



HAL
open science

A comparison of neural control of the biarticular gastrocnemius muscles between knee flexion and ankle plantar flexion

Raphaël Hamard, Jeroen Aeles, Simon Avrillon, Taylor J M Dick, François Hug

► To cite this version:

Raphaël Hamard, Jeroen Aeles, Simon Avrillon, Taylor J M Dick, François Hug. A comparison of neural control of the biarticular gastrocnemius muscles between knee flexion and ankle plantar flexion. *Journal of Applied Physiology*, 2023, 135 (2), pp.394-404. 10.1152/jappphysiol.00075.2023 . hal-04395083

HAL Id: hal-04395083

<https://hal.science/hal-04395083>

Submitted on 16 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

16 **Abstract**

17 We aimed to determine whether the neural control of the biarticular gastrocnemius medialis
18 (GM) and lateralis (GL) muscles is joint-specific, i.e, whether their control differs between
19 isolated knee flexion and ankle plantar flexion tasks. Twenty-one male participants performed
20 isometric knee flexion and ankle plantar flexion tasks while we recorded high-density surface
21 electromyography (HDsEMG). First, we estimated the distribution of activation both within-
22 and between-muscles using two complementary approaches: surface EMG amplitude and
23 motor unit activity identified from HDsEMG decomposition. Second, we estimated the level of
24 common synaptic input between GM and GL motor units using a coherence analysis. The
25 distribution of EMG amplitude between GM and GL was not different between tasks, which
26 was confirmed by the analysis of motor units discharge rate. Even though there was a significant
27 proximal shift in GM and GL EMG amplitude during knee flexion compared to ankle plantar
28 flexion, the magnitude of this shift was small and not confirmed via the inspection of the spatial
29 distribution of motor unit action potentials. A significant coherence between GM and GL motor
30 units was only observed for four (knee flexion) and three (ankle plantar flexion) participants,
31 with no difference in the level of coherence between the two tasks. We were able to track only
32 a few motor units across tasks, which raises the question as to whether the same motor units
33 were activated across tasks. Our results suggest that the neural control of the GM and GL
34 muscles is similar across their two main functions.

35 **Keywords:** coherence; activation; triceps surae; electromyography; motor units; common
36 drive.

37 **New & Noteworthy**

38 Several studies have focused on the neural strategies used to control the gastrocnemius medialis
39 (GM) and lateralis (GL) during ankle plantar flexion. However, their secondary function, i.e.
40 knee flexion, is not often explored. We observed a robustness of the GM and GL activation
41 strategy across tasks, which was further confirmed with an analysis of the motor unit discharge
42 characteristics. The level of common synaptic input between GM and GL motor units was low,
43 regardless of the task.

44

45 **Introduction**

46 Muscle coordination is for a large part determined by how skeletal muscles are activated by the
47 central nervous system. It appears that muscle activation is not always evenly distributed among
48 muscles from the same anatomical group. For example, within the quadriceps, during
49 submaximal isometric knee extension, some individuals activate the vastus lateralis (VL) more
50 than the vastus medialis (VM), whereas others use the reverse strategy (1–4). This distribution
51 of muscle activation between VL and VM is robust across different tasks such as walking and
52 pedalling (3). An imbalance in the distribution of activation also exists between the biarticular
53 gastrocnemius medialis (GM) and gastrocnemius lateralis (GL) during ankle plantar flexion. In
54 this case, the activation is almost always biased towards the GM, with a large amount of
55 variation in the degree of this imbalance across individuals (2, 3). The imbalance of activation
56 between GM and GL is related to their differences in maximal force-generating capacity (2).
57 Specifically, the greater the physiological cross-sectional area (PCSA) of GM compared to the
58 PCSA of the GL, the stronger the bias in muscle activation towards the GM. In contrast to what
59 is observed across different tasks for VL and VM (3), the strategy used to control the biarticular
60 GM and GL seems more variable across tasks. For example, foot position (i.e. external versus
61 internal rotation) induces a modification in the distribution of activation between the
62 gastrocnemii (5–7). This more flexible control of the GM and GL muscles is associated with a
63 relatively low level of, if any, common synaptic input between these muscles (8, 9). Even
64 though such a flexible control may be needed to manipulate the ankle joint in the frontal plane
65 where the GM and GL generate opposite moment (10, 11), it occurs at the expense of the
66 number of dimensions that need to be controlled by the nervous system.

67 Previous studies have focused on the neural strategies used to control the GM and GL during
68 ankle plantar flexion (12–14). However, as biarticular muscles, they also cross the knee joint
69 and contribute to the knee flexion moment (15). This second function is not often explored,
70 leaving a knowledge gap in our understanding of the control and function of the gastrocnemii,
71 and more broadly of biarticular muscles. There is evidence that the activation of biarticular
72 muscles is joint-specific. For example, hip flexion is associated with a preferential recruitment
73 of the proximal portion of the rectus femoris muscle whereas knee extension is associated with
74 more distal activation of the rectus femoris (16–18). There is no consensus on whether such
75 task-specific regional activation occurs in the human GL and GM muscles. Region-specific
76 functional roles of GM have been suggested in some (18), but not all studies (19). Yet, the task-
77 specific modulation of common synaptic input has not been examined in biarticular muscles.

78 Specifically, if the low level of common synaptic input between GM and GL observed during
79 ankle plantar flexion (8) is necessary to control additional degrees of freedom of the ankle, then
80 it is possible that these muscles receive a higher level of common synaptic input during a task
81 whereby these muscles control only one degree of freedom, e.g. knee flexion. This may
82 decrease the number of degrees of freedom that needs to be controlled by the central nervous
83 system.

84 In this study we determined whether the neural control of the GM and GL differs between a
85 knee flexion and an ankle plantar flexion task performed at the same relative activation level
86 and at the same joint configuration. The main aim was to estimate the distribution of activation
87 between the GM and GL using two complementary approaches: the amplitude of surface high-
88 density electromyographic signals (HDsEMG) and the discharge rate of individual motor units
89 identified from decomposed HDsEMG signals. Based on the observation that there is a coupling
90 between the distribution of activation and the distribution of force-generating capacity (2), we
91 hypothesized that the distribution of activation would be robust across tasks where no specific
92 control of moment in the frontal plane is required. The second aim was to investigate the
93 regional activation within gastrocnemii from the amplitude of HDsEMG signals and from the
94 spatial location of the motor units action potentials. We hypothesized that the regional
95 activation would remain similar across knee flexion and ankle plantar flexion based on the
96 limited possibility to regionally recruit motor units over the gastrocnemius muscle (19). Third,
97 we aimed to compare the level of common synaptic input within each muscle and between GM
98 and GL across tasks using a coherence analysis applied to motor unit spike trains. We
99 hypothesized that the motor units extracted from the GM and GL would show a higher
100 coherence during knee flexion compared to ankle plantar flexion, which may be an effect of the
101 higher common synaptic input. This would reduce the number of dimensions to be controlled
102 during knee flexion.

