $\rho = 0.98$ with a 2-mm IED at
404 30% MVC, and from $\rho = 0.85$ with an 8-mm IED to $\rho = 0.93$ with a 2-mm IED at 50% MVC (Figure
405 9A). All the motor units identified with the 8-mm and 4-mm IED were also identified with the 4-mm
406 and 2-mm IED grids, respectively. Finally, more motor units with an early recruitment were identified
407 when increasing the density from 8- to 4-mm IED (blue vs black trains in Figure 9B), and from 4- to 2-
408 mm IED (red trains in Figure 9B).

409

410 Discussion

411 This study systematically investigated how the design parameters of grids of surface EMG electrodes
412 (grid size and electrode density) impact the number and the properties of the motor units identified with
413 EMG decomposition. Using a combination of computational and experimental analyses, we found that
414 larger and denser grids of electrodes than conventionally used reveal a larger sample of identified motor
415 units. As most of the motor units that were not identified with less dense and smaller grids had an early
416 recruitment threshold, we concluded that denser grids allow to identify smaller motor units. This is due
417 to a better spatial sampling of MUAPs over the grid, which in turn improves the discrimination of motor
418 units with a unique set of MUAPs among active motor units. These results clarify the direction for
419 designing new grids of electrodes that could span across the entire surface of the muscle of interest while
420 keeping a high density of electrodes, with IED as low as 2 mm. Identifying large sets of small and large
421 motor units is relevant in many research areas related to motor control, such as the investigation of
422 synergies (Hug et al., 2022), neuromuscular modelling (Caillet et al., 2022c), or human-machine
423 interfacing (Farina et al., 2021).

424

425 The number N of identified motor units increased across participants with the density of electrodes
426 (Figure 4; Figure 8C), the size of the grid (Figure 6), and the number of electrodes (Figure 6). On
427 average, 30 and 19 motor units were identified with the ‘conventional’ 64-electrode grid (8-mm IED,
428 32 cm² surface area) at 30% and 50% MVC, respectively, which is consistent with several previous
429 studies using similar grid designs (Del Vecchio et al., 2020). By increasing the density of electrodes and
430 size of the grid to reach a total of 256 electrodes separated by a 4-mm IED, we identified on average 56
431 and 45 motor units at 30% and 50% MVC, respectively. We even reached 79 and 59 motor units for one
432 subject (Figure 3), which is substantially more than the numbers of motor units usually reported in
433 studies with similar methods, and twice those obtained with grids of 64 electrodes in this study. Our
434 computational and experimental analyses showed that the size of the grid is a key factor contributing to
435 the higher number of identified motor units (Figure 2B; Figure 6). According to our simulations,
436 increasing the size of the grid increases the number of theoretically identifiable motor units, i.e., the
437 number of motor units with unique sets of MUAPs across electrodes (Figure 2B). These differences
438 between MUAPs result from the anatomical and physiological differences between adjacent motor units,
439 such as the length of their fibers, the spread of the end plates, or their conduction velocity, as well as
440 from the properties of the tissues separating the fibers from each recording electrode (Farina et al., 2004).
441 Larger grids better sample these differences across electrodes, revealing the unique profiles of each
442 motor unit action potentials (Farina et al., 2008). The density of electrodes was also a critical factor to
443 increase the number of identified motor units (Figure 4; Figure 4-2C). Dense grids especially allowed
444 to better identify early recruited motor units. Classically, the decomposition algorithms tend to converge

445 towards the large and superficial motor units that contribute to most of the energy of the EMG signals
446 (Farina and Holobar, 2016). Conversely, action potentials of the smallest motor units tend to have lower
447 energy and are masked by the potentials of the larger units. These factors explain the lowest
448 representation of low-threshold motor units in available HD-EMG datasets (Caillet et al., 2023).
449 Increasing the density of electrodes would therefore enable to better sample the action potential profiles
450 of these early recruited motor units across multiple electrodes, enabling their identification. However,
451 we observed that increasing the density did not reveal additional early recruited motor units during
452 contractions at 50% MVC (Figure 7D). This is potentially due to the higher energy of the MUAPs of
453 the motor units recruited between 30% and 50% MVC. Additionally, we also showed in one subject that
454 synthetically increasing the density of electrodes by resampling EMG signals with spatial interpolation
455 does not have the same effect as with denser grids. In this example, 4 and 19 motor units were identified
456 from the interpolated grid with a 4-mm and 2-mm IED, respectively, vs. 19 and 24 motor units with the
457 experimentally recorded signals. All the motor units identified with the interpolated grid were also
458 identified with the experimentally recorded signals (Figure 4-2).

459

460 The number of identified motor units N monotonically increased with the density of electrodes (Figure
461 4BD), the size of the grid (Figure 6BD) and the number of electrodes (Figure 6), following significant
462 logarithmic trendlines. Remarkably, very similar logarithmic tendencies were obtained at both 30% and
463 50% MVC in all the analyses. Altogether, these trendlines suggested that the normalized number of
464 identified motor units \bar{N} would grow with an electrode density beyond a 4-mm IED. We experimentally
465 tested this hypothesis by designing a new prototyped grid of 256 electrodes separated by an IED of 2
466 mm. As predicted, more motor units were identified with a 2 mm than with a 4 mm IED, following at
467 30% MVC the same rate of increase as predicted by the logarithmic trendlines (Figure 4-2C) between
468 4-mm and 2-mm IED. This increase may plateau with higher electrode densities, as the level of
469 correlation between the profiles of MUAPs detected over adjacent electrodes tended to 1 (Figure 4-2A).
470 Therefore, the high level of similarity between signals recorded from adjacent electrodes in ultra-dense
471 grids (IED < 2 mm) may limit the percentage of identifiable motor units (Farina and Holobar, 2016).
472 According to these results, we consider that optimal designs of surface grids of electrodes for identifying
473 individual motor units would involve a surface that covers the muscle of interest with an IED as low as
474 2 mm.

475

476 Another important factor for the accuracy of the discharge times estimated for each individual motor
477 unit is the quality of the motor unit pulse trains, estimated by the PNR (Holobar et al., 2014) or the
478 silhouette value. In this study, we found that the quality of the identified motor units (i.e., decomposition
479 accuracy) increased when increasing the density of electrodes or the size of the grid, with PNR reaching

480 on average 37-38 dB across participants with the grid of 256 electrodes (Figure 4-3). A greater average
481 PNR implies the need of less manual editing following the automatic decomposition (Hug et al., 2021b).
482 The better estimates of motor unit pulse trains depend on the better signal to noise ratio following the
483 inversion of the mixing matrix, since the pulse train of each motor unit is computed by projecting the
484 extended, whitened signals on the separation vector (Holobar and Farina, 2014; Farina and Holobar,
485 2016; Negro et al., 2016). Likewise, the PNR substantially increased after we computationally increased
486 the number of electrodes by spatially resampling the EMG signals. This practical result is of interest for
487 most of the physiological studies that require a lengthy processing time to visually inspect and manually
488 edit the discharge times estimated from the pulse trains of all the motor units (Hug et al., 2021b).

489

490 Finally, we increased both the total number and the percentage of early recruited motor units identified
491 by independently decomposing subsets of 64 electrodes within the grids of 256 electrodes, compared to
492 the simultaneous decomposition of all available observations (Figure 7B, C). This was likely due to the
493 lower ratio of large motor units sampled by each subset of electrodes, allowing the algorithm to converge
494 to smaller motor units that contributed to the signal (Figure 7B, C). Importantly, it should be noted that
495 the simulation results were obtained independently of a specific decomposition algorithm, as previously
496 proposed by Farina et al (2008). On the other hand, the experimental results are based on a specific
497 algorithm. Interestingly, however, the simulation and laboratory results were fully consistent and in
498 agreement, indicating that the difference in shape of the spatially sampled MUAPs is the main factor
499 influencing EMG decomposition.

