

Differential behavior of distinct motoneuron pools that innervate the triceps surae

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(Article begins on next page)

1 **Title:** Differential behaviour of distinct motoneuron pools that innervate the triceps surae

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21 Abstract

22
23 It has been shown that when humans lean in various directions, the central nervous system
24 (CNS) recruits different motoneuron pools for task completion; common units that are active
25 during different leaning directions, and unique units that are active in only one leaning direction.
26 We used high-density surface electromyography (HD-sEMG) to examine if motor unit (MU)
27 firing behaviour was dependent on leaning direction, muscle (medial and lateral gastrocnemius;
28 soleus), limits of stability, or whether a MU is considered common or unique. Fourteen healthy
29 participants stood on a force platform and maintained their center of pressure in five different
30 leaning directions. HD-sEMG recordings were decomposed into MU action potentials and the
31 average firing rate (AFR), coefficient of variation (CoV_{ISI}) and firing intermittency were
32 calculated on the MU spike trains. During the 30-90° leaning directions both unique units and
33 common units had higher firing rates ($F = 31.31, p < 0.0001$). However, the unique units
34 achieved higher firing rates compared to the common units (mean estimate difference = 3.48 Hz,
35 $p < 0.0001$). The CoV_{ISI} increased across directions for the unique units but not for the common
36 units ($F = 23.65, p < 0.0001$). Finally, intermittent activation of MUs was dependent on the
37 leaning direction ($F = 11.15, p < 0.0001$), with less intermittent activity occurring during
38 diagonal and forward-leaning directions. These results provide evidence that the CNS can
39 preferentially control separate motoneuron pools within the ankle plantarflexors during voluntary
40 leaning tasks for the maintenance of standing balance.

41

42 New & Noteworthy

43 In this study, we demonstrate that the different sub-populations of motor units within the three
44 muscles comprising the ankle plantarflexors behave differently during multi-directional leaning.

45 Our results suggest that the central nervous system has the capability to control distinct sub-
46 populations of motor units to meet the force requirements necessary for leaning. This may allow
47 for a precise, efficient, and flexible control strategy for the maintenance of standing balance.

48

49 **Keywords**

50 High-density surface electromyography, Motor units, Firing rates, Plantarflexors, Postural
51 control

52

53 **Introduction**

54 Control of the ankle plantarflexor muscles is fundamental for motor tasks such as quiet
55 standing and leaning; these tasks are accomplished largely by the medial and lateral
56 gastrocnemius (MG and LG) as well as the soleus (SOL) muscles (1-3). The motor unit (MU)
57 behaviour of these muscles during various non-weight bearing contractions have been well
58 documented (4-10). However, motor units may behave differently during postural control than
59 during non-weight bearing contractions (11).

60 Evidence is mounting for differential MU behaviour during standing compared to that
61 observed from non-weight bearing contractions. During quiet standing, the common modulation
62 of MU discharge rates in the SOL is higher compared to voluntary isometric contractions (12)
63 reflecting a stronger common drive during standing than seated contractions. Further, there is
64 greater inhibition of MUs of the MG muscle in response to sural nerve stimulation when
65 individuals are standing compared to lying supine (13). The relative contribution of recruitment
66 or rate coding to ankle plantarflexor force production depends on the posture. That is, when
67 responding to perturbations of increasing intensity during standing, MU recruitment in the

68 gastrocnemius appears to be the main mechanism of force gradation (14), whereas in a sitting
69 position both rate coding and recruitment are used to modulate force gradation in the
70 gastrocnemius (15). Taken together, it appears that the MUs of the plantarflexors behave
71 differently during postural control than during non-weight bearing contractions.

72 For a MU to be recruited, synaptic current must reach the soma of a motoneuron for it to
73 discharge and initiate an action potential (16). When the distribution of synaptic current occurs
74 uniformly across the motoneuron pool, the first MUs to discharge are those with the small size
75 and high input resistance (17). Unequal distribution of synaptic current however may influence
76 MU recruitment. In previous work, we have shown that the ankle plantarflexor muscles have
77 unequal, regionally specific activation in response to directionally induced perturbations (18).
78 Further, we observed the spatial recruitment of MUs within the ankle plantarflexor muscles
79 during a multidirectional leaning task (19). Our findings indicate that the CNS possesses the
80 ability to recruit two distinct subpopulations of MUs. During the different directional
81 movements, *common* MUs are recruited from the MG and SOL, putatively to meet the baseline
82 torque requirements. As the directionality of the force requirements for body stabilization
83 changes, *unique* MUs in separate locations were recruited in the plantarflexor muscles. We
84 defined common units as MUs that were matched between different leaning directions identified
85 by waveforms that were indistinguishable from each other (19, 20). Unique units were MUs that
86 were not matched between leaning directions and were distinguishable from each other (19, 20).
87 The CNS may rely on the uneven spatial distribution of synaptic inputs to control recruitment;
88 however, whether the MU firing behaviour is influenced by this nonuniform recruitment pattern
89 is unknown.

90 An interesting consideration of MU firing behaviour in the plantarflexors is the
91 intermittent firing patterns observed during standing. Evidence from ultrasound and surface
92 EMG has shown intermittent activity of the MG muscle while subjects stand at ease (3, 21, 22).
93 Observations from intramuscular EMG have determined that postural activation of the MG MUs
94 occurs intermittently, specifically at different body sway positions (23). Taken together, there
95 appears to be intermittent activation of MUs in the MG that is associated with the position and
96 velocity of the standing body. What is still unknown is whether this intermittent firing behaviour
97 continues as task difficulty increases from quiet stance to more demanding postural tasks.

98 The purpose of this study was to investigate the MU firing behaviours of the MG, LG,
99 and SOL during a multi-directional leaning task. It was hypothesized that: a) MU firing rate
100 would be higher in leaning directions that required higher force production; b) based on our
101 previous finding of common and unique units across leaning directions (19), MU firing
102 behaviour would be dependent on whether the MU was common or unique; and c) intermittency
103 would be more evident in the gastrocnemius, compared to the SOL, and would decrease in
104 response to forward-leaning directions.

105

106 **Methods**

107 *Participants and Experimental Protocol*

108 Fourteen healthy adults participated in this study (7 females, 7 males; mean \pm SD: body
109 mass 70 ± 15.3 kg; height 168 ± 9.3 cm; age 25 ± 2.5 years). Participants were excluded if they
110 had any health conditions that negatively impacted balance (e.g., musculoskeletal disorders,
111 neuropathies, dizziness), or previous injury to their lower legs within the past 6 months. The
112 experimental protocol was approved by the institutional Ethics Committee (Research Ethics