103 **Methods**

104 Participants

105 Twenty-one physically active males participated in this study (mean \pm standard deviation; age:
106 27.3 ± 4.3 years, height: 176 ± 24 cm, body mass: 77.1 ± 12.9 kg). Of note, we recruited only
107 males because we failed to identify enough GL motor units on females during our pilot testing.
108 Participants had no history of lower leg pain that had limited function that required time off
109 work or sport, or a consultation with a health practitioner in the previous six months. They
110 provided informed written consent prior to the experimental session. The study was approved
111 by the institutional research ethics committee of Nantes University (CERNI n°28022022-1).

112 Experimental design

113 The experimental protocol consisted of isometric knee flexion and ankle plantar flexion
114 contractions. For both knee flexion and ankle plantar flexion, participants laid prone on a
115 dynamometer bed (Biodex System 3 Pro, Biodex Medical, Shirley, NY) with their right knee
116 and ankle secured at 160° (180° degrees equals a fully-extended knee) and 90° (foot
117 perpendicular to the shank), respectively. During the knee flexion task, we used a custom brace
118 to secure the ankle at 90° . Two inextensible straps immobilized the back and the right knee of
119 the participants.

120 Participants performed the knee flexion and ankle plantar flexion tasks in a randomized order.
121 The procedure was similar for knee flexion and ankle plantar flexion tasks. Participants began
122 with a standardized and progressive warm-up which consists in isometric knee flexion or ankle
123 plantar flexion. After 2 min of rest, they performed three maximal isometric voluntary
124 contractions (MVC), held for 3 s, with 120 s of rest between each contraction. Peak torque was
125 considered as the maximal torque value obtained through a moving average with a window of
126 250 ms. To compare the neural control of GM and GL between the knee flexion and ankle
127 plantar flexion tasks, it was important to match the averaged activation between tasks. We
128 observed during our pilot testing that these muscles exhibit a lower activation during knee
129 flexion than ankle plantar flexion for the same relative task-specific peak torque. Then, it was
130 necessary to use higher contraction intensities for knee flexion. Therefore, participants
131 performed submaximal isometric contractions, presented in a randomized order, at the
132 following intensities: 30%, 40%, and 50% of peak torque for knee flexion and 20%, 30%, and
133 40% of peak torque for ankle plantar flexion, and the best match of activation between these
134 relative torque levels was determined *a posteriori* as further described below. Of note, we chose

135 to match the average GM-GL activation *a posteriori* instead of asking the participants to
136 produce a specific activation level using biofeedback as it would have likely modified the
137 natural coordination strategies. For each intensity, participants performed three trapezoidal
138 isometric contractions with 60 s of rest between each contraction. The contraction consisted of
139 a 5 s ramp-up, 20 s steady contraction at the torque target and a 5 s ramp-down. A monitor
140 displayed the target torque levels and the real-time torque output to the participants, digitized
141 at 2048 Hz and provided by the HDsEMG acquisition system (EMG-Quattrocento; 400-channel
142 EMG amplifier, OT Bioelettronica, Italy).

143 Surface electromyography recordings

144 HDsEMG signals from the GM and the GL of the right leg were recorded using two-
145 dimensional adhesive grids of 64 electrodes (13×5 electrodes with one electrode absent on a
146 corner, gold-coated, interelectrode distance: 8 mm; GR08MM1305 OT Bioelettronica, Italy).
147 First, we identified the muscle boundaries using B-mode ultrasound (Aixplorer, Supersonic
148 Imagine, France) such that we could place the HDsEMG grid in the center of the muscle belly
149 to minimize crosstalk. B-mode ultrasound was also used to define the fascicle orientation and
150 align the grid in the direction of the fascicles. Then we shaved, cleansed, and abraded the skin
151 using abrasive gel to reduce the skin-electrode impedance. A reference electrode (Kendall
152 Medi-Trace, Canada) and a strap ground electrode were placed over the right tibia and around
153 the left ankle, respectively. Finally, we secured the electrodes with elastic bandages to ensure
154 good skin-electrode contact throughout the experiment. The multichannel HDsEMG
155 acquisition system (EMG-Quattrocento, 400 channel EMG amplifier; OT Bioelettronica, Italy)
156 band-pass filtered (10-500 Hz) and digitized the recorded monopolar signals at a sampling rate
157 of 2048 Hz.

158 Data analysis

159 *Global surface electromyography*

160 Analyses were performed offline following the experiment in Matlab (R2018b, The
161 MathWorks, Natick, MA) using custom-written scripts. First, the signals were band-pass
162 filtered (20-500 Hz). Then, we visually checked the HDsEMG signals to remove channels with
163 excessive noise or artefacts. To estimate the normalized electromyography (EMG) amplitude
164 from the HDsEMG signals, we calculated the differential signals by taking the difference
165 between adjacent electrodes in the proximo-distal direction, which resulted in 59 differential
166 signals. When the signal from an electrode was excluded because of noise or artefacts, we

167 linearly interpolated the EMG amplitude from all the adjacent electrodes. For the MVC trials,
168 we averaged the rectified signals over the whole grid and the maximal value of a 500 ms moving
169 mean from the three MVC was considered as the maximal EMG value (EMG_{max}). For the
170 submaximal contractions, each differential signal was rectified and then normalized to EMG_{max}
171 measured during ankle plantar flexion. We used EMG_{max} measured during ankle plantar flexion
172 as this value was systematically higher than that measured during knee flexion. Of note, as our
173 aim was to compare EMG amplitude across tasks within a person and muscle, without removing
174 electrodes, the value used to normalize the signals does not impact the outcomes (20). Finally,
175 we calculated the average of these submaximal, normalized EMG values first over the 3
176 plateaus for each channel and then over the 59 signals to get one representative value per
177 muscle.

178 Normalized HDsEMG values were used to find an appropriate match for the comparison
179 between knee flexion and ankle plantar flexion. To this end, we averaged the normalized
180 HDsEMG value between the GM and GL for each intensity and each task, to obtain a single
181 'gastrocnemius activation value' per intensity and per task. For further analyses, we only used
182 the knee flexion intensity and the ankle plantar flexion intensity that exhibited the closest
183 normalized gastrocnemius activation between the two tasks. Second, we used the HDsEMG
184 normalized values to calculate the $GM/(GM+GL)$ ratio of activation during both the knee
185 flexion and the ankle plantar flexion tasks. Third, we assessed the regional activation for each
186 muscle and for each task by calculating the barycenter of the normalized EMG amplitude across
187 the 2D EMG grid (Fig. 1).

188 *EMG decomposition*

189 We used the convolutive blind source separation method to decompose the HDsEMG signals
190 into individual motor unit spiking activity (21). Of note, this analysis was only performed on
191 the contractions that were matched between tasks in terms of normalized EMG amplitude (see
192 above). The automatic decomposition was performed on the plateau of the trapezoidal
193 contraction that exhibited the highest EMG amplitude. Then, we manually edited all the motor
194 units according to previously published procedures (22, 23). The goal of this manual editing
195 was to remove the false positive spikes identified by the automatic processing and to add the
196 false negative spikes that were not identified by the automatic selection process. The motor unit
197 filters derived from the processing of this plateau were then reapplied on the other plateaus.
198 Consistent with previous studies, for further analyses, we retained the motor units that had a
199 pulse-to-noise ratio above 30 dB (24, 25).