500

501 Conclusion

502 By increasing the density and the number of electrodes, and the size of the grids, we increased the
503 number of theoretically identifiable and experimentally identified motor units from the surface EMG
504 signals. The identified motor units had pulse trains with high PNR, limiting the manual processing time.
505 Moreover, we identified a higher percentage of early recruited motor units, which are classically filtered
506 out with the conventional grid designs. In this way, a maximum of 79 motor units (PNR > 28 dB; mean:
507 36 dB), including 40% of early recruited motor units, were identified, which is substantially greater than
508 the samples previously reported with smaller and less dense grids. From these results, we encourage
509 researchers to develop and apply larger and denser EMG grids to cover the muscle of interest with IEDs
510 as small as 2 mm. This approach should increase the sample of motor units that can be experimentally
511 investigated with non-invasive techniques.

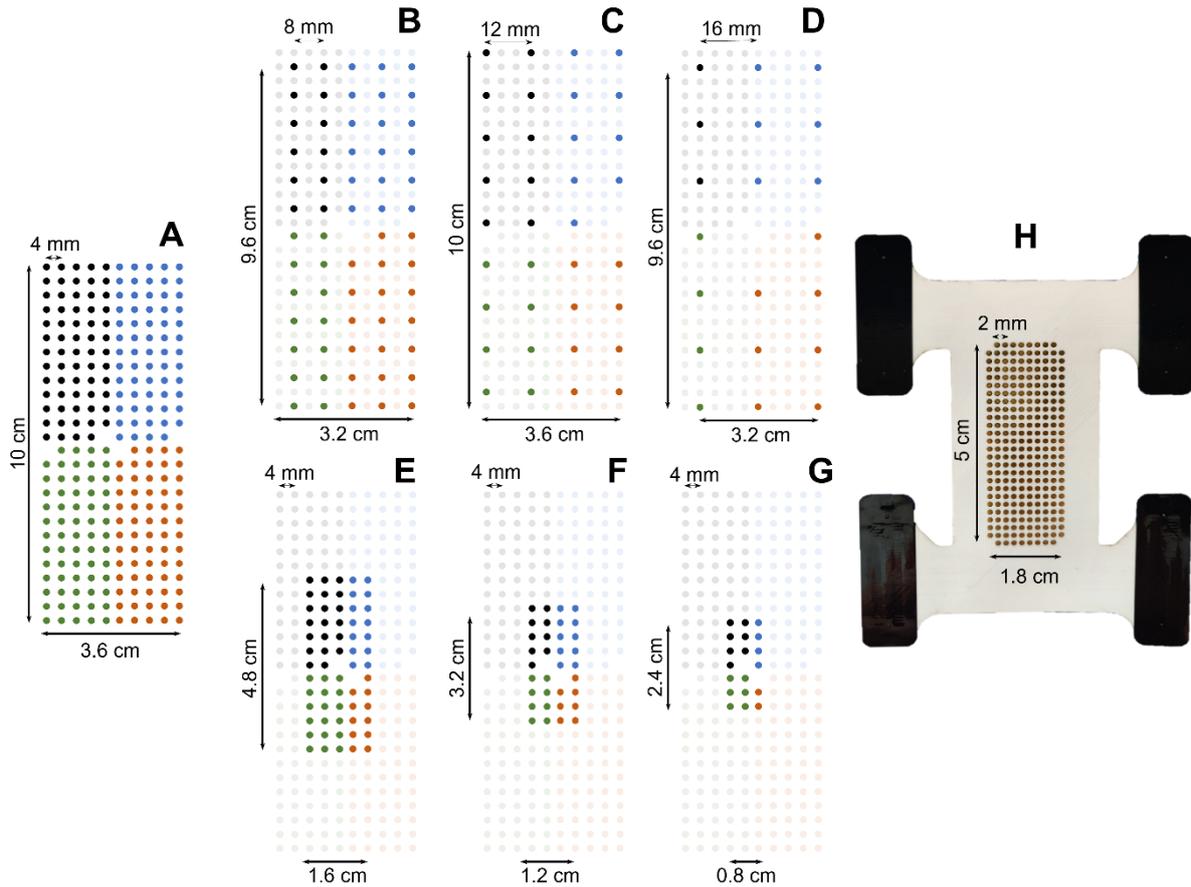
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Figures

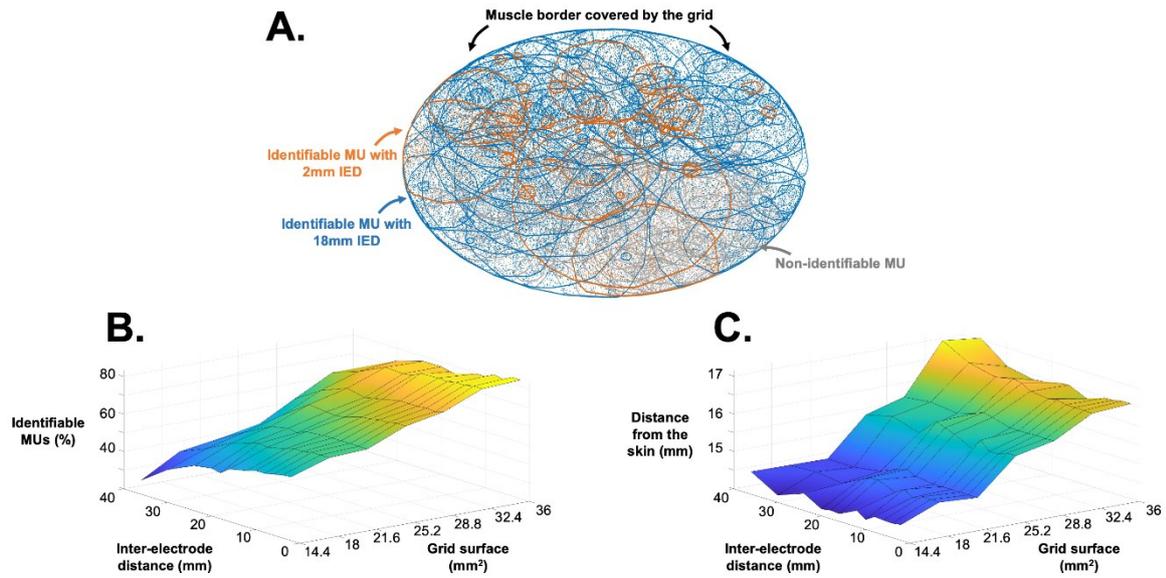


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588 Figure 1: The eight grid configurations considered in this study. From the first grid of 256 electrodes
589 (A, grid size: 36 cm², IED: 4 mm), six shallower and smaller grids (B-G) were artificially obtained by
590 discarding the relevant electrodes. (B,C,D) Density analysis: 8, 12, and 16mm IED. (E,F,G) Size
591 analysis: 7.7, 3.6, and 2 cm² surface area. (H) The ultra-dense prototyped grid of 256 electrodes (grid
592 size: 9 cm², IED: 2 mm).