113 Board Number: 110471) and conformed to the latest amendment of the Declaration of Helsinki.

114 Participants provided written, informed consent before participating in the study.

115 Experimental data used in this study were part of a larger study examining the spatial
116 location of MU recruitment (19). Briefly, standing in their natural bipedal stance on a
117 piezoelectric force platform (9286AA Kistler, Zurich Switzerland), participants were instructed
118 to lean in different directions. To identify the limit of stability (LoS), participants were instructed
119 to lean about their ankles anteriorly, posteriorly, and laterally to both sides as far as they could
120 without taking a step. This 4-way LoS test has been shown to be more reliable in testing LoS
121 compared to other tests (24). After completing the 4-way LoS test, participants performed a quiet
122 stance trial for 30s. Then, while provided with visual feedback of their center of pressure (CoP),
123 participants were asked to lean in 5 different directions. Circular targets for each direction, 0°,
124 30°, 60°, 90° and 120°, counter-clockwise from the mediolateral axis crossing the average CoP
125 position were computed during the 30s of quiet stance. Each target was displayed one at a time
126 on a screen in front of the participant (Figure 1A, B). The targets appeared over an ellipse with
127 semiaxes corresponding to 30% LoS (or 40% LoS) in the anterior-posterior and medio-lateral
128 directions (Figure 1B). The resulting ellipses representing 60% LoS and 80% LoS, respectively,
129 imposed progressively greater contraction intensities, given the CoP distance from the ankle
130 scales with the ankle torque (25). Participants leaned toward each of the targets, maintained their
131 CoP position in the target for 35s, and then returned to their quiet stance position. After leaning
132 in a specific direction, a rest period of 15s was provided. An example of the CoP trace can be
133 found in Figure 1C. During the leaning task, participants were instructed to keep their legs and
134 body straight and lean like a pole. Participants moved in a smooth manner until their CoP
135 position was in the target for a total duration of 45s (5s towards the target, 35s in the target, and

136 5s back to their natural bipedal stance). To familiarize themselves with the procedure,
137 participants first conducted an unrecorded practice trial. If the participant did not maintain the
138 instructed body position or was unable to keep their CoP steady in the target for more than 25s,
139 the trial was discarded and repeated. The trial was retained and analyzed once the correct leaning
140 posture was maintained.

141

142 *High-Density Surface EMG Electrode Placement*

143 Placement of the High-Density surface Electromyography (HD-sEMG) was guided using
144 an ultrasound imaging system (LogicScan 64 LT-1T; Telemed, Vilnius, Lithuania). With
145 participants lying prone and the ankle in the neutral position, the medial and lateral edges of the
146 gastrocnemius and SOL, the insertion of the MG and LG on the Achilles tendon, the fascial
147 space between the MG and LG, and the distal edges between the two muscles were identified
148 and marked on the skin. The skin was abraded and cleaned before grid placement. Semi-
149 disposable grids with 32 electrodes at an interelectrode distance of 10mm (LISiN, Torino, Italy)
150 were affixed to each of the MG and LG (one grid on each muscle). The grids were placed on the
151 proximal belly of the muscle, 10mm above the distal edges of the superficial aponeurosis of the
152 MG and LG to avoid EMG detection from the distal region of gastrocnemius where propagation
153 of action potentials is observed along the muscle fibres (18, 19, 26). A 64-electrode matrix,
154 comprised of two adjacent 32-electrode semi-disposable grids with an interelectrode distance of
155 10mm (LISiN, Torino, Italy), was placed over the SOL muscle. The 64-electrode grid was
156 centered on the Achilles tendon and placed 10mm below the distal insertion of the MG (Figure
157 1D). The grids were held in place with bio-adhesive foam, and conductive paste ensured optimal

158 contact between the skin and the electrodes. Three reference electrodes were placed on the
159 medial malleolus (SOL), the patella (MG), and the fibular head (LG).

160

161 *HD-sEMG and Forceplate Recordings*

162 Signals were detected in a monopolar configuration using a modular, wireless HD-sEMG
163 amplifier (27). This device is composed of four modules (one for each gastrocnemius head, and
164 two for SOL), each detecting, conditioning, and transmitting 32 EMG signals (amplification:
165 192V/V; sampling frequency: 2048 Hz; 16-bit A/D Converter). A Wi-Fi link from the EMG
166 acquisition modules to a personal computer enabled the detected signals to be transmitted,
167 visualized, and stored in real-time.

168 CoP coordinates in the sagittal and frontal planes were computed from the ground
169 reaction forces, sampled at 2048 Hz using a 16-bit A/D Converter (± 2.5 V input voltage range).
170 A pulse generator (LISiN, Torino, Italy) sent a signal at 3Hz (0V – 3.3 V amplitude) to both
171 EMG amplifier and force platform to synchronize the systems. Signals were aligned on the first,
172 rising edge of the trigger signal.

173

174 **Data Analysis**

175 *Motor unit analysis*

176 All EMG data analyses were performed using MATLAB R2020b (The MathWorks, Inc.,
177 Natick, MA, USA). Single-differential EMGs between pairs of adjacent electrodes along each
178 column of the matrix were computed from the monopolar signals and visually inspected for
179 quality. Channels presenting contact problems or power line interference were linearly
180 interpolated with the data collected by the surrounding 8 channels. After verifying the quality of

181 all EMG signals, monopolar signals were filtered (Butterworth, 2nd order, 20-350 Hz) and
182 decomposed with the blind source separation method (28) implemented in the DEMUSE tool
183 software (v. 4.9; The University of Maribor, Slovenia). Signals for each leaning direction and
184 LoS condition were decomposed separately, over the central 20s of the 35s period when CoP was
185 maintained at the target location. This decomposition procedure can identify MU discharges over
186 a wide range of contraction intensities and has been extensively validated using experimental and
187 simulated signals (29, 30). After decomposition of the EMG signals, the firing instants of
188 identified MUs were used to trigger and average single differential EMGs over 30ms epochs,
189 providing the surface representation of single MU action potentials (31). The results of the
190 decomposition were inspected visually, according to Power et al. (32), and noisy waveforms
191 were discarded. Examples of retained MUs are shown in Figure 2.

192 For each retained MU, the average firing rate (AFR) was calculated. Interspike intervals
193 (ISIs) < 25ms were discarded for subsequent analysis because these ISIs are likely due to
194 decomposition errors (30) and would have a large impact on the AFR. The coefficient of
195 variation of the ISI (CoV_{ISI}) was calculated as the standard deviation of the ISI divided by the
196 mean ISI and is reported as a percentage.

197 To determine whether the MUs were activated intermittently, an intermittency index was
198 established. It has been shown previously that very frequently, the postural MUs in the MG
199 muscle discharge below the physiological minimal tonic rate (<4 Hz) during quiet stance (23).
200 These occurrences of discharge rates lower than 4 Hz are considered as representing instances of
201 intermittent recruitment of MU (i.e., units were successively de-recruited and re-recruited). To
202 standardize the amount of intermittent activity for a single MU, we calculated the degree of
203 intermittency:

204
$$\text{Degree of Intermittency} = \frac{\text{Number of intermittent occurrences}}{\text{Time MU was active (s)}}$$

205 where the “*Number of intermittent occurrences*” is the number of cases in which the MU
 206 firing rate is below 4 Hz, and the “*Time MU was active*” is the total time that the MU was
 207 actively recruited, from the instance of the first firing to the last firing of the corresponding MU.
 208 This procedure standardized the intermittency of MU activity and allowed comparisons between
 209 different MUs by evaluating the number of intermittent occurrences per second.