200 *Assessment of crosstalk*

201 We ensured that each motor unit identified in a HDsEMG grid did not originate from crosstalk,
202 that is, if any unit identified in one muscle actually belonged to the other recorded muscle. To
203 do this, the firing times of each motor unit of the testing muscle were used to extract the
204 waveform of each motor unit potential in all 59 differential EMG channels of the grid for each
205 of the two muscles (26). For each channel and each grid separately, peak-to-peak amplitude of
206 the motor unit action potential was determined. We then compared the maximal amplitude
207 between the grids, under the assumption that the action potential amplitude should be largest in
208 the signal obtained from the electrodes closest to the discharging muscle fibres. If the amplitude
209 in the other grid exceeded the amplitude of the testing muscle, it was deemed that the motor
210 unit was identified due to crosstalk, and it was removed from the analyses (26, 27).

211 *Assessment of motor unit discharge rate and spatial location*

212 For this specific analysis, we retained only the participants that had at least three motor units
213 identified for each muscle and for each task. For the GM, only 19 and 18 participants met this
214 requirement for the knee flexion and ankle plantar flexion, respectively. For the GL, only nine
215 and 12 participants met this requirement for the knee flexion and ankle plantar flexion,
216 respectively. First, we calculated the average discharge rate of each motor unit over each plateau
217 for both the knee flexion and ankle plantar flexion tasks. We considered only the instantaneous
218 discharge rates between 5 pps and 20 pps (28). Then, we averaged the values across the three
219 contractions to obtain a single value for each task. Second, we determined the spatial location
220 of the action potential of each motor unit over the grid of electrodes. For this purpose, we used
221 the shape of the motor unit action potentials obtained from the spike-trigger averaged
222 differential EMG signals of the 59 channels. Then, we calculated the peak-to-peak amplitude
223 of the motor unit action potential for each channel and we determined the barycenter of the
224 peak-to-peak amplitude for each grid, i.e. muscle.

225 *Coherence analysis*

226 To estimate the amount of common synaptic input within each muscle and between muscles,
227 we performed a coherence analysis on cumulative motor unit spike trains. We first maximized
228 the number of motor units and the duration used for the coherence analysis for each participant.
229 Specifically, we discarded the portions of the signal where not enough motor units were
230 discharging simultaneously, and we also discarded the motor units that had discharge patterns
231 that were too intermittent during the torque plateau (interspike interval > 500 ms). For the
232 within-muscle coherence, we retained only the participants when they had at least four motor

233 units identified per muscle, which allowed us to calculate the coherence between two groups of
234 two motor units. For the GM, only 18 participants met this requirement for both the knee flexion
235 and ankle plantar flexion. For the GL, only five and ten participants met this requirement for
236 the knee flexion and ankle plantar flexion, respectively. For the between-muscle coherence, we
237 retained only the participants that had at least three motor units identified per muscle and per
238 task. Only nine participants met this requirement. As coherence is affected by the duration on
239 which it is calculated, we used the same duration for both tasks, i.e. on average 23 ± 9 s, within
240 a participant. The within-muscle coherence was performed on 12 ± 6 and 6 ± 3 motor units for
241 GM and GL, respectively during knee flexion and on 14 ± 6 and 9 ± 6 motor units for GM and
242 GL, respectively during ankle plantar flexion. The between-muscle coherence was performed
243 on 11 ± 6 and 5 ± 3 motor units for GM and GL, respectively during knee flexion and on $17 \pm$
244 6 and 9 ± 7 motor units for GM and GL, respectively during ankle plantar flexion.

245 We calculated the magnitude-squared coherence using the Welch periodogram with
246 nonoverlapping windows of 1 s (1, 26, 27, 29). This analysis was performed on two equal-sized
247 groups of cumulative spike trains. We used a constant number ($n=2$ and $n=3$ for the within-
248 muscle coherence and the between-muscle coherence, respectively) of motor units to calculate
249 the cumulative spike trains as the number of motor units affects the level of coherence.
250 However, to consider all the identified motor units in our analysis, we tested all the unique
251 combinations of motor units from each participant's entire available motor unit pool, with the
252 maximal number of permutations set to 100. We considered the pooled coherence of these 100
253 random permutations for further analysis and we focused on the coherence within the delta band
254 (0-5 Hz), which reflects the presence of common synaptic input relevant for force modulation
255 (25, 26). Consistent with previous studies (25, 26, 30), we transformed the coherence values to
256 a standard z-score:

$$257 \quad \text{COH z score} = \sqrt{2L} \times \text{atanh} \sqrt{\text{COH}} - \text{bias} \quad \text{Eq. 1}$$

258 Where COH is coherence, L is the number of time segments considered for the coherence
259 analysis, and bias is calculated as the mean COH z score between 250 and 500 Hz where no
260 coherence is expected. As classically done, the significance threshold for the Z-score
261 transformed coherence was set at 1.65, based on a one-tailed 95% confidence interval (25).

262 *Motor unit matching*

263 We assessed the proportion of motor units which were identified during both tasks. To do this,
264 we tracked motor units across knee flexion and ankle plantar flexion tasks using the spatio-

265 temporal properties of the action potential waveforms within the 2D grids similar to previous
266 studies (31, 32). Briefly, we first identified the motor unit action potential waveform from
267 differential signals for each electrode using the spike-triggered averaging technique previously
268 described. The motor unit waveforms from the 59 electrodes were concatenated. We then
269 performed cross-correlations between the waveforms of motor units. We considered motor
270 units within a pairs as matched across tasks when they exhibited a coefficient of correlation
271 higher than 0.75 (32). We visually checked the matched motor unit action potentials to ensure
272 the quality of the matching and removed incorrectly matched units when necessary (Fig. 1).

273 Statistical analysis

274 All statistical analyses were performed with R (v4.3.0, Vienna, Austria). First, we confirmed
275 whether all the data were normally distributed using Kolgomorov-Smirnoff test. To compare
276 the average gastrocnemius activation across the matched knee flexion and ankle plantar flexion
277 tasks, we performed a two-tailed paired t-test. We used linear mixed effects models (lme.R
278 function from the nlme package (33)) to compare (i) the overall normalized EMG amplitude
279 between muscles and between tasks; (ii) the mean GM and GL discharge rate between tasks,
280 (iii) the regional activation, determined as the x and the y coordinates of the barycenter of EMG
281 amplitude, between tasks for each muscle and (iv) the spatial location of motor units,
282 determined as the x and the y coordinates of the barycenter of motor unit action potentials,
283 between tasks for each muscle. Of note, regarding the regional activation of EMG and the
284 spatial location of motor unit action potentials, we did not interpret the main effect of muscle
285 as the barycenter's coordinates were determined with respect to the grid location, which
286 precludes any meaningful comparison between muscles. In all models, muscle (GM, GL) and
287 task (knee flexion, ankle plantar flexion) were specified as fixed factors with participant as a
288 random factor.

289 To determine whether the distribution of activation was correlated across tasks at the population
290 level, we calculated the Pearson correlation coefficient on the GM/(GM+GL) ratio of activation.
291 Finally, to compare the level of common synaptic input between tasks, we used a two-tailed
292 paired t-test on the mean z-score values between 0 and 5 Hz. For all tests, the level of
293 significance was set at $p < 0.05$.