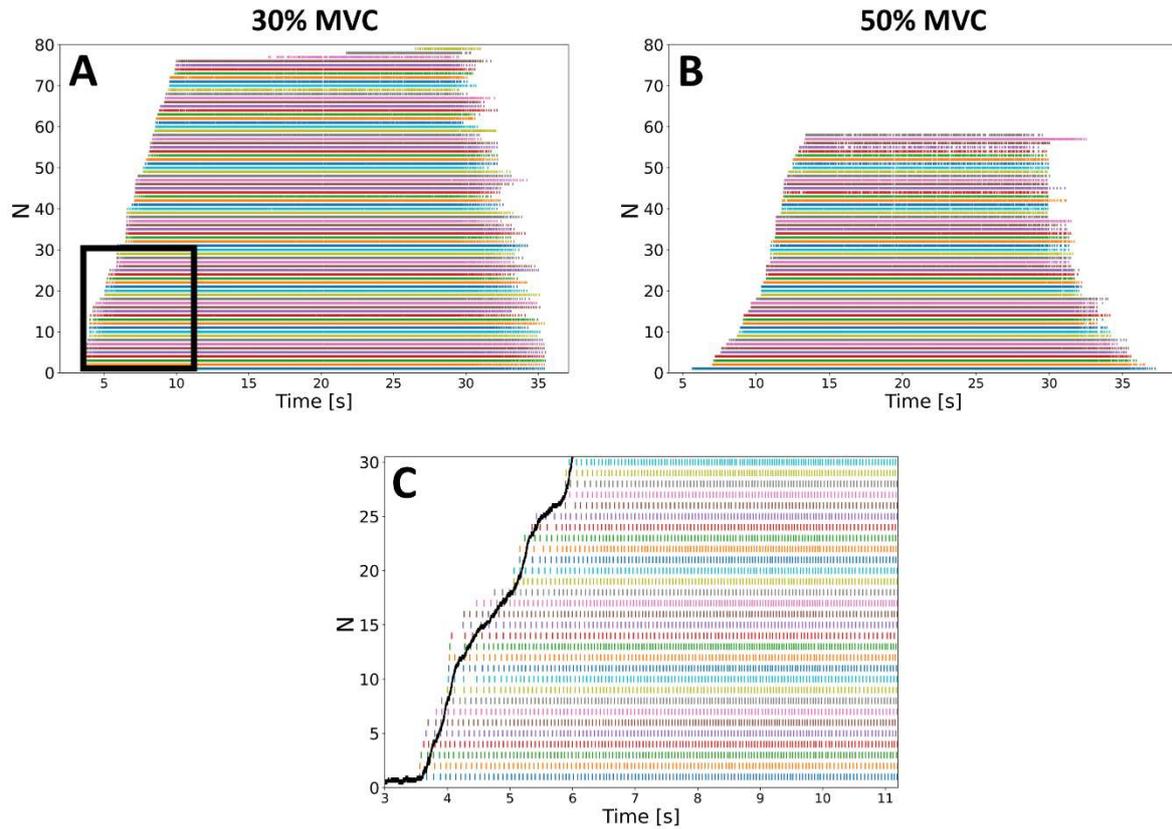
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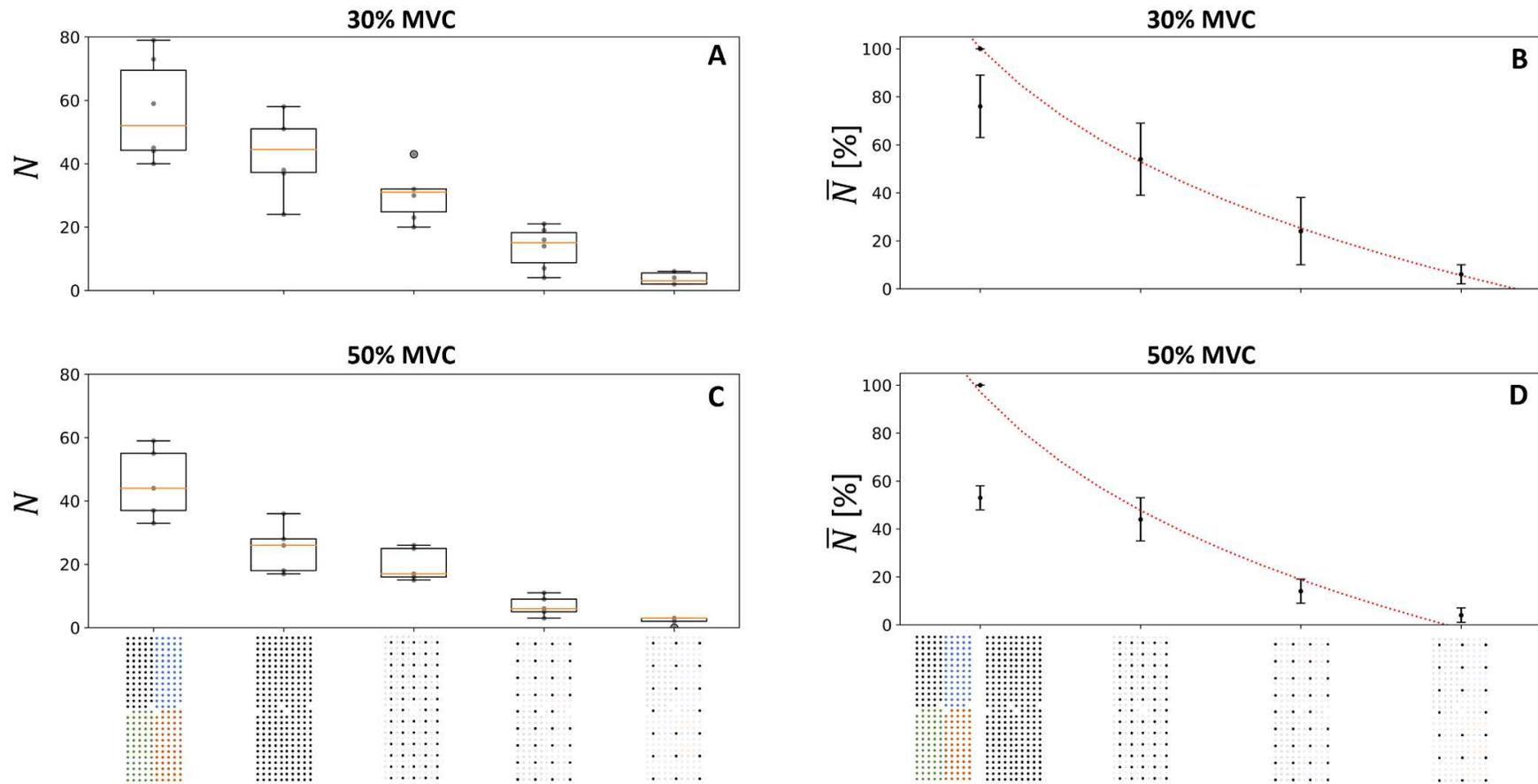
596 Figure 2: Results from the 200 simulated motor units with 84 configurations of grids of electrodes. (A)
597 Each solid line represents a motor unit territory, the scatters being the muscle fibers. Blues lines are
598 the theoretically identifiable motor units with a grid of 21.6 cm² and an interelectrode distance (IED)
599 of 18 mm, while the orange lines are the motor units revealed with a grid of 21.6 cm² and an IED of
600 2mm. Grey lines represent the non-identifiable motor units. The percentage of theoretically
601 identifiable motor units (B) and their distance from the skin (C) are reported for the 84 configurations.