210

211 *Motor unit tracking*

212 A MU tracking analysis was conducted separately for the 60% and 80% LoS conditions.
 213 MU action potentials that were common between the different leaning directions were identified
 214 by focusing on both the shape and amplitude of the waveform of MU action potentials. For each
 215 retained MU, the average rectified value (ARV) was calculated across 30ms epochs centered on
 216 individual action potentials. Each retained MU was paired to all other MUs identified during
 217 different leaning directions within the same muscle and LoS condition for each participant (e.g.,
 218 a MU identified in the 0° leaning direction in the 60% LoS condition within the MG is compared
 219 to all other units identified in the MG at the 30°, 60°, 90°, and 120° leaning directions of the 60%
 220 LoS condition within a single participant). The action potentials for each pair of MUs were
 221 aligned in time by maximizing their cross-correlation function. The mean square difference was
 222 calculated between the two sets of time-aligned action potentials, averaged across channels, and
 223 then normalized with respect to the mean ARV of the two sets of action potentials. This
 224 normalization procedure is utilized to reduce the bias of waveform amplitude between two MU
 225 pairs (33). Finally, pairs of MU action potentials with a mean square difference smaller than 10%
 226 were considered common (19, 20). An expert operator visually confirmed all MUs that were

227 identified as common by the algorithm. This provided us with a group of MUs that will be
228 referred to as *common* units that were present in two leaning conditions or more. When a MU
229 had a mean square difference greater than 10% with all MUs it was paired with, the MU was
230 considered to be a uniquely recruited unit not found in other leaning directions. These MUs will
231 be called *unique* units from this point. An example of a pair of retained *common* MUs is
232 provided in Figure 3.

233

234 **Statistical Analysis**

235 All statistical analyses were conducted in R (v.5.12.1, R Development Core Team, 2009).
236 To analyze MU behaviour, we performed a multilevel mixed model linear regression analysis
237 using the package lme4 (v. 1.1-27.1) (34). As previously discussed in detail (35, 36), linear
238 mixed models have several advantages that are particularly suitable for MU experimental
239 designs: 1) they allow the whole sample of extracted MUs to be analyzed and not just the mean
240 observations for each subject and direction. This allows better evaluation of the data variance
241 compared to conventional ANOVA statistics; 2) they account for the non-independence of
242 observations (i.e., different MUs coming from the same participant) with correlated error; 3) they
243 separately treat the effects caused by the experimental manipulation (fixed effects) and those that
244 were not (random effects), increasing the generalizability of the findings. Linear mixed
245 modelling computes and utilizes estimated marginal means of the data. Further, in a nested or
246 hierarchical data structure, averaging is performed separately in each nesting group (in our case,
247 by participant). This procedure creates estimated marginal means that are based on the statistical
248 model, not directly from the raw data, and therefore the estimated marginal means are different
249 than the means of the raw data.

250 *Motor Unit Behaviour*

251 Diagnostic plots of the residuals from all models were inspected for violations of the
252 assumptions of normality and homoscedasticity. The AFR and CoV_{ISI} data met the assumptions,
253 however, the degree of intermittency did not meet homoscedasticity and normality of residuals
254 assumptions. The intermittency values were transformed by a $\log(x + 1)$ transformation for the
255 data to fit the assumptions of the model. The aggregated data set were analyzed by three separate
256 linear mixed-effects models, using the fixed effects of leaning direction (0° , 30° , 60° , 90° , 120°),
257 LoS (60% and 80%), muscle (MG, LG, and SOL), and MU subgroup (unique or common units).
258 Starting with the maximal random structure, including by-participant and by-item random slopes
259 and intercepts, this structure was then reduced to the optimal structure that could be supported by
260 the data following the steps of Bates (2015). Likelihood ratio tests were used to find the optimal
261 model structure; the random slope model improved the model. We considered the random
262 intercept over participants as a random factor and the random slope of the MU subgroup and
263 muscle. We could not include a random slope of leaning direction or LoS condition because of
264 singular fit violation (i.e., multiple collinearities).

265 Statistical significance of fixed effects was determined using type III Wald F tests with
266 Kenward-Rogers degrees of freedom with the ANOVA function from R's *car* package (v.
267 3.0.12). Post hoc pairwise comparisons (with Bonferroni's correction) were performed using the
268 estimated-means contrast, as employed in R's *emmeans* package (v. 1.7.2). Post hoc comparisons
269 were applied between each leaning direction (all-pairwise comparisons), and within the same
270 leaning direction for the LoS condition, muscle group, and MU subgroup. Confidence intervals
271 around the parameter estimate differences were calculated via parametric bootstrapping with

272 5000 iterations. The post hoc results are reported with the mean estimated difference (M), 95%
273 confidence intervals (CI) and adjusted p -values.

274 *Balance Performance*

275 To determine whether the participants' balance performance during the leaning task
276 differed across the leaning directions, the CoP 95% confidence ellipse area was compared using
277 a one-way repeated measures ANOVA for 60% and 80% LoS separately. The 95% confidence
278 ellipse area is the area of the smallest ellipse containing 95% of the data points collected during
279 the central 20s of the 35s task (when CoP was maintained in the target). It is used to interpret
280 sway displacement and is a measure of performance during postural tasks (37).

281

282 **Results**

283 The total number of identified MUs for each muscle and LoS condition can be found in
284 Table 1. There were no differences in the CoP 95% confidence ellipse area in any leaning
285 directions (p -values ranging from 0.23 to 0.94), indicating that participants performed the
286 leaning task appropriately and similarly among leaning directions.

287 *Average Firing Rate*

288 Individual MU Average Firing Rates (AFRs) and associated distributions for the MG, LG
289 and SOL during the 5 leaning directions are depicted in Figure 4. The AFR of the common units
290 is distributed unimodally (with a rightward skew) with a peak occurring at approximately 8-12
291 Hz. The AFR of the unique units, on the other hand, had two peaks occurring at approximately
292 12-15 Hz and 22-24 Hz. However, this second peak was modest in the unique MUs of the SOL
293 muscle. Note that, in gastrocnemius, a larger proportion of the higher AFRs occurred during the

294 30°-90° leaning directions (indicated by the density of the symbols at the higher firing
295 frequencies in Figure 4).

296 The estimates of the MU AFR generated by the statistical model are depicted in Figure 5.
297 There were main effects of direction ($F = 31.31, p < 0.0001$), muscle ($F = 29.35, p < 0.0001$) and
298 MU subgroup ($F = 254.53, p < 0.0001$) on the AFR. There was no statistically significant effect
299 of LoS on AFR, suggesting that recruitment of MUs is utilized to meet the force requirements of
300 the higher contraction intensities. The higher number of unique units during the 80% LoS
301 condition than in the 60% LoS condition confirms this (Table 1). No interaction effects were
302 observed.

303 A detailed description of the post hoc test results (including 95% CI and p -values) are
304 reported in Table 2. Post hoc tests for the leaning direction showed that for all three muscles and
305 MU subgroups the AFR was higher at 30°, 60°, 90° directions compared to the 0° and 120°
306 leaning directions, 30° (0°: $M = 1.65$ Hz; 120°: $M = 2.32$ Hz), 60° (0°: $M = 1.52$ Hz; 120°: $M =$
307 2.15 Hz) and 90° (0°: $M = 1.27$ Hz; 120°: $M = 2.09$ Hz) leaning directions. The post hoc tests for
308 the muscle effect showed that during each of the leaning directions the MUs in the MG ($M =$
309 1.44 Hz) and LG ($M = 1.39$ Hz) were discharging with higher AFRs compared to the SOL.
310 Finally, the post hoc test for MU subgroup showed that unique units were discharging with
311 higher firing rates compared to the common units during all leaning directions and muscle
312 groups ($M = 3.48$ Hz).

313

314 *Coefficient of Variation of the Interspike Interval*

315 The estimates of the CoV_{ISI} are displayed in Figure 6. The CoV_{ISI} values increased across
316 leaning directions ($F = 23.64, p < 0.0001$), and were larger during the 80% LoS condition ($F =$

317 30.26, $p < 0.0001$). The directional effect was dependent on the MU subgroup, indicated by the
 318 interaction between direction and MU subgroup ($F = 23.58$, $p = 0.0005$). Additionally, there was
 319 muscle effects ($F = 47.97$, $p < 0.001$) however, these effects were expected based on previous
 320 literature.