294 **Results**

295 Global surface EMG

296 *EMG amplitude*

297 We first matched the knee flexion and ankle plantar flexion tasks based on the average
298 gastrocnemius activation. In most of the participants (n=6/21), the best match was obtained by
299 using an intensity of 40% and 30% of the task-relative peak torque for knee flexion and ankle
300 plantar flexion, respectively. This led to an average gastrocnemius EMG amplitude of $16.1 \pm$
301 6.3% of EMG_{max} for knee flexion and $17.1 \pm 5.1\%$ of EMG_{max} for ankle plantar flexion ($p =$
302 0.061 ; Fig. 2). The average within-participant difference in average gastrocnemius EMG
303 amplitude between knee flexion and ankle plantar flexion was $2.0 \pm 1.7\%$ of EMG_{max} .

304 For the separate muscles, the GM EMG amplitude was $21.9 \pm 8.0\%$ and $21.8 \pm 5.6\%$ of EMG_{max}
305 during knee flexion and ankle plantar flexion, respectively (Fig. 3). The GL EMG amplitude
306 was $10.3 \pm 7.2\%$ and $12.4 \pm 6.2\%$ of EMG_{max} during knee flexion and ankle plantar flexion,
307 respectively. There was a main effect of muscle ($p < 0.001$) but no main effect of task ($p =$
308 0.294) nor a muscle \times task interaction ($p = 0.250$). Specifically, normalized GM EMG amplitude
309 was significantly higher than normalized GL EMG amplitude, leading to a GM/(GM+GL) ratio
310 of $65.0 \pm 10.0\%$ and $70.7 \pm 12.9\%$ in knee flexion and ankle plantar flexion, respectively. The
311 GM/(GM+GL) ratio measured during knee flexion was correlated to that measured during
312 plantar flexion ($R = 0.604$; $p = 0.004$; Fig. 4). This demonstrates that participants who exhibited
313 a greater bias of activation to GM during knee flexion also exhibited a greater bias of activation
314 to GM during ankle plantar flexion.

315 As indicated in the methods section, the aforementioned results were derived from values
316 averaged over the entire torque plateaus. Even though GM and GL EMG amplitude was
317 relatively constant during the plateau for ankle plantar flexion, GM and GL EMG amplitude
318 increased over the torque plateau for knee flexion (Fig. 5). To ensure that these different profiles
319 did not impact the results, we repeated the analysis for the knee flexion task considering only
320 the second half of each plateau, where EMG amplitude was the highest during knee flexion.
321 Results were similar to those presented above, with an average difference in average
322 gastrocnemius EMG amplitude between the matched knee flexion and ankle plantar flexion
323 task of $3.1 \pm 2.8\%$ of EMG_{max} . The GM/(GM+GL) ratio of EMG amplitude was correlated
324 between tasks ($R = 0.591$; $p = 0.005$). Results was therefore consistent between the initial

325 method and this revised method that only considered the part of the knee flexion plateau where
326 GM and GL are substantially activated.

327 *Regional activation*

328 When considering the proximo-distal (y axis) distribution of EMG amplitude, there was a main
329 effect of task ($p = 0.019$) but no muscle \times task interaction ($p = 0.116$). Specifically, the EMG
330 amplitude was located 1.0 ± 1.4 mm more proximally on the EMG grid during knee flexion
331 than during ankle plantar flexion, regardless of the muscle. Of note, this difference of 1 mm,
332 albeit significant, is relatively small. When considering the medio-lateral (x axis) distribution
333 of EMG amplitude, there was no main effect of task ($p = 0.157$) nor a muscle \times task interaction
334 ($p = 0.252$).

335 Motor unit discharge characteristics

336 We decomposed the HDsEMG signals into the activity of individual motor units. After the
337 crosstalk assessment, four GL motor units were considered as crosstalk motor units and
338 therefore discarded for further analyses. For the GM, we identified on average 14.0 ± 8.1 (range:
339 5-31) motor units and 16.3 ± 6.9 (range: 6-32) motor units per participant during knee flexion
340 and ankle plantar flexion, respectively. For the GL, we identified on average 7.2 ± 6.6 (range:
341 3-24) motor units and 8.9 ± 6.9 (range: 3-23) motor units per participant for knee flexion and
342 ankle plantar flexion, respectively. Among these motor units, only a small proportion were
343 identified during both tasks: $5.8 \pm 10.6\%$ for GM ($n = 0.7 \pm 1.2$ motor units per participant) and
344 $7.5 \pm 11.8\%$ for GL ($n = 1.0 \pm 2.0$ motor units per participant). As this proportion is small, the
345 following results are reported for the whole population of identified units, regardless of whether
346 they could be matched between conditions.

347 *Average discharge rate*

348 The discharge rate of GM motor units was 11.6 ± 2.3 pps during knee flexion and 10.1 ± 1.5
349 pps during ankle plantar flexion. The discharge rate of GL motor units was 10.8 ± 1.7 pps during
350 knee flexion and 10.3 ± 1.8 pps during ankle plantar flexion. There was a significant effect of
351 task ($p < 0.001$), but no effect of muscle ($p = 0.388$) nor task \times muscle interaction ($p = 0.0848$).
352 Specifically, the discharge rate was significantly higher during knee flexion compared to ankle
353 plantar flexion, regardless of the muscle.

354 *Spatial localization of identified motor units*

355 The linear mixed effect model did not reveal a main effect of task ($p = 0.820$) or a muscle \times task
356 interaction ($p=0.295$) on the proximo-distal location of the identified motor units. Similar
357 results were observed for the medio-lateral location of motor units, with no main effect of task
358 ($p = 0.399$) or a muscle \times task interaction ($p = 0.120$).

359 Within- and between-muscle coherence

360 For both the within-muscle coherence and the between-muscle coherence, we considered the
361 mean coherence value over the delta band (0-5 Hz) as an index of the level of common synaptic
362 input received by GM and GL. When considering the coherence within the GM muscle, we
363 observed a significant z-score for 16 out of 18 participants during knee flexion and for all the
364 participants (n=18) during ankle plantar flexion (Fig. 6). When considering the coherence
365 within the GL muscle, we observed a significant z-score for four participants out of five during
366 knee flexion and for all the participants (n=10) during ankle plantar flexion. This indicates that
367 motor neurons innervating each muscle received a significant level of common synaptic inputs.
368 Of note, the small number of participants considered for the GL muscle precluded any robust
369 statistical analysis to test the effect of task/muscle; but this was not part of our main aims.

370 A significant z-score was only observed for four and three participants (out of nine participants)
371 during the knee flexion and ankle plantar flexion task, respectively (Fig. 7). This indicates that
372 most of the participants did not exhibit a significant level of common synaptic input between
373 motor neurons innervating the GM and GL muscles. The mean coherence value over the delta
374 band (as a z-score) was not different between tasks (knee flexion: 1.7 ± 1.2 , and ankle plantar
375 flexion: 1.4 ± 1.3 ; $p = 0.570$).