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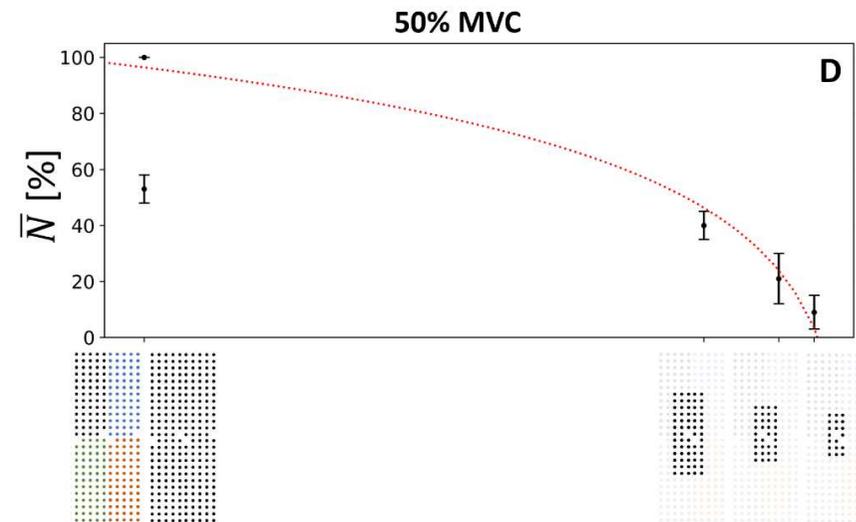
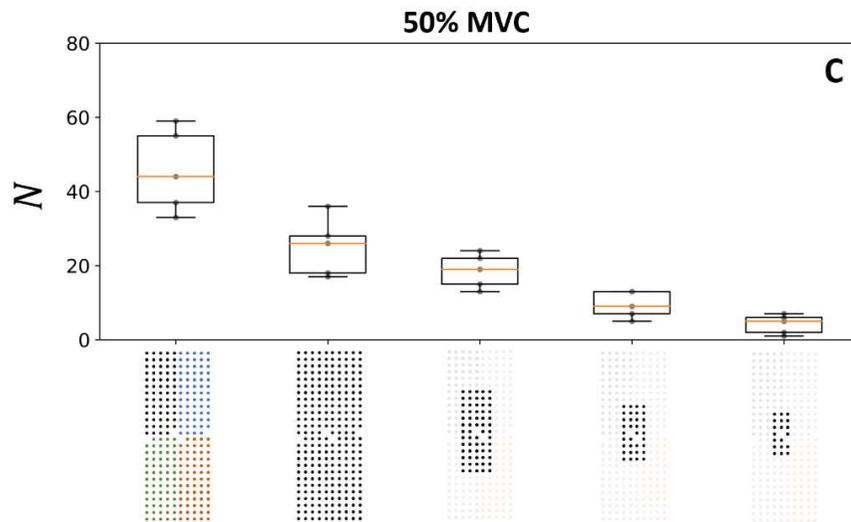
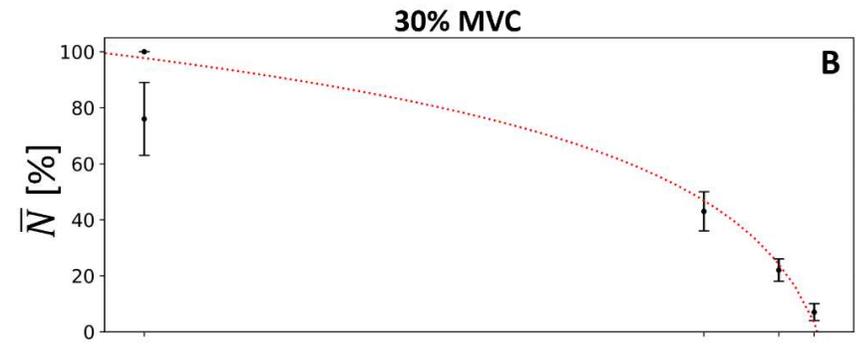
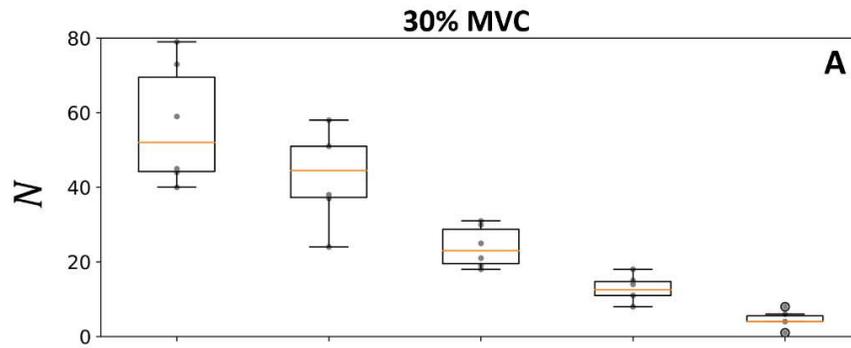
603 Figure 3: Discharge times of the maximum number of motor units identified in one participant (S1) at
604 30% (A) and 50% MVC (B), with 79 and 58 identified motor units, respectively. The motor units were
605 identified with separated decompositions of the four grids of 64 electrodes (4 mm IED). (C) Discharge
606 times of the 30 first recruited motor units during the ascending ramp of force (black curve) at 30%
607 MVC (black box in A).

608



609

610 Figure 4: Effect of the electrode density on the number of identified motor units N at 30% (A, B) and 50% MVC (C, D). The boxplots in the left column report the absolute
 611 number N of identified motor units per participant (grey dots) and the median (orange line), quartiles, and 95%-range across participants. In the right column, the normalized
 612 number of motor units \bar{N} logarithmically decreases with interelectrode distance d (4, 8, 12, and 16mm in abscissa) as $\bar{N} = 195 - 68 \log(d)$ ($r^2 = 1.0, p = 2.5 \cdot 10^{-5}$) at 30%
 613 MVC (B) and $\bar{N} = 196 - 71 \log(d)$ ($r^2 = 0.99, p = 0.001$) at 50% MVC (D). The standard deviation of \bar{N} across subjects is displayed with vertical bars. Moreover, the
 614 quality of the motor unit pulse trains (i.e., decomposition accuracy, estimated by the PNR) increased when increasing the density of electrodes (see Figure 4-3 for more details).
 615 Two decomposition procedures were considered for the 256-electrode condition; the grid of 256 black electrodes indicates that the 256 signals were simultaneously decomposed
 616 and the grid of 256 electrodes of four different colors indicates that four subsets of 64 electrodes were decomposed. To maintain consistency with the computational study, the
 617 trendlines were fitted with the 4*64 condition, which returned the higher number of identified motor units (see Figure 4-1 for the other fitting condition). It is worth noting that
 618 computationally increasing the density of electrodes by resampling the EMG signals with a spatial interpolation did not reveal any previously hidden motor units (Figure 4-2).