321 A detailed description of the post hoc test results (including 95% CI and p -values) are
 322 reported in Table 3. In the unique units there was an increase in CoV_{ISI} during the 60° ($M = 2\%$)
 323 leaning direction compared to the 0° target. In addition, the CoV_{ISI} during the 30° ($M = 4\%$), 60°
 324 ($M = 5\%$) and 90° ($M = 4\%$) leaning directions were higher compared to the 120° leaning
 325 direction. The post hoc test for the MU subgroup interaction showed that the unique units'
 326 CoV_{ISI} increased higher from the 0° - 60° ($M = 1.5\%$) and 120° - 60° ($M = 4\%$) leaning direction
 327 compared to the common. The post hoc test for LoS showed higher CoV_{ISI} values during the
 328 80% LoS condition at all leaning directions in all three muscles of both MU subgroups ($M =$
 329 2%). The MG (Unique: $M = 3\%$; Common: $M = 6\%$) and LG (Unique: $M = 4\%$; Common: $M =$
 330 4%) had higher CoV_{ISI} than the SOL in both MU subgroups during all leaning directions.
 331 Finally, the unique units had higher CoV_{ISI} than the common units during all leaning directions
 332 ($M = 7.1\%$).

333

334 *Degree of Intermittency*

335 The estimates of the degree of intermittency described by the model are reported in
 336 Figure 7. There was a main effect of direction ($F = 11.15$, $p < 0.0001$), muscle ($F = 10.33$, $p <$
 337 0.0001) and MU subgroup ($F = 38.37$, $p < 0.001$) on the degree of intermittency, but no effect of
 338 LoS condition. The muscle effect was dependent on the common units indicated by the muscle x
 339 MU subgroup interaction ($F = 3.67$, $p = 0.02$).

340 A detailed description of the post hoc test results (including 95% CI and p-values) are
341 reported in Table 4. Post hoc tests for the leaning direction showed that in the unique units for all
342 three muscles there was a decrease in the degree of intermittency compared to the 0° and 120°
343 leaning directions during the 30° (0°: M = 0.16; 120°: M = 0.21), 60° (0°: M = 0.13; 120°: M =
344 0.18) and 90° (0°: M = 0.18; 120°: M = 0.21) leaning directions. In addition, the post hoc test for
345 the MU subgroup showed that the unique units had more intermittent activity compared to the
346 common units during the 0° (M = 0.13) and 120° (M = 0.28) leaning directions. Finally, the post
347 hoc test for the muscle interaction showed that the MG common units had more intermittent
348 recruitment compared to the SOL common units during all leaning directions (M = 0.07).

349

350 Discussion

351 This study examined the motor control of two sub-populations of MUs (common and
352 unique MUs) within the ankle plantarflexors to meet the force requirements in different
353 directions during a voluntary multidirectional leaning task. The AFR of both common and
354 unique units was higher during the leaning directions that required higher forces. However, even
355 though the 80% LoS condition had greater force requirements, the AFR did not differ between
356 LoS conditions, relying on recruitment instead. The two MU subgroups displayed different
357 patterns of MU variability. The directional effect of CoV_{ISI} was dependent on the two sub-
358 populations of MUs. The unique units had higher CoV_{ISI} , especially in the 30-90° directions,
359 whereas the common units CoV_{ISI} remained relatively constant, while intermittent firing
360 behaviour was higher across the leaning directions for the unique units.

361

362 *Differential behaviour of motor unit subgroups*

363 Across the leaning directions, the unique and common units discharged at higher AFRs
364 when force requirements were largest (during 30°-90° directions). When analyzing the
365 differences between the MU subgroups, the unique units discharged at higher AFR compared to
366 the common units during all leaning directions (Figure 5). The distributions of the AFR (Figure
367 4) provide a better indication of the differences seen. There was a shift towards higher firing
368 frequencies in the distribution of AFR for the unique units during the 30°, 60°, and 90° leaning
369 directions that was not observed in the common units. The shift (or lack thereof) in the AFR
370 depending on the MU subgroups may suggest differential control of the distinct subgroups.
371 Further evidence supporting differential control is the strikingly different patterns of the CoV_{ISI}
372 between the two MU subgroups (Figure 6). The directional effect on CoV_{ISI} was dependent on
373 the MU subgroups; with unique units modulating their CoV_{ISI} while the common remained
374 relatively constant. Further, the unique units had larger CoV_{ISI} when force requirements were
375 largest, whereas the common units fired with a lower CoV_{ISI} across the directions.

376 The differences in the AFR and CoV_{ISI} between the unique and common units suggest a
377 degree of selectivity of synaptic inputs. If the synaptic input was distributed uniformly across
378 common and unique units, we would have expected a similar pattern of firing. A recent study by
379 Hug et al. (38) provides support for selectivity of synaptic inputs controlling firing behaviours.
380 During volitional contractions, the modulations in the firing rates of concurrently active MUs
381 have been shown to be correlated and are believed to reflect a common drive (12, 39). However,
382 Hug and colleagues have recently shown that muscles from the same anatomical muscle group
383 (e.g. the ankle plantarflexors) do not share the same common drive during isometric and standing
384 heel-raise contractions (38). It is therefore possible that there are selective synaptic inputs that
385 can control separate motoneuron pools with the ankle plantarflexors.

386 In our previous study (19), we postulated that common units are recruited to produce a
387 baseline tonic level of force production. Once the task exceeded the capabilities of the common
388 units, recruitment of unique units with fibres grouped in different spatial locations were utilized
389 to meet the force requirements of the task. It is interesting to note that there were no statistically
390 significant differences in AFR between the LoS conditions. This may indicate that to achieve the
391 force required for the 80% LoS, recruitment of additional MUs is utilized over rate coding. The
392 results of our study provide indirect evidence for this hypothesis with more MUs identified
393 during the 80% LoS condition compared to the 60% condition (Table 1).

394 At first glance, it may appear that this organization is violating the size principle that has
395 been very well established in previous studies (40-46). However, the organization we describe
396 exists in harmony with the size principle. Henneman's size principle states that recruitment will
397 depend on the size of motor neurons receiving the *same amount* of synaptic input (47). This
398 implies that when a common input is sent to a motoneuron pool, recruitment will be governed by
399 the size of the units. However, when separate synaptic inputs are sent to different motoneuron
400 pools, the size principle will be governed separately within the pools. In fact, it has been
401 previously shown that different motoneuron pools may be controlled differentially when the
402 muscle contributes to different actions (48-51). Although the triceps surae are normally
403 considered to strictly plantarflex, there is evidence suggesting that MG and LG are also
404 responsible for providing inversion and eversion torques, respectively (18, 52, 53). Further, we
405 have previously demonstrated there is an uneven distribution of activation in the ankle
406 plantarflexors during postural control (18, 19). As the synaptic input to the ankle plantarflexors is
407 nonuniform and the muscles can contribute to different actions, it follows that the organization
408 we describe exists within the parameters of the size principle.