376

377 **Discussion**

378 We aimed to determine whether the neural control of GM and GL differs between their two
379 main functions in the sagittal plane, i.e., isolated knee flexion and ankle plantar flexion. In
380 accordance with our first hypothesis, we observed that the bias of activation towards GM,
381 already reported during ankle plantar flexion (3, 12), persisted during knee flexion. The
382 robustness of the GM and GL activation strategy across knee flexion and ankle plantar flexion
383 was further confirmed by the analyses of the motor unit discharge rate and the spatial location
384 of EMG amplitude and identified motor units. Contrary to our hypothesis, the level of common
385 synaptic input between GM and GL remained relatively low during knee flexion, with no
386 significant difference between the tasks. Overall, our results suggest that the neural control of
387 the gastrocnemius muscles at a similar overall level of activation, is consistent across their two
388 main functions.

389 We observed a higher normalized EMG amplitude for GM compared to GL during ankle plantar
390 flexion, with a GM/(GM+GL) ratio of $65.0 \pm 10.0\%$, which is consistent with previous studies
391 ($68.8 \pm 11.9\%$ in Crouzier et al., 2019; $65.0 \pm 13.2\%$ in Crouzier et al., 2018). The current study
392 extends this observation to knee flexion, during which this imbalance of activation persisted.
393 Interestingly, the distribution of activation, assessed through the GM/(GM+GL) ratio, was
394 correlated between tasks, providing further support for the existence of an individual muscle
395 activation strategy, robust across time and tasks (3, 34). Even though the origin of this
396 individual strategy is mostly unknown, preliminary evidence suggests that it could be explained
397 by a neuromechanical coupling between the activation a muscle receives and its biomechanical
398 characteristics (2, 4, 35). Specifically, previous studies reported a significant positive
399 correlation between the distribution of activation across synergist muscles and their distribution
400 of PCSA (2, 4). Considering that muscle PCSA provides an index of muscle force-generating
401 capacity (36), this observation aligns particularly well with the theory that activation strategies
402 are selected such that the sum of muscle stresses cubed is minimized (37). The consistency of
403 the distribution of muscle activation that we observed between knee flexion and ankle plantar
404 flexion may be explained by this coupling with the distribution of PCSA, which remained
405 unchanged across tasks.

406 To further probe the neural strategy, we confirmed our results with a more direct estimate of
407 the neural drive sent to the muscles. To this end, we decomposed HDsEMG signals into
408 individual motor units activity. The absence of significant muscle \times task interaction confirms
409 that the distribution of neural drive between muscles was not different between tasks. However,

410 we observed a significant effect of task, with a higher discharge rate during knee flexion
411 compared to plantar flexion, regardless of the muscle. When interpreting this result, it is
412 important to keep in mind that the time-varying profile of activation varied between tasks (Fig.
413 5) with a gradual increase during knee flexion, accompanied by recruitment of motor units
414 during the plateau of the torque profile. This led to higher peak discharge rates during knee
415 flexion, which in turn could have biased the mean toward higher values. It is also possible that
416 this difference in discharge rate is explained by the recruitment of different motor units (with
417 different intrinsic properties) between tasks. This result should be verified from matched motor
418 units across tasks, which was impossible in our study, as discussed below.

419 Previous studies have suggested that the regional activation of biarticular muscles may be task
420 dependent (17, 18). This hypothesis was based on evidence showing task-dependent regional
421 activation of the rectus femoris, as well as possible regional activation (38) and spatial
422 localization of the motor unit within the GM (39). At first glance, our results seem in agreement
423 with this hypothesis, with a significant proximal shift of EMG amplitude during knee flexion
424 compared to ankle plantar flexion, regardless of the muscle. However, the difference was
425 relatively small (1 mm) in comparison with the inter-electrode distance (8 mm), raising the
426 question of the physiological meaningfulness of this shift in EMG amplitude. Given the lack of
427 selectivity of global surface EMG (40), it is important to ensure that this small, albeit
428 significant, shift was not induced by crosstalk from distant muscles, such as the monoarticular
429 hip extensor muscles. Different gearing or fascicle shortening between knee flexion and ankle
430 plantar flexion can also explain these small differences. Importantly, a similar analysis
431 performed at the motor unit level did not confirm the existence of a significant shift, as observed
432 from global surface EMG. Even though the number of identified motor units is relatively
433 modest in regard to the total number of active units, such an analysis provides a more direct
434 estimate of the spatial location of muscle activation. This result aligns with data obtained from
435 direct recordings of motor unit activity made using fine-wire electrodes, where motor units
436 recruited in knee flexion or in ankle plantar flexion were not localized in a specific muscle
437 region (19). It is also in agreement with a study using high-density EMG which reported no
438 task-related change in regional activation within the biarticular biceps brachii muscle (41).
439 Moreover, the absence of regional activation is consistent with the high level of common
440 synaptic input within each muscle that we observed, which likely limits the possibility of joint-
441 specific motor units recruitment. Taken together, our results suggest that, although regional
442 activation within the GM has been previously observed (38, 39), the distribution of activation

443 between and within GM and GL is robust regardless of the main function for which they are
444 used, *i.e.* knee flexion or ankle plantar flexion.

445 In addition to the distribution of activation, understanding the control of synergist muscles
446 requires knowledge of the level of common synaptic input received by their motor units. To
447 explore this, we calculated the coherence between cumulative spike trains within each muscle
448 and between muscles. In accordance with recent work (8, 9), we observed an absence of
449 significant coherence between GM and GL motor units for most of the participants during ankle
450 plantar flexion, which is interpreted as the presence of little, if any, common input. Of note,
451 when the coherence exceeded the significant threshold in some participants, its amplitude was
452 relatively modest when compared to other muscle pairs such as the VL and VM (1, 25). It is
453 thought that this low level of common synaptic input is required to dissociate GM and GL
454 activation to comply with secondary roles such as modulation of the ankle moment in the frontal
455 plane (8). Because such a secondary role is likely not required for knee flexion, we hypothesized
456 that the level of common synaptic input would be higher in knee flexion compared to ankle
457 plantar flexion. However, this was not observed which is consistent with recent results showing
458 a consistent low level of common synaptic input to motor neurons innervating the GM and GL
459 muscles across ankle plantar flexion tasks performed at different ankle angles (42). Together,
460 this suggests that the nervous system has a limited ability to modify the level of common
461 synaptic input to motor neurons across tasks, even when such a change would reduce the
462 dimensions to be controlled independently. Nevertheless, it is important to consider that the
463 between-muscle coherence has been calculated on a relatively small number of participants.