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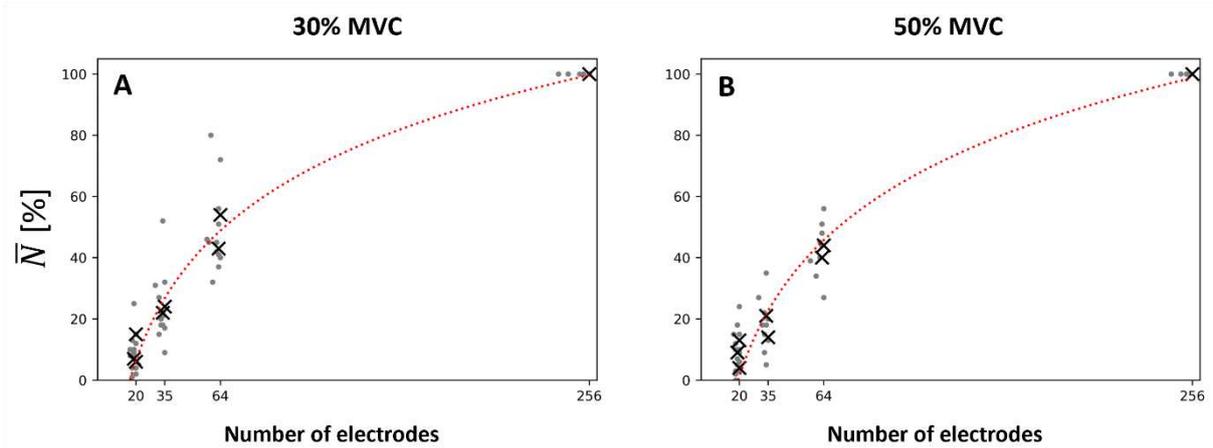
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Figure 5: Effect of the size of the grid on the number of identified motor units N at 30% (A, B) and 50% MVC (C, D). The boxplots in the left column report the absolute number N of identified motor units per participant (grey dots) and the median (orange line), quartiles, and 95%-range across participants. In the right column, the normalized number of motor units \bar{N} logarithmically decreases with the size of the grid s (2, 3.8, 7.7, and 36 cm² in abscissa) as $\bar{N} = -20 + 33 \log(s)$ ($r^2 = 0.99, p = 3.0 \cdot 10^{-4}$) at 30% MVC (B), and $\bar{N} = -19 + 32 \log(s)$ ($r^2 = 0.98, p = 0.001$) at 50% MVC (D). The standard deviation of \bar{N} across subjects is displayed with vertical bars. Moreover, the quality of the identified motor unit pulse trains (i.e., decomposition accuracy, estimated by the PNR) increased when increasing the size of the grid (see Figure 4-3 for more details). Two decomposition procedures were considered for the 256-electrode condition; the grid of 256 black electrodes indicates that the 256 signals were simultaneously decomposed and the grid of 256 electrodes of four different colors indicates that four subsets of 64 electrodes were decomposed. To maintain consistency with the computational study, the trendlines were fitted with the 4*64 condition, which returned the higher number of identified motor units (see Figure 4-1 for the other fitting condition).

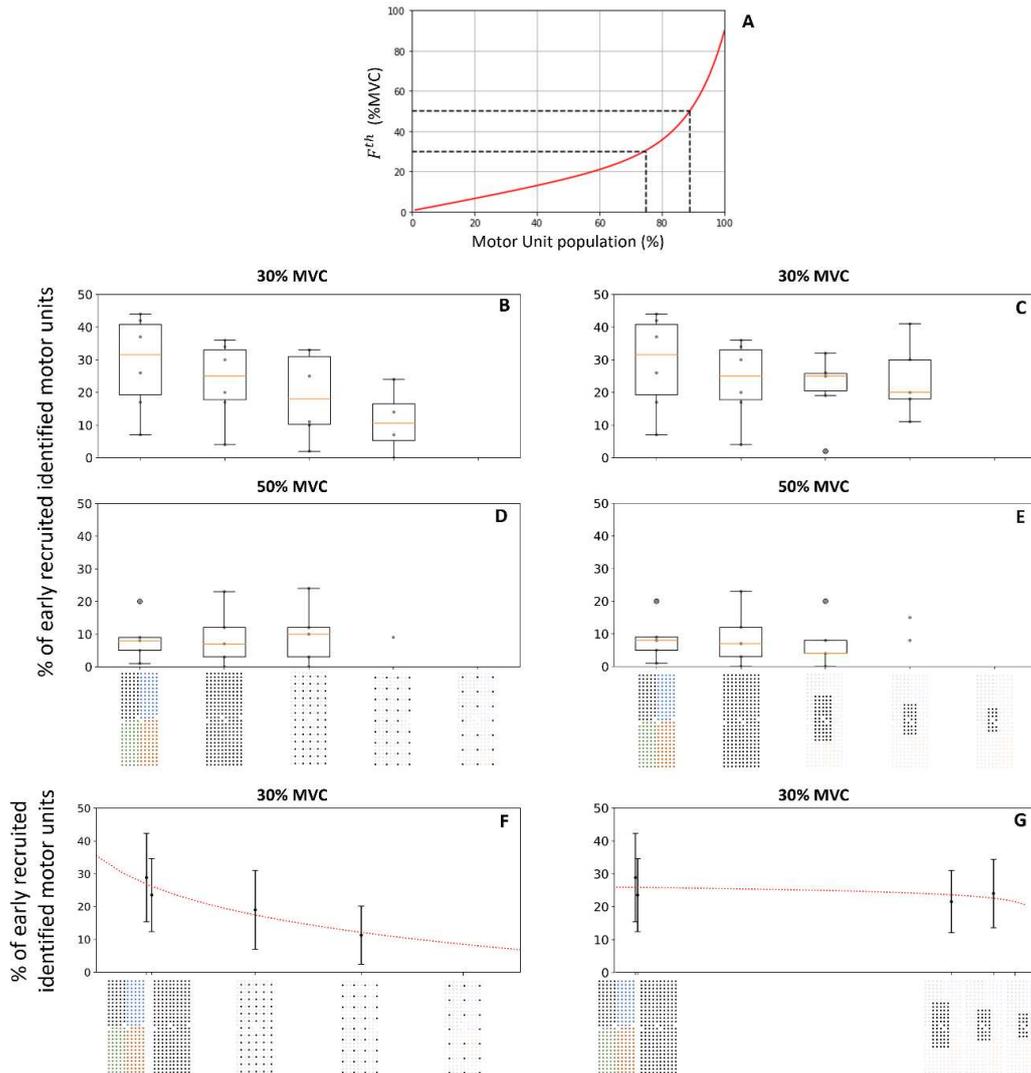


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629 Figure 6: Effect of the number n of electrodes on the normalized number \bar{N} of identified motor units at
630 30% (A) and 50% MVC (B). The discrete results per participant are displayed with grey data points.
631 The average values \bar{N} per condition are displayed with black crosses. Weighted logarithmic trendlines
632 were fitted to the data and returned (A) $\bar{N} = -104 + 37 \log(n)$ ($r^2 = 0.98, p = 0.018$), and (B) $\bar{N} =$
633 $-113 + 38 \log(n)$ ($r^2 = 0.95, p = 0.016$). Two decomposition procedures were considered for the
634 256-electrode condition; the grid of 256 black electrodes indicates that the 256 signals were
635 simultaneously decomposed and the grid of 256 electrodes of four different colors indicates that four
636 subsets of 64 electrodes were decomposed. To maintain consistency with the computational study, the
637 trendlines were fitted with the 4*64 condition, which returned the higher number of identified motor
638 units (see Figure 4-1 for the other fitting condition).