409 While selective synaptic inputs to the distinct motoneuron pools is supported in the
410 literature (18, 19, 38, 54-56), there is the possibility that the differential behaviour of the MU
411 subgroups may derive from the interaction between spinal mechanisms and muscle mechanics.
412 Neither spinal mechanisms (57) nor mechanical features alone can maintain upright standing in
413 humans (25, 58). However, the *combination* of spinal mechanisms and mechanical features of
414 the ankle plantarflexors without cortical input have been demonstrated in simulations to be
415 adequate for the maintenance of upright stance (57). The changing mechanical properties of the
416 muscles during the different leaning directions (i.e. nonuniform variations in length across the
417 different parts of the muscles) may interact with spinal proprioceptive activity (e.g. muscle
418 spindles, Golgi tendon organs, and interneurons) distributing nonuniformly on the motoneuron
419 pools. It should be noted that selective synaptic inputs, spinal mechanisms, and mechanical
420 properties may all interact to produce the differential behaviour output. Future research is
421 required to determine whether cortical, subcortical, and/or spinal contributions mediate the
422 differential behaviour observed.

423 *Shifts in the distribution of the AFR*

424 The distribution of AFRs in unique units showed peaks at lower frequency during the 0°
425 and 120° leaning directions and peaks at higher frequency during the 60°-90° (Figure 4). The
426 common units displayed lower frequency peaks for all leaning directions (Figure 4). To our
427 knowledge, this is the first study to demonstrate different AFR distributions for separate sub-
428 populations of MUs within the same muscle in humans. Previous studies have investigated
429 similar characteristics in animal models that may help explain our findings. Tansey and
430 Botterman (59) assessed MU firing rate behaviours during muscle contractions evoked by
431 stimulation of the mesencephalic locomotor region in cats. The distribution of instantaneous

432 firing rates between slow-twitch and fast-twitch units was different. Both types of MUs
433 displayed unimodal distribution patterns, but at opposite ends of the distribution pattern; slow-
434 twitch units had distribution peaks near 20 Hz, whereas fast-twitch had distribution peaks near
435 40 Hz. In our study, it is possible that the common units were comprised of mostly slow-twitch
436 units, whereas the unique units were both slow and fast-twitch units. Given that slow-twitch
437 MUs tend to fire more tonically, this explanation fits well with the idea that the common units
438 are first recruited to create baseline, stable torque (see above).

439 *Coefficient of Variation of the Interspike Interval and Degree of Intermittency*

440 There are subtle but important differences between the CoV_{ISI} and the degree of
441 intermittency. CoV_{ISI} is a measure of the variability of the MU firing instants and is associated
442 with the steadiness of the MU firings with a higher CoV_{ISI} being indicative of fluctuating
443 (unsteady) firing intervals. The CoV_{ISI} has been utilized and investigated extensively and is
444 generally used to describe the modulation of MU discharge times and force output steadiness
445 (60-63). The degree of intermittency is a standardized value measuring the extent to which the
446 firing rate has dropped below a physiological threshold of 4 Hz and determines whether a unit is
447 recruited sporadically. A low degree of intermittency is indicative of less MU derecruitment
448 occurrences, and therefore less sporadic (continuous) activity. The degree of intermittency has
449 been investigated to a lesser extent compared to CoV_{ISI} but is believed to be due to the
450 physiological properties of the muscle (e.g. recruitment thresholds), the time constant of the
451 standing body (22, 23), the neural drive delivered to the motoneuron pool, or a combination of
452 the three (22, 23, 57, 64).

453 Unique and common units were observed to have different patterns of CoV_{ISI} , but similar
454 patterns of the degree of intermittency across directions. Interestingly, during the leaning task,

455 the unique units CoV_{ISI} increased across the directions and the degree of intermittency decreased.
456 This observation demonstrates that the unique units had higher firing variability but are behaving
457 less sporadically as the leaning directions shifted towards 90° . Contrastingly, common units
458 CoV_{ISI} remained constant across directions, while their degree of intermittency decreased,
459 however this decrease was not found to be statistically significant. This is suggestive of a
460 steadier firing pattern and a more continuous activation compared to the unique units. These
461 observations may illustrate two things. First, it is possible that the higher CoV_{ISI} may be a
462 response to the fluctuations in participant's CoP during the leaning task. As the unique units are
463 recruited in optimal locations (see above), the CNS may be modulating the activity of the units
464 producing the most efficient torques. Second, it may provide evidence of different mechanisms
465 or synaptic inputs controlling the two constructs. One might assume if the CoV_{ISI} and degree of
466 intermittency were similarly controlled, they would follow the same trend (as MU firings
467 become more variable, they also become more intermittent) and they would have the same effect
468 in both MU subgroups. We observed an opposite trend (more variable firings and less
469 intermittency), and different effects in the unique and common units CoV_{ISI} . While speculative,
470 this may suggest that the constructs are controlled by different mechanisms (e.g., physiological
471 organization vs synaptic inputs) or by a certain degree of independent synaptic inputs. Future
472 studies are required to confirm these potential explanations.

473 The degree of intermittency of both MU subgroups had a similar response to the
474 directions, however the unique units were more intermittent compared to the common units. It
475 has been reported that an intermittent control pattern allows for precise control (65) while
476 minimizing the neural cost compared to a constant signal (64). This efficiency allows the CNS to
477 reduce the degrees of freedom of standing balance (64). While these models were derived during

478 quiet standing, our results provide more evidence that intermittent control could be used to
479 reduce the degrees of freedom during more demanding tasks.

480 The intermittent behaviour in the unique units is modulated based on direction. As the
481 leaning directions shifted towards the 90° target angle (directly forward movement), the degree
482 of intermittency of the ankle plantarflexors decreased. This is in agreement with other studies in
483 which intermittency has been observed to decrease during forward sway (12, 23). However,
484 previous studies have only investigated standing balance where directional information is
485 minimal. As mentioned, the 90° target angle requires maintaining the CoP position far forward
486 from its average position during quiet standing and thus imposing greater demands for
487 plantarflexion ankle torque. Given that leaning directly forward is controlled mostly by the ankle
488 plantarflexors with little help from other musculature (66, 67), it is not surprising that the degree
489 of intermittency decreased at 90° leaning direction. At this forward CoP position, fluctuations in
490 ankle torque necessary to compensate for the bodily sways would be superimposed on high,
491 average plantarflexion torque. As the leaning directions shift clockwise and counter clockwise,
492 more hip musculature may be recruited (67) that increases the feedback from group II muscle
493 spindles of the hip musculature received by CNS. Group II afferents can evoke opposite effects
494 in motor neurons that innervate flexor and extensor muscles in heteronymous connections (68,
495 69). This feedback system may allow the ankle plantarflexor to become more intermittent when
496 the leaning task is being assisted by other muscles to maintain the low metabolic cost of
497 standing.

498

499 *Possible Limitations*

500 While impossible to utilize with our methodology, the ability to record recruitment
501 thresholds for different MUs would have aided our interpretation of the findings. While other
502 studies provide information regarding slow twitch and fast twitch MUs, being able to record
503 recruitment thresholds would help confirm our hypothesis. In this study, the recruitment of motor
504 units occurs during a dynamic movement to the target location. Currently, decomposition of
505 HDs-EMG during dynamic movements has not been validated (70). Further development of
506 dynamic decomposition of HDs-EMG needs to be completed before we can utilize this
507 technique.