464 In this study, we also attempted to determine whether the same motor units were activated
465 between knee flexion and ankle plantar flexion. Given that the activation level was matched
466 between tasks, and based on the size principle (43), one would expect that the same units were
467 active during both tasks. However, our approach was able to track only a small proportion of
468 units between tasks (< 8%). There are three potential explanations. First, even though we
469 matched the mean gastrocnemius activation level across tasks, small (~2%) inevitable
470 differences were observed, which could have led to the recruitment of different motor units
471 across tasks. However, given the small differences in activation between tasks, only a very
472 small proportion of units would have differed across tasks. Second, the small number of tracked
473 units may be explained by limitations of our approach to track motor units. However, this
474 approach was used in previous studies whereby motor units were matched across days (32, 44).
475 For instance, at 30% of knee extension MVC, more than 40% of the motor units from the VL

476 and VM muscles were matched between days (32). Despite the lower leg position remaining
477 similar between tasks, we cannot rule out the possibility that the muscles moved with respect
478 to the EMG grids, for example, owing to complex force transmission processes with other
479 synergist muscles such as the soleus. However, it is unlikely that it substantially affected the
480 relative muscles' position. This is confirmed by the absence of change in the barycenter of
481 motor unit action potentials across tasks. Thus, we believe that our experimental procedures
482 provided the ideal conditions to track motor units across tasks. To be sure that we did not fail
483 to identify motor units, we performed a supplementary matching round with a lower threshold
484 (0.60). Even with this lower threshold, the proportion of matched motor units remained low;
485 i.e. $16.8 \pm 11.0\%$ for the GM and $13.5 \pm 13.2\%$ for the GL. In addition, we used a secondary
486 analysis whereby we reapplied the motor unit filters obtained in one task to the other task (45).
487 The use of this approach led to very similar results, that is, motor units that were successfully
488 tracked across tasks were also identified by application of the motor unit filter, but motor units
489 that were not tracked across tasks could not be identified by the application of the motor unit
490 filter. Finally, even though we cannot rule out that the small proportion of tracked motor units
491 is explained by methodological limitations, it remains possible that different motor units were
492 recruited in knee flexion and in ankle plantar flexion. Using a more direct approach, a previous
493 study observed that over the population of GM motor units identified from fine-wire EMG
494 during knee flexion and ankle plantar flexion tasks, only 41% were recruited during both tasks
495 (19). This echoes previous work which challenges Heneman's size principle by reporting
496 divergent recruitment orders across motor neurons innervating the same muscle (46–48). This
497 is also consistent with a recently proposed framework whereby motor units are grouped into
498 functional clusters such that motor units from the same pool (muscle) can receive different
499 common inputs (49). However, given the methodological limitations mentioned above, further
500 studies are needed to explore whether different motor units are recruited during knee flexion
501 and ankle plantar flexion.

502 In conclusion, our results demonstrate that the within- and between-muscle distribution of
503 activation is robust regardless of the function for which the GM and GL muscles are used, that
504 is, knee flexion or ankle plantar flexion. Specifically, the GM muscle was activated at a higher
505 level than the GL muscle during submaximal contractions, regardless of the task. Even though
506 this strategy leads to a force imbalance between these muscles, it likely contributes to minimize
507 the overall muscle stress, a well-established motor control strategy. Although the presence of
508 strong common input between GM and GL motor units would serve to reduce the computational

509 load associated with the control of these muscles, it was not observed. This low level of
510 common input could allow GM and GL to be independently controlled during ankle plantar
511 flexion to comply with secondary goals, such as producing opposing ankle moments in the
512 frontal plane. However, the functional benefit of a low level of common input in knee flexion
513 remains unclear.

514 **Acknowledgements**

515 Jeroen Aeles is supported by a Marie Skłodowska-Curie Actions Individual Fellowship funded
516 by the European Union (I-MUSCLE, 101063675). This study was supported by the French
517 national research agency (ANR-19-CE17-002-01, COMMODE project, to François Hug).

518 **Authors contributions**

519 **Raphaël Hamard:** Investigation, Data curation, Writing – review & editing, Writing – original
520 draft, Visualization, Validation, Software, Methodology, Formal analysis.

521 **Jeroen Aeles:** Writing – review & editing, Supervision, Software, Formal analysis,
522 Conceptualization.

523 **Simon Avrillon:** Writing – review & editing, Methodology, Software, Formal analysis.

524 **Taylor J.M. Dick:** Writing – review & editing, Validation, Supervision, Project administration,
525 Investigation, Conceptualization.

526 **François Hug:** Writing – review & editing, Validation, Supervision, Resources, Project
527 administration, Methodology, Investigation, Funding acquisition, Formal analysis,
528 Conceptualization.

529

530 **Data availability**

531 Supplementary data to this article can be found online at [10.6084/m9.figshare.23206430](https://doi.org/10.6084/m9.figshare.23206430).

532

533 **Figures legends**

534

535 **Figure 1: HDsEMG electrode location, regional activation estimation and motor units matching.**

536 Panel A depicts the location of the grids of electrodes over the gastrocnemius medialis and
537 gastrocnemius lateralis muscles. Panel B depicts an individual example of the distribution of GM EMG
538 amplitude over the grid of electrodes for both knee flexion and ankle plantar flexion. Panel C depicts an
539 individual example of the distribution of the motor unit action potentials over the grid of electrodes
540 during knee flexion (black) and ankle plantar flexion (red). In this specific example, the same motor unit
541 was identified during both tasks, which was only possible for $5.1 \pm 7.8\%$ of the GM motor units and 7.5
542 $\pm 11.8\%$ of the GM motor units and. GM, gastrocnemius medialis; GL, gastrocnemius lateralis; ARV,
543 average and rectified EMG.

544

545 **Figure 2: Average gastrocnemius activation during the matched submaximal knee flexion and** 546 **ankle plantar flexion.**

547 The EMG amplitude was estimated during the submaximal torque plateaus. EMG was normalized to the maximal EMG value during maximal isometric voluntary contractions. Each color represents a participant, with the mean shown in black. The individual data points are displaced along the x axis for visual purposes. A paired t-test revealed that the mean GM-GL EMG was not different between knee flexion and ankle plantar flexion ($p = 0.061$). $n = 21$.

551

552 **Figure 3: GM and GL EMG amplitude measured during submaximal knee flexion and ankle** 553 **plantar flexion.**

554 The EMG amplitude was estimated using the average and rectified value of the EMG during the submaximal torque plateau. EMG was normalized to the maximal isometric voluntary contraction. Each color represents a participant, with the mean shown in black. The individual data points are displaced along the abscissa axis for a visual purpose. The linear mixed effect model revealed that GM EMG was higher than GL EMG ($p < 0.001$), but there was no main effect of task ($p = 0.294$) nor muscle \times task interaction ($p = 0.250$). GM: Gastrocnemius medialis; GL: Gastrocnemius lateralis. $n = 21$.

560

561 **Figure 4: Relationship between the distribution of activation during knee flexion and the** 562 **distribution of activation during ankle plantar flexion.**

563 The distribution of activation was estimated using the GM/(GM+GL) ratio of activation. The dashed line corresponds to the $x=y$ equation. Each datapoint represents a participant. The Pearson correlation coefficient was statistically significant ($R = 0.604$; $p = 0.004$). GM: Gastrocnemius medialis; GL: Gastrocnemius lateralis. $n = 21$.

566

567 **Figure 5: GM and GL EMG patterns during knee flexion (left panel) and ankle plantar flexion** 568 **(right panel) for one representative participant.**

Y axis displays both the EMG amplitude (% of

569 EMG_{max}) and the torque (% of task-specific peak torque). Even though GM and GL EMG amplitude
570 was relatively constant during the plateau for ankle plantar flexion, GM and GL EMG amplitude
571 increased over the torque plateau for knee flexion in most of the participants. GM: Gastrocnemius
572 medialis; GL: Gastrocnemius lateralis.