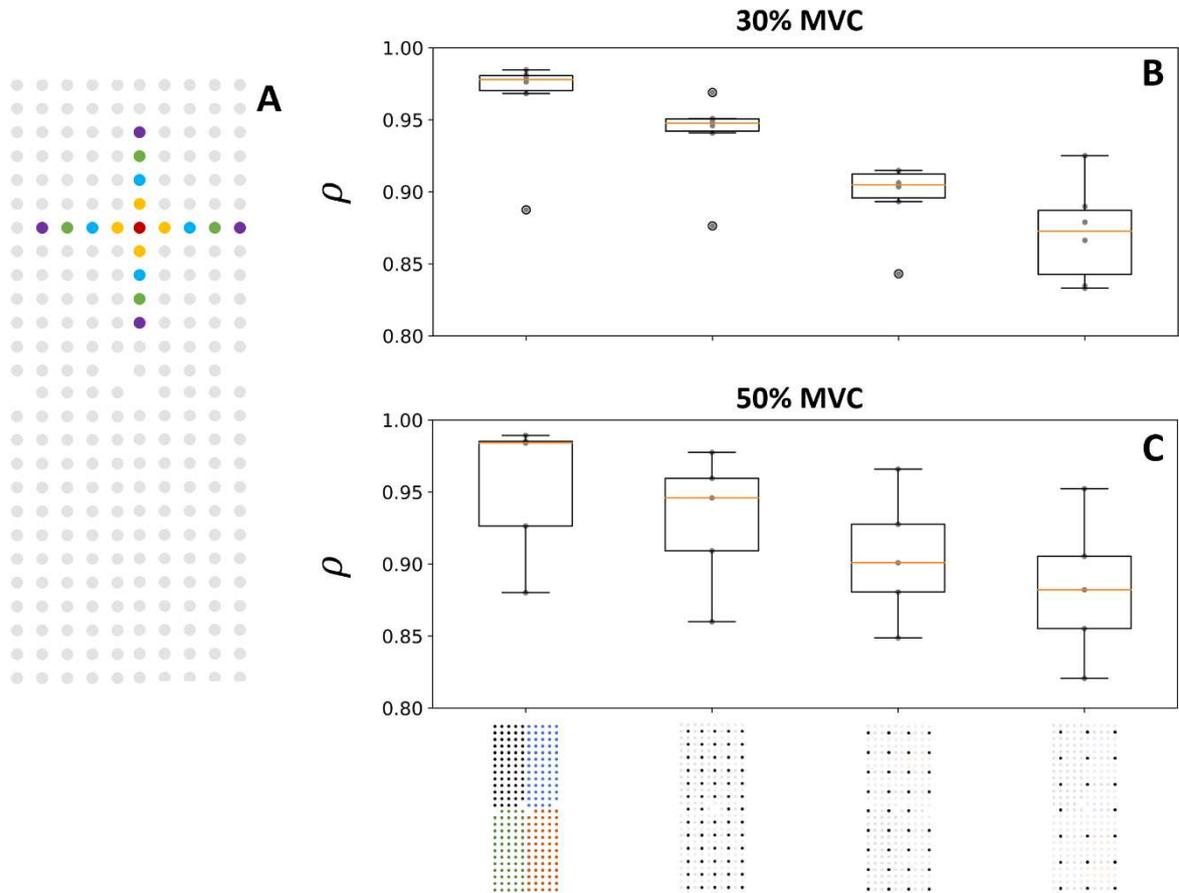
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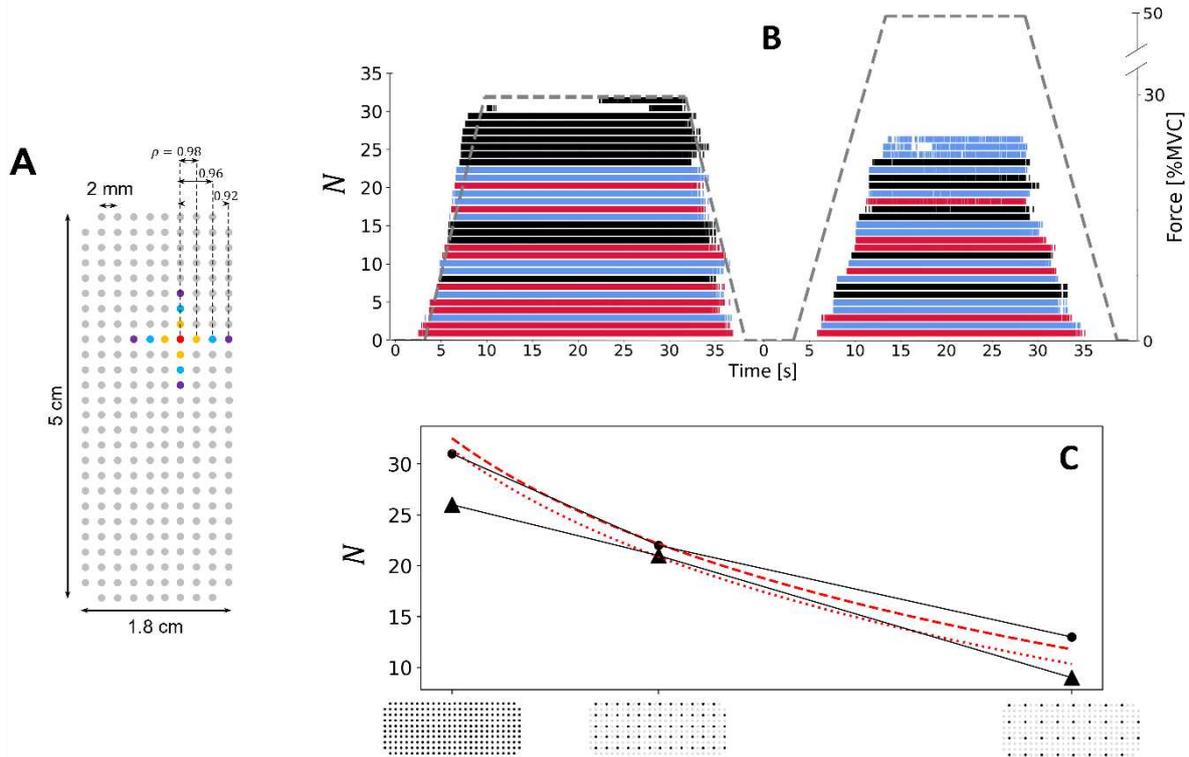
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642 Figure 7: (A) Typical frequency distribution of motor unit force recruitment thresholds in a human TA.
 643 The black dashed lines denote the theoretical portions of the population of motor units recruited at 30%
 644 and 50% MVC. Effect of the grid density (B, D, F) and grid size (C, E, G) on the percentage of early
 645 recruited motor units identified at 30% (B, C, F, G) and 50% MVC (D, E). The boxplots report the
 646 results per participant (grey dots) and the median (orange line), quartiles, and 95%-range across
 647 participants. (F) At 30% MVC, the percentage of early recruited identified motor units logarithmically
 648 decreases with interelectrode distance d (4, 8, 12, and 16mm in abscissa) as $44.6 - 13.1 \log(d)$ ($r^2 =$
 649 $0.91, p = 2.8 \cdot 10^{-3}$). (G) At 30% MVC, the percentage of early recruited identified motor units does
 650 not vary with the size of the grid s (2, 3.8, 7.7, and 36 cm^2 in abscissa), the logarithmic trendline fitting
 651 ($20.5 + 1.2 \log(s)$) returning a negligible slope and low $r^2 = 0.28$ ($p = 8 \cdot 10^{-4}$). The standard
 652 deviation across subjects is displayed with vertical bars. Two decomposition procedures were
 653 considered for the 256-electrode condition; the grid of 256 black electrodes indicates that the 256 signals
 654 were simultaneously decomposed and the grid of 256 electrodes of four different colors indicates that
 655 four subsets of 64 electrodes were decomposed. To maintain consistency with the computational study,
 656 the trendlines were fitted with the 4*64 condition, which returned the higher number of identified motor
 657 units (see Figure 4-1 for the other fitting condition). We did not report the results when five or fewer
 658 motor units were identified in one condition for three or more participants.