508 The contraction during the leaning task was not completely isometric; this caused slight
509 changes in muscle length during maintenance of the CoP within the targets. We nevertheless do
510 not expect these changes, which have been reported to be smaller than 50 μm (22), to have
511 affected the identification of firing instants. Indeed, the pulse-to-noise ratio, a metric indicating
512 the quality of EMG decomposition (71), was consistently high across all MUs analysed ($>28\text{dB}$).
513 Changes in muscle length across leaning directions were however presumably greater than those
514 occurring during each leaning direction. For this reason, we raised the error threshold for
515 matching units to 10% (19, 20), compensating for shape changes of common MUs waveform
516 during different leaning directions.

517

518 **Conclusion**

519 This study provides evidence that there is differential behaviour of distinct motoneuron
520 pools within the ankle plantarflexors utilized during postural control. Further research is
521 necessary to determine the precise mechanism of the differential behaviour of distinct
522 motoneuron subpopulations.

523

524 **Conflict of interest**

525 The authors have no conflicts of interest to declare

526

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529

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 708

709 Figure Legends

710 Figure 1

711 Schematic of the experimental set-up for the multi-directional leaning task. A. A participant
 712 standing on a force platform observing their centre of pressure movements towards the leaning
 713 targets in real time. B. The screen displaying participant's centre of pressure, leaning targets and
 714 the 60% (broken line) and 80% (solid line) limits of stability (LoS). The grey circles represent
 715 the 5 different leaning directions (0°, 30°, 60°, 90°, and 120°) placed along the ellipses
 716 calculated from the participant's limit of stability measures. Only one target was displayed at a
 717 time during the experiment. C. Placement of the high-density surface EMG sensors over the
 718 medial (MG) and lateral (LG) gastrocnemius (two 32 electrode grids) and the soleus (SOL; a
 719 single 64 electrode grid). D. Center of pressure (CoP) of a single participant during the multi-
 720 directional leaning task.

721

722 Figure 2

723 Examples of motor unit action potentials from each plantarflexor muscle of a single participant
 724 during the 30° leaning direction. Medial and lateral gastrocnemius (MG and LG) are displayed
 725 on the top. Soleus (SOL) is displayed on the bottom. The vertical and horizontal axes represent
 726 the channel rows (vertical axes) and columns (horizontal axes) from the high-density surface
 727 EMG.

728

729 Figure 3

730 Example of motor unit pairs that were matched (A), and two motor unit (MU) pairs that were
 731 untracked (B and C). The reference MU is depicted in red, and the paired MU in black. A
 732 displays a motor unit that was considered common, with the mean square error (MSE) = 6%. B
 733 and C display motor unit pairs that were considered unique, with MSE close to the 10%
 734 threshold (B: MSE = 16%) and very far from the threshold (C: MSE = 80%). Note that in C the
 735 locations of the motor units are different. The vertical and horizontal axes represent the channel
 736 rows and columns numbers from the high-density surface EMG, respectively.

737

738 Figure 4

739 Violin plots for each leaning direction of the average firing rate (AFR) distribution of the motor
 740 units (MUs) for all participants combined, identified during both 60% and 80% limits of stability

741 conditions overlaid by the MU AFR for the unique (left column; A, B) and common (right
 742 column; C, D) units in the medial and lateral gastrocnemius (MG: white circles, LG: black
 743 circles; A, C) and soleus (SOL: white circles; B, D). The number of MUs for each direction are
 744 indicated under each violin plot. A small amount of swarm was added to the x-axis for clarity of
 745 display. The horizontal location of the individual MU AFR does not have an effect on the
 746 distributions of the violin plots. There is a shift toward higher frequency in the AFR distribution
 747 for the unique units during the 30°-90° leaning directions.

748

749 Figure 5

750 Average firing rates (AFR) for unique (circles) and common (triangles) motor units during 60%
 751 (grey) and 80% (black) limits of stability (LoS) reported for the medial gastrocnemius (MG),
 752 lateral gastrocnemius (LG), and soleus (SOL). The data are estimated means and 95%
 753 confidence intervals. The estimates are calculated from the linear mixed model, with subjects as
 754 random intercepts, and fixed effects of leaning direction, muscle, limit of stability, and motor
 755 unit subgroup. Main effects are reported, no interactions were observed. The AFR was
 756 significantly higher for both unique and common units in medial and lateral gastrocnemius as
 757 compared to soleus (see Table 2). * Indicates significantly different from the 0° and 120° leaning
 758 directions for both 60% and 80% LoS ($p < 0.001$). † Indicates significantly different between
 759 unique and common units ($p < 0.001$).

760

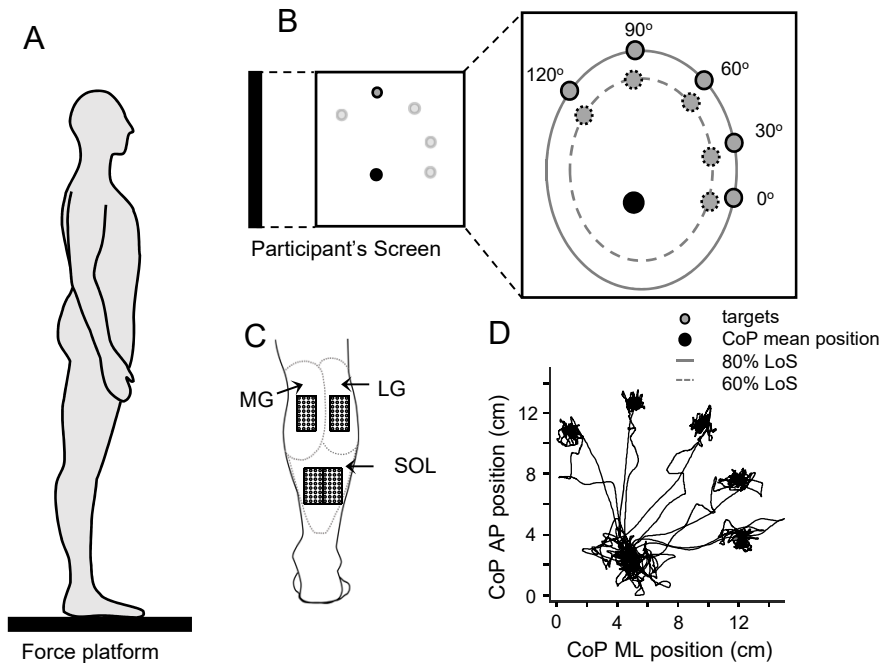
761 Figure 6

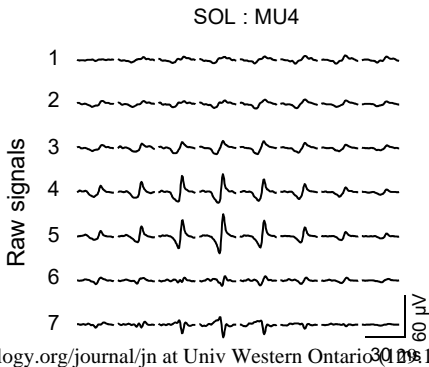
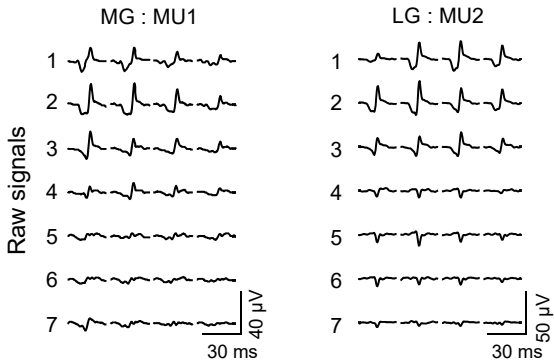
762 Interspike intervals coefficient of variation (CoV_{ISI}) for unique (circles) and common (triangles)
 763 motor units during 60% (grey) and 80% (black) limits of stability (LoS) reported for the medial
 764 gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL). The data are estimated
 765 means and 95% confidence intervals. The estimates are calculated from the linear mixed models,
 766 with a participant as a random intercept, and fixed effects of leaning direction, muscle, limit of
 767 stability and motor unit subgroup. The CoV_{ISI} was significantly higher for both unique and
 768 common units in the medial and lateral gastrocnemius as compared to soleus (see Table 3). × and
 769 * indicate significantly different from the 0° and 120° leaning direction, respectively, for both
 770 60% and 80% LoS. † Indicates significantly different CoV_{ISI} between unique and common units.
 771 ‡ Indicates significantly different CoV_{ISI} between 60% and 80% LoS for both unique and
 772 common units.