573

574 **Figure 6: Within-muscle coherence during knee flexion and ankle plantar flexion.** The right and
575 left panels represent the within-muscle coherence for the GM and GL, respectively. Each panel depicts
576 the average z-score value between 0 and 5 Hz for each participant for the two tasks. Each color
577 corresponds to one participant. The red horizontal dashed line indicates the significant threshold, which
578 is set at 1.65 (95% confidence limit). The mean group is depicted in black. n=18 for the GM. n=5 and
579 n=10 for the GL during knee flexion and ankle plantar flexion, respectively.

580

581 **Figure 7: Between-muscle coherence during knee flexion and ankle plantar flexion.** The left and
582 middle panels represent the z-scores between 0 and 50 Hz during knee flexion and ankle plantar flexion
583 respectively for each participant. The magnified inset depicts the z-score between 0 and 5 Hz. The right
584 panel represents the average z-score value between 0 and 5 Hz for each participant for the two tasks.
585 Each color corresponds to one participant. The red horizontal dashed line indicates the significant
586 threshold, which is set at 1.65 (95% confidence limit). Within the right panel, the mean group is depicted
587 in black. The two-tailed paired t-test revealed no difference in the z-score between knee flexion and
588 ankle plantar flexion ($p = 0.570$), n=9.

589

590

591

592

593 **References**

- 594 1. **Avrillon S, Del Vecchio A, Farina D, Pons JL, Vogel C, Umehara J, Hug F.** Individual
595 differences in the neural strategies to control the lateral and medial head of the quadriceps
596 during a mechanically constrained task. *Journal of Applied Physiology* 130: 269–281,
597 2021.
- 598 2. **Crouzier M, Lacourpaille L, Nordez A, Tucker K, Hug F.** Neuromechanical coupling
599 within the human triceps surae and its consequence on individual force-sharing strategies.
600 *J Exp Biol* 221: jeb187260, 2018.
- 601 3. **Crouzier M, Hug F, Dorel S, Deschamps T, Tucker K, Lacourpaille L.** Do individual
602 differences in the distribution of activation between synergist muscles reflect individual
603 strategies? *Exp Brain Res* 237: 625–635, 2019.
- 604 4. **Hug F, Goupille C, Baum D, Raiteri BJ, Hodges PW, Tucker K.** Nature of the coupling
605 between neural drive and force-generating capacity in the human quadriceps muscle. *Proc*
606 *Biol Sci* 282, 2015.
- 607 5. **Cibulka M, Wenthe A, Boyle Z, Callier D, Schwerdt A, Jarman D, Strube MJ.**
608 Variation in medial and lateral gastrocnemius muscle activity with foot position. *Int J*
609 *Sports Phys Ther* 12: 233–241, 2017.
- 610 6. **Marcori AJ, Moura TBMA, Okazaki VHA.** Gastrocnemius muscle activation during
611 plantar flexion with different feet positioning in physically active young men. *Isokinetics*
612 *and Exercise Science* 25: 121–125, 2017.
- 613 7. **Riemann BL, Limbaugh GK, Eitner JD, LeFavi RG.** Medial and Lateral
614 Gastrocnemius Activation Differences During Heel-Raise Exercise with Three Different
615 Foot Positions. *The Journal of Strength & Conditioning Research* 25: 634–639, 2011.
- 616 8. **Hug F, Del Vecchio A, Avrillon S, Farina D, Tucker K.** Muscles from the same muscle
617 group do not necessarily share common drive: evidence from the human triceps surae.
618 *Journal of Applied Physiology* 130: 342–354, 2021.
- 619 9. **Rossato J, Tucker K, Avrillon S, Lacourpaille L, Holobar A, Hug F.** Less common
620 synaptic input between muscles from the same group allows for more flexible coordination
621 strategies during a fatiguing task. *Journal of Neurophysiology* 127: 421–433, 2022.
- 622 10. **Lee SSM, Piazza SJ.** Inversion–eversion moment arms of gastrocnemius and tibialis
623 anterior measured in vivo. *Journal of Biomechanics* 41: 3366–3370, 2008.
- 624 11. **Vieira TMM, Minetto MA, Hodson-Tole EF, Botter A.** How much does the human
625 medial gastrocnemius muscle contribute to ankle torques outside the sagittal plane?
626 *Human Movement Science* 32: 753–767, 2013.
- 627 12. **Kinugasa R, Kawakami Y, Fukunaga T.** Muscle activation and its distribution within
628 human triceps surae muscles. *Journal of Applied Physiology* 99: 1149–1156, 2005.
- 629 13. **Masood T, Bojsen-Møller J, Kalliokoski KK, Kirjavainen A, Äärimaa V, Peter**
630 **Magnusson S, Finni T.** Differential contributions of ankle plantarflexors during

- 631 submaximal isometric muscle action: A PET and EMG study. *Journal of*
632 *Electromyography and Kinesiology* 24: 367–374, 2014.
- 633 14. **Segal RL, Song AW.** Nonuniform Activity of Human Calf Muscles During an Exercise
634 Task. *Archives of Physical Medicine and Rehabilitation* 86: 2013–2017, 2005.
- 635 15. **Li L, Landin D, Grodesky J, Myers J.** The function of gastrocnemius as a knee flexor
636 at selected knee and ankle angles. *Journal of Electromyography and Kinesiology* 12: 385–
637 390, 2002.
- 638 16. **Miyamoto N, Wakahara T, Kawakami Y.** Task-dependent inhomogeneous muscle
639 activities within the bi-articular human rectus femoris muscle. *Plos one* 7: e34269, 2012.
- 640 17. **Watanabe K, Kouzaki M, Moritani T.** Regional neuromuscular regulation within
641 human rectus femoris muscle during gait in young and elderly men. *Journal of*
642 *Biomechanics* 49: 19–25, 2016.
- 643 18. **Watanabe K, Vieira TM, Gallina A, Kouzaki M, Moritani T.** Novel Insights Into
644 Biarticular Muscle Actions Gained From High-Density Electromyogram. *Exerc Sport Sci*
645 *Rev* 49: 179–187, 2021.
- 646 19. **Héroux ME, Brown HJ, Inglis JT, Siegmund GP, Blouin J-S.** Motor units in the human
647 medial gastrocnemius muscle are not spatially localized or functionally grouped. *The*
648 *Journal of Physiology* 593: 3711–3726, 2015.
- 649 20. **Besomi M, Hodges PW, Clancy EA, Van Dieën J, Hug F, Lowery M, Merletti R,**
650 **Søgaard K, Wrigley T, Besier T, Carson RG, Disselhorst-Klug C, Enoka RM, Falla**
651 **D, Farina D, Gandevia S, Holobar A, Kiernan MC, McGill K, Perreault E, Rothwell**
652 **JC, Tucker K.** Consensus for experimental design in electromyography (CEDE) project:
653 Amplitude normalization matrix. *Journal of Electromyography and Kinesiology* 53:
654 102438, 2020.
- 655 21. **Negro F, Muceli S, Castronovo AM, Holobar A, Farina D.** Multi-channel intramuscular
656 and surface EMG decomposition by convolutive blind source separation. *J Neural Eng*
657 13: 026027, 2016.
- 658 22. **Del Vecchio A, Holobar A, Falla D, Felici F, Enoka RM, Farina D.** Tutorial: Analysis
659 of motor unit discharge characteristics from high-density surface EMG signals. *Journal of*
660 *Electromyography and Kinesiology* 53: 102426, 2020.
- 661 23. **Hug F, Avrillon S, Del Vecchio A, Casolo A, Ibanez J, Nuccio S, Rossato J, Holobar**
662 **A, Farina D.** Analysis of motor unit spike trains estimated from high-density surface
663 electromyography is highly reliable across operators. *Journal of Electromyography and*
664 *Kinesiology* 58: 102548, 2021.
- 665 24. **Holobar A, Minetto MA, Farina D.** Accurate identification of motor unit discharge
666 patterns from high-density surface EMG and validation with a novel signal-based
667 performance metric. *Journal of neural engineering* 11: 016008, 2014.
- 668 25. **Laine CM, Martinez-Valdes E, Falla D, Mayer F, Farina D.** Motor Neuron Pools of
669 Synergistic Thigh Muscles Share Most of Their Synaptic Input. *J Neurosci* 35: 12207–
670 12216, 2015.