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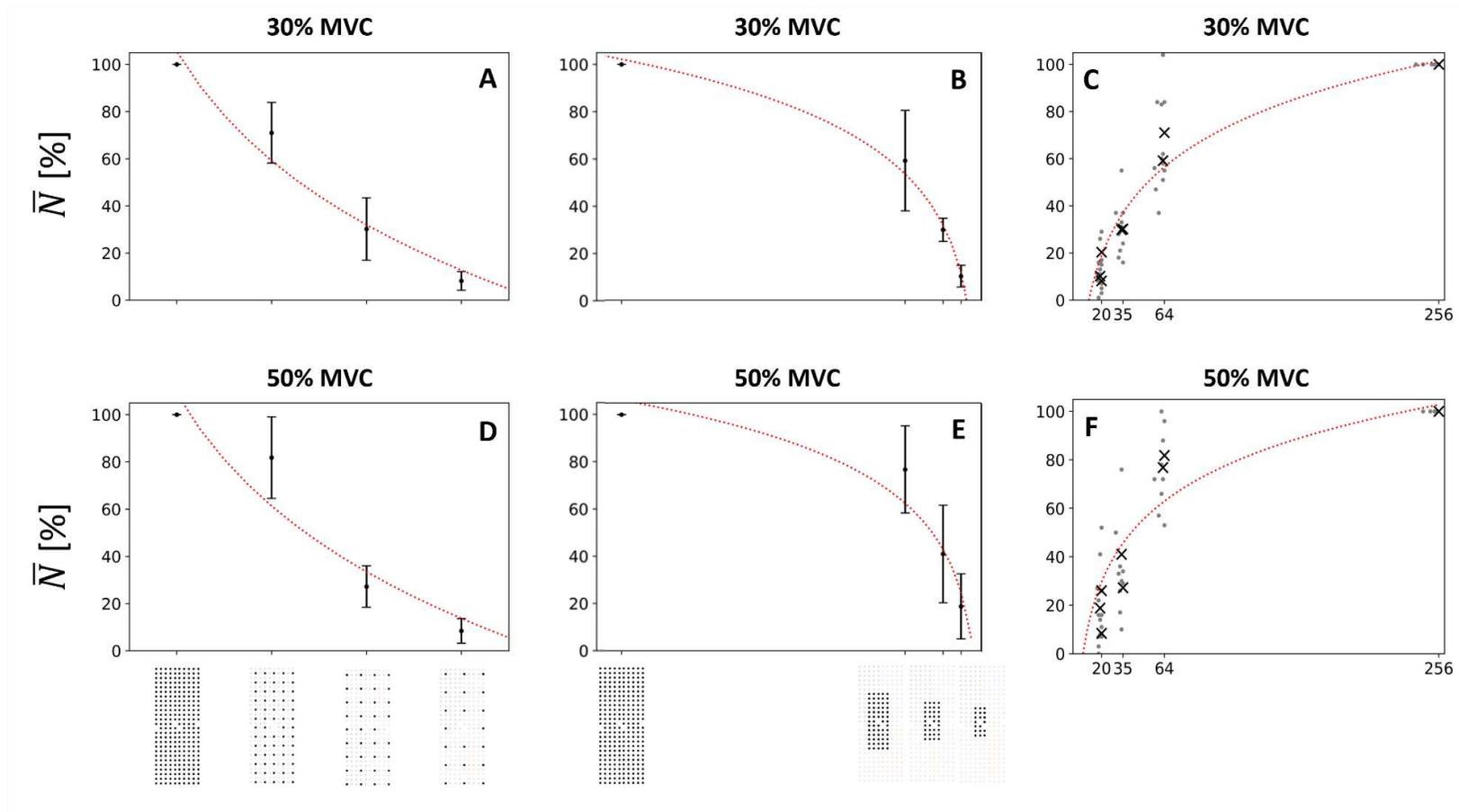
Figure 8: Effect of the electrode density on the correlation ρ between the profiles of motor unit action potentials (MUAP) detected over adjacent electrodes (A) at 30% (B) and 50% MVC (C). The profile of the MUAP detected over the red electrode was compared to those detected over the four adjacent electrodes separated by a 4 (orange), 8 (blue), 12 (green) and 16 (purple) mm IED (A). The boxplots denote the correlation coefficient ρ per participant (grey dots) and the median (orange line), quartiles, and 95%-range across participants.



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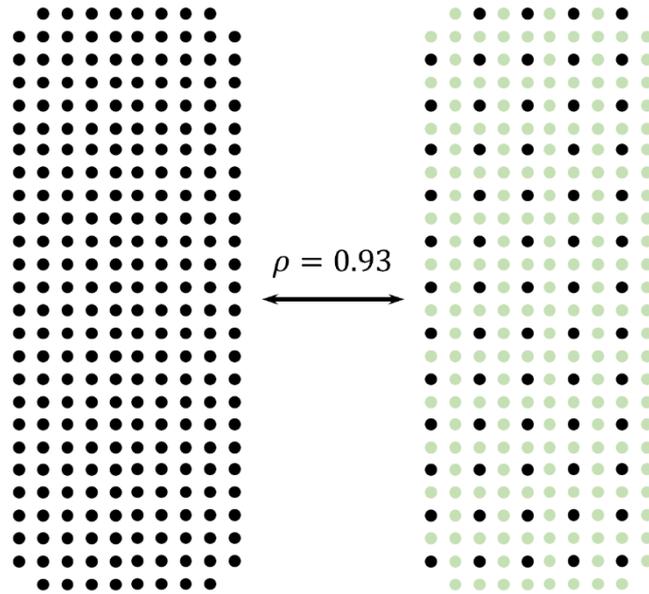
669 Figure 9: Results for the ultra-dense prototyped grid (2 mm IED, 5 x 1.8 cm, 256 electrodes). (A)
670 Description of the ultra-dense grid, where grey circles represent the electrodes. On average, the
671 correlation between the profiles of MUAPs detected over electrodes separated by an IED of 2 mm
672 (orange), 4 mm (blue), and 8 mm (purple) reached $\rho = 0.98$, 0.96, and 0.92 at 30% MVC, respectively,
673 and 0.93, 0.88, and 0.85 at 50% MVC, respectively. (B) Series of discharge times for motor units
674 identified at 30% (left) and 50% MVC (right). The dark ticks represent the discharge times identified
675 with a grid of electrodes with an 8-mm IED. The discharge times in blue were additionally identified
676 with a grid of electrodes with a 4-mm IED, and the discharge times in red were additionally identified
677 with a grid of electrodes with a 2-mm IED. All the pulse trains identified with one grid were also
678 identified with the denser grids. (C) Effect of electrode density on the number of identified motor units
679 at 30% (scatters) and 50% MVC (triangles). The trendlines from the density analysis in Figure 4B, D are
680 also reported (red dotted lines). To maintain consistency with the other results, the grid was decomposed
681 as four independent subsets of 64 electrodes, as explained in the Methods, to identify the higher number
682 of motor units.