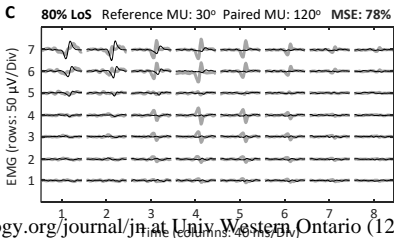
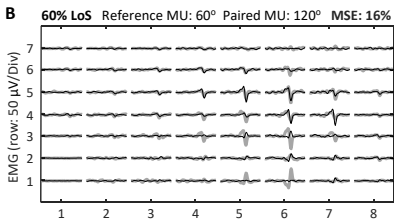
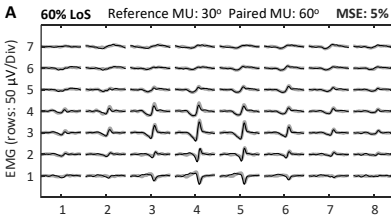
773

774 Figure 7

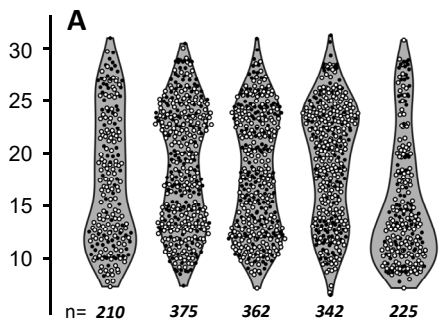
775 Degree of intermittency for unique (circles) and common (triangles) motor units. Only the 60%
 776 (grey) limits of stability (LoS) are reported for the medial gastrocnemius (MG), lateral
 777 gastrocnemius (LG), and soleus (SOL). The 80% LoS had near identical values, and has been
 778 omitted for clarity. The data are estimated means and 95% confidence intervals for the log
 779 transformed values. The estimates are calculated from the linear mixed model with participants
 780 as a random intercept, and fixed effects of leaning direction, muscle, limit of stability and motor
 781 unit subgroup. * Indicates significantly different from the 0° and 120° leaning directions for all
 782 muscles ($p < 0.001$). † Indicates significantly different degree of intermittency between unique
 783 and common units. The degree of intermittency was significantly higher for the common units in
 784 the medial gastrocnemius as compared to soleus (see Table 4).



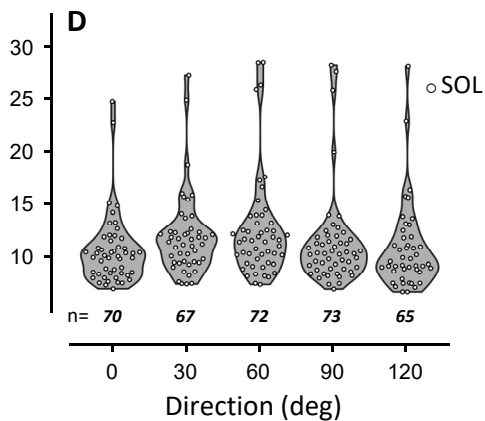
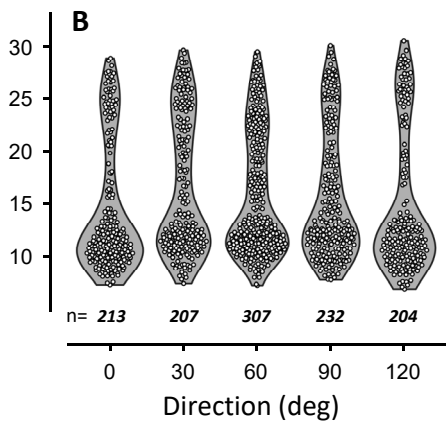
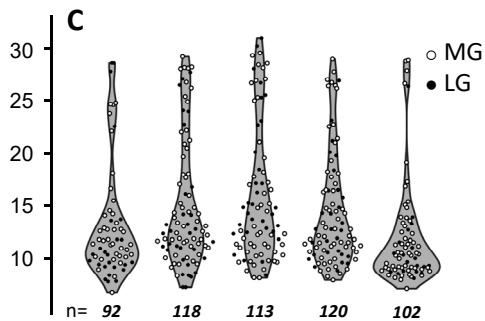