- 671 26. **Del Vecchio A, Germer CM, Elias LA, Fu Q, Fine J, Santello M, Farina D.** The human
672 central nervous system transmits common synaptic inputs to distinct motor neuron pools
673 during non-synergistic digit actions. *The Journal of Physiology* 597: 5935–5948, 2019.
- 674 27. **Aeles J, Sarcher A, Hug F.** Common synaptic input between motor units from the lateral
675 and medial posterior soleus compartments does not differ from that within each
676 compartment. *Journal of Applied Physiology* 134: 105–115, 2023.
- 677 28. **Mazzo MR, Weinman LE, Giustino V, Mclagan B, Maldonado J, Enoka RM.**
678 Changes in neural drive to calf muscles during steady submaximal contractions after
679 repeated static stretches. *The Journal of Physiology* 599: 4321–4336, 2021.
- 680 29. **Negro F, Yavuz UŞ, Farina D.** The human motor neuron pools receive a dominant slow-
681 varying common synaptic input. *The Journal of physiology* 594: 5491–5505, 2016.
- 682 30. **Laine CM, Valero-Cuevas FJ.** Intermuscular coherence reflects functional coordination.
683 *Journal of Neurophysiology* 118: 1775–1783, 2017.
- 684 31. **Del Vecchio A, Farina D.** Interfacing the neural output of the spinal cord: robust and
685 reliable longitudinal identification of motor neurons in humans. *Journal of neural*
686 *engineering* 17: 016003, 2019.
- 687 32. **Martinez-Valdes E, Negro F, Laine CM, Falla D, Mayer F, Farina D.** Tracking motor
688 units longitudinally across experimental sessions with high-density surface
689 electromyography. *The Journal of physiology* 595: 1479–1496, 2017.
- 690 33. **Pinheiro JC, Bates DM, DebRoy SS, Sarkar D.** Nlme: linear and nonlinear mixed
691 effects models. R package version 31-110 3: 1–113, 2013.
- 692 34. **Hug F, Vogel C, Tucker K, Dorel S, Deschamps T, Le Carpentier É, Lacourpaille L.**
693 Individuals have unique muscle activation signatures as revealed during gait and pedaling.
694 *Journal of Applied Physiology* 127: 1165–1174, 2019.
- 695 35. **Hudson AL, Taylor JL, Gandevia SC, Butler JE.** Coupling between mechanical and
696 neural behaviour in the human first dorsal interosseous muscle. *The Journal of Physiology*
697 587: 917–925, 2009.
- 698 36. **Powell PL, Roy RR, Kanim P, Bello MA, Edgerton VR.** Predictability of skeletal
699 muscle tension from architectural determinations in guinea pig hindlimbs. *Journal of*
700 *Applied Physiology* 57: 1715–1721, 1984.
- 701 37. **Crowninshield RD, Brand RA.** A physiologically based criterion of muscle force
702 prediction in locomotion. *Journal of Biomechanics* 14: 793–801, 1981.
- 703 38. **Hodson-Tole EF, Loram ID, Vieira TMM.** Myoelectric activity along human
704 gastrocnemius medialis: Different spatial distributions of postural and electrically elicited
705 surface potentials. *Journal of Electromyography and Kinesiology* 23: 43–50, 2013.
- 706 39. **Vieira TM, Botter A, Minetto MA, Hodson-Tole EF.** Spatial variation of compound
707 muscle action potentials across human gastrocnemius medialis. *Journal of*
708 *Neurophysiology* 114: 1617–1627, 2015.

- 709 40. **Farina D, Zennaro D, Pozzo M, Merletti R, Läubli T.** Single motor unit and spectral
710 surface EMG analysis during low-force, sustained contractions of the upper trapezius
711 muscle. *Eur J Appl Physiol* 96: 157–164, 2006.
- 712 41. **Borzelli D, Gazzoni M, Botter A, Gastaldi L, d’Avella A, Vieira TM.** Contraction level,
713 but not force direction or wrist position, affects the spatial distribution of motor unit
714 recruitment in the biceps brachii muscle. *Eur J Appl Physiol* 120: 853–860, 2020.
- 715 42. **Levine J, Avrillon S, Farina D, Hug F, Pons JL.** Two motor neuron synergies, invariant
716 across ankle joint angles, activate the triceps surae during plantarflexion. bioRxiv:
717 2022.11.11.516183, 2022.
- 718 43. **Henneman E.** Relation between size of neurons and their susceptibility to discharge.
719 *Science* 126: 1345–1347, 1957.
- 720 44. **Del Vecchio A, Casolo A, Negro F, Scorcelletti M, Bazzucchi I, Enoka R, Felici F,**
721 **Farina D.** The increase in muscle force after 4 weeks of strength training is mediated by
722 adaptations in motor unit recruitment and rate coding. *The Journal of Physiology* 597:
723 1873–1887, 2019.
- 724 45. **Frančić A, Holobar A.** On the Reuse of Motor Unit Filters in High Density Surface
725 Electromyograms Recorded at Different Contraction Levels. *IEEE Access* 9: 115227–
726 115236, 2021.
- 727 46. **Desmedt JE, Godaux E.** Ballistic contractions in man: characteristic recruitment pattern
728 of single motor units of the tibialis anterior muscle. *The Journal of physiology* 264: 673–
729 693, 1977.
- 730 47. **Marshall NJ, Glaser JI, Trautmann EM, Amematsro EA, Perkins SM, Shadlen MN,**
731 **Abbott LF, Cunningham JP, Churchland MM.** Flexible neural control of motor units.
732 *Nature neuroscience* 25: 1492–1504, 2022.
- 733 48. **Tucker K, Butler J, Graven-Nielsen T, Riek S, Hodges P.** Motor unit recruitment
734 strategies are altered during deep-tissue pain. *Journal of Neuroscience* 29: 10820–10826,
735 2009.
- 736 49. **Hug F, Avrillon S, Ibáñez J, Farina D.** Common synaptic input, synergies and size
737 principle: Control of spinal motor neurons for movement generation. *The Journal of*
738 *Physiology* 601: 11–20, 2023.
- 739