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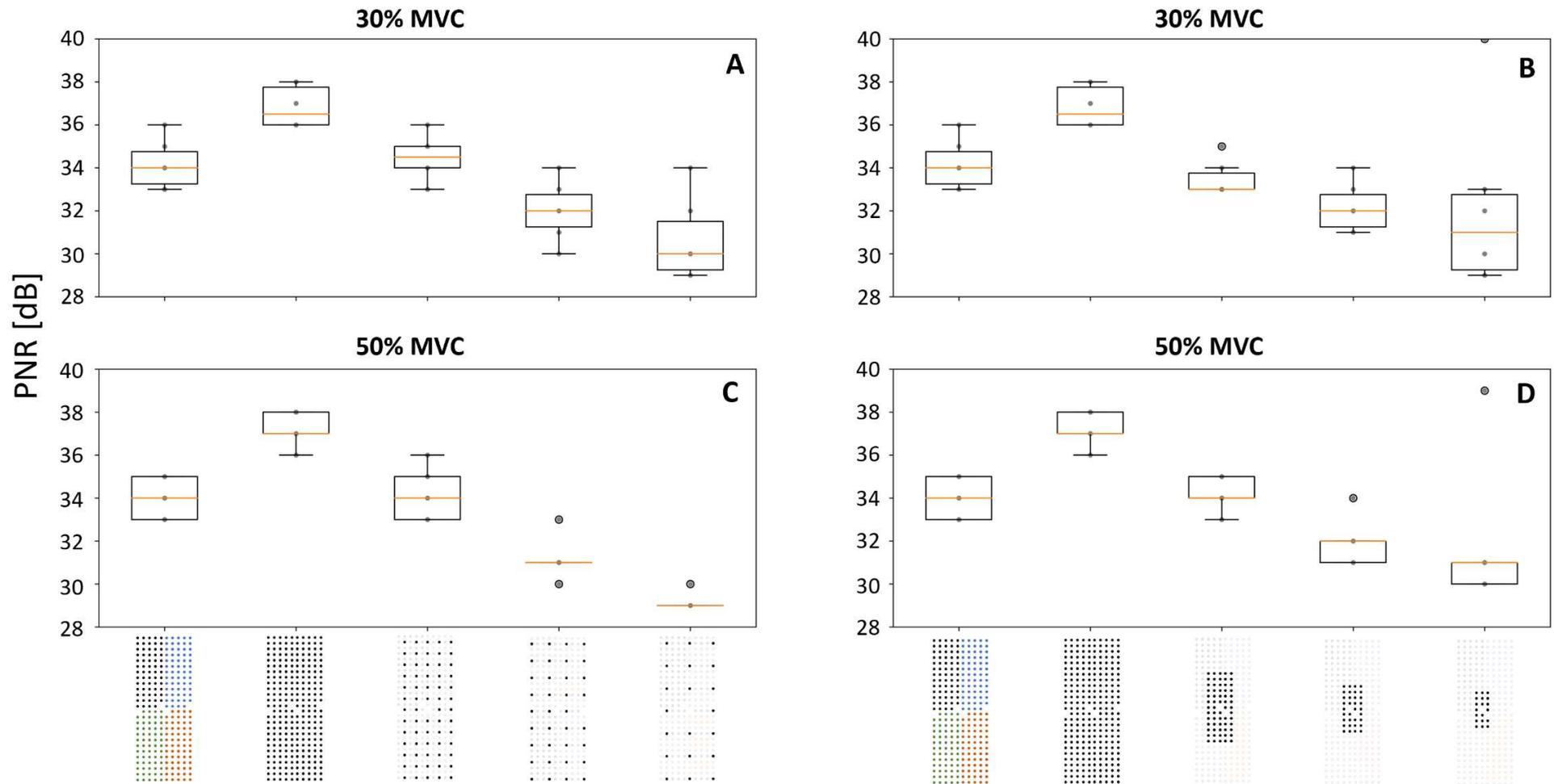
685 Figure 4-1. Effect of the density of the grid (A, D), the size of the grid (B, E), and the number of electrodes (C, F) on the normalized number \bar{N} of identified motor units at 30%
 686 (A, B, C) and 50% MVC (D, E, F). \bar{N} was estimated after decomposing the full grid of 256 electrodes and manually editing the motor unit pulse trains. Vertical bars (A, B, D,
 687 E) are the standard deviation of \bar{N} across subjects, scatters are the individual data points, and crosses are their mean (C, F). Logarithmic trendlines were fitted between the
 688 averaged values \bar{N} and IED, grid size, and number of channels, as in Figures 4, 5, and 6 of the main document. Here, the trendlines were fitted with the values obtained from
 689 the decomposition of the full grid of 256 electrodes. Consistent with the results provided in the main document, \bar{N} increased with electrode density (d), grid size (s), and with
 690 the number of electrodes (n) following statistically significant logarithmic trendlines ($p < 0.05$). At 30% MVC, $\bar{N} = 198 - 67 \log(d)$ ($r^2 = 0.92$), $\bar{N} = -10 + 31 \log(s)$ ($r^2 = 0.98$), and
 691 $\bar{N} = -78 + 32 \log(n)$ ($r^2 = 0.90$). At 50% MVC, $\bar{N} = 204 - 69 \log(d)$ ($r^2 = 0.92$), $\bar{N} = 5 + 28 \log(s)$ ($r^2 = 0.98$), and $\bar{N} = -57 + 29 \log(n)$ ($r^2 = 0.90$). It is noteworthy that the trendlines exhibited more pronounced plateaus (lower b value in the $y = a + b \cdot \log(x)$ trendlines) with the decomposition of
 692 the full grid of 256 electrodes than with the decomposition of subsets of 64 electrodes.
 693



694

695 Figure 4-2. Correlation ρ between experimentally recorded (Left, black) and interpolated (Right, green)
696 EMG signals (Right, black). Using the ultra-dense grid of 256 electrodes (2-mm IED) at 30% MVC, we
697 spatially interpolated down-sampled montages of 4x9 electrodes with an IED of 8 mm and 5x13
698 electrodes with an IED of 4 mm to generate 5x13 (4-mm IED) and 10x26 (2-mm IED) grids of
699 electrodes, respectively. In these interpolated grids, 25% of the signals were therefore experimentally
700 recorded (Right, black) and 75% interpolated (Right, green). After comparing interpolated and
701 experimentally recorded grids of electrodes, we observed that a better signal reconstruction was obtained
702 with the 2-mm IED, with a correlation coefficient of $\rho = 0.93 \pm 0.09$ between recorded and interpolated
703 signals. We identified 4 and 19 motor units from the interpolated grid with a 4-mm and 2-mm IED,
704 respectively, vs. 19 and 24 motor units with the experimentally recorded signals. We only identified the
705 same motor units as identified with the original less dense grids used to generate the interpolation. These
706 results indicate that interpolation is not sufficient to reconstruct signals from a lower spatial sampling.
707 This may be due to the spatial bandwidth which is greater than the inverse of the minimal interelectrode
708 distance used or to the edge effects of the interpolation due to the relatively small size of the grid.

709



710

711 Figure 4-3. Effect of the electrode density (A, C) and grid size (B, D) on the average PNR across the identified spike trains at 30% MVC (A, B) and 50% MVC (C, D). The
 712 boxplots report the average PNRs per participant (grey dots) and the median (orange line), quartiles, and 95%-range across participants. We calculated the average PNR value
 713 for the motor unit spike trains (PNR > 28 dB) identified in each subject and condition. The average PNR across identified motor units increased together with both the density
 714 and the size of the grid. The lowest PNR values were observed with 16 mm-IED (30 ± 1.8 dB at 30% MVC and 29 ± 1.2 dB at 50% MVC) and with a grid of 2 cm^2 (31 ± 0.9
 715 dB at 30% MVC and 30 ± 0.9 dB at 50% MVC). The highest PNR was observed with 4 mm-IED and a grid of 36 cm^2 (36 ± 0.7 dB at 30% MVC and 37 ± 0.7 dB at 50% MVC),
 716 enabling the operators to quickly edit the identified motor units.