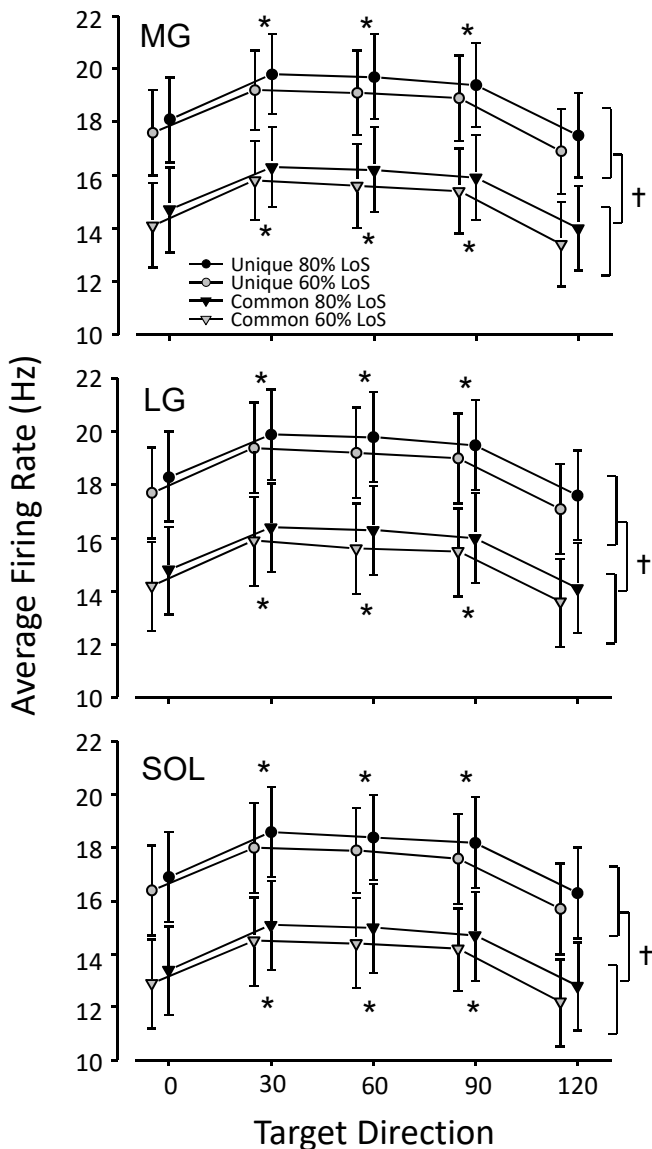


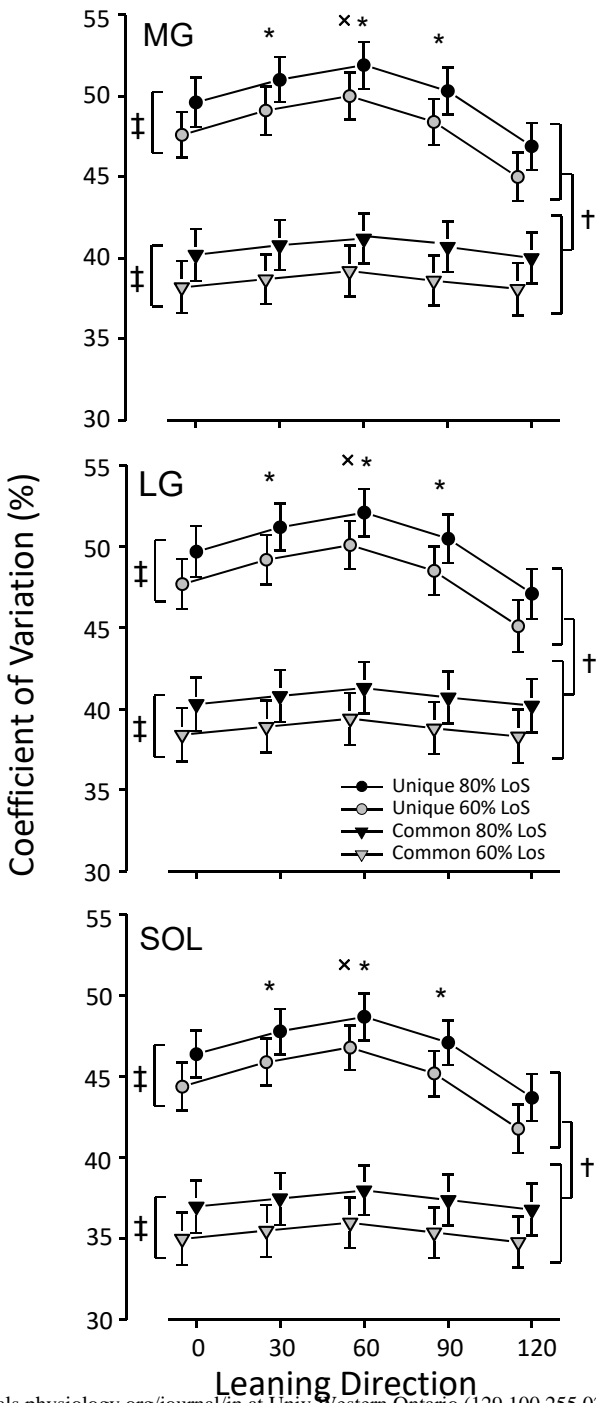
Unique MUs



Common MUs







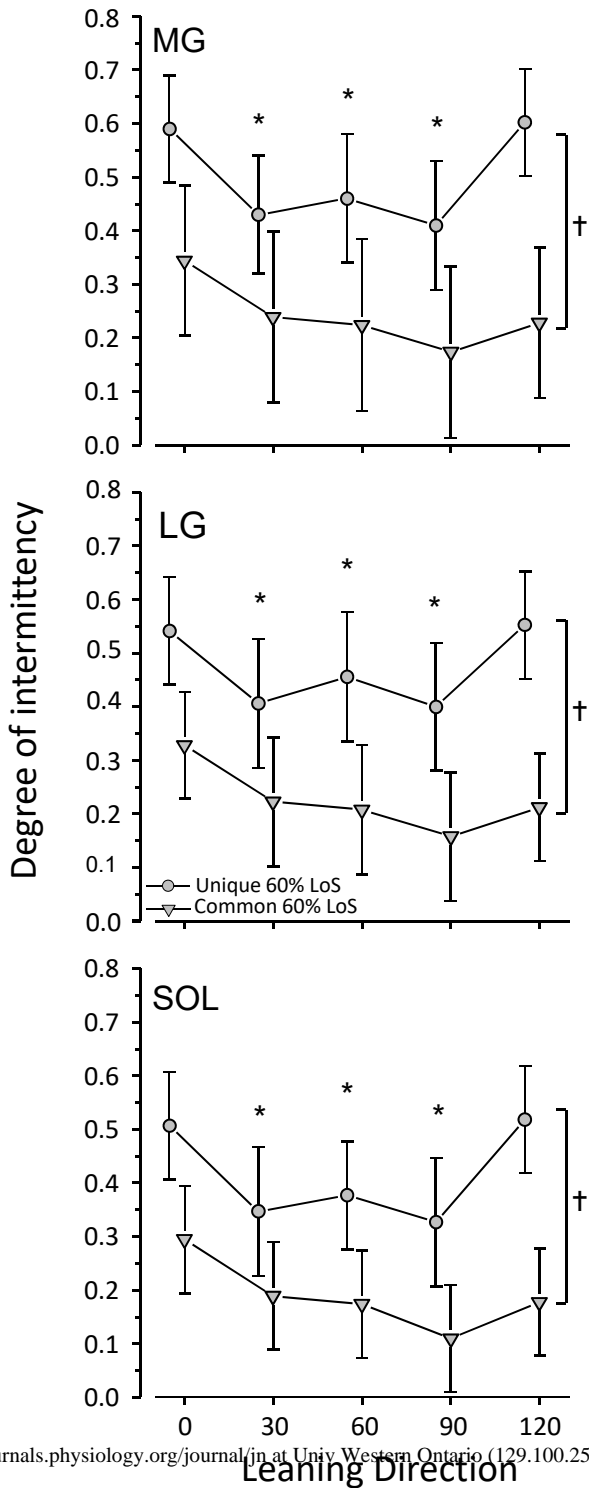


Table 1. Number of identified motor units

Muscle	Medial Gastrocnemius	Lateral Gastrocnemius	Soleus
Total	60%: 649 units, 44 ± 27 (24-59)	60%: 324 units, 20 ± 17 (6-48)	60%: 616 units, 42 ± 25 (26-52)
	80%: 684 units, 47 ± 28 (25-63)	80%: 402 units, 25 ± 19 (10-52)	80%: 894 units, 56 ± 30 (28-82)
Unique	60%: 468 units, 31 ± 20 (13-52)	60%: 227 units, 15 ± 10 (4-32)	60%: 448 units, 32 ± 19 (23-48)
	80%: 520 units, 36 ± 21 (13-58)	80%: 299 units, 21 ± 14 (5-43)	80%: 715 units, 51 ± 32 (33-78)
Common	60%: 181 units, 13 ± 11 (4-30)	60%: 97 units, 5 ± 3 (1-12)	60%: 168 units, 12 ± 6 (5-23)
	80%: 164 units, 11 ± 10 (5-22)	80%: 103 units, 5 ± 5 (1-13)	80%: 179 units, 14 ± 8 (6-24)

Values are group totals, mean ± standard deviation and (minimum-maximum range) across participants. 60% and 80% correspond to the limit of stability condition.

Table 2: Post hoc results from the linear mixed model using average firing rate as the dependent variable.

<i>Predictors</i>	Comparison	Estimated difference (Hz)	95% CI	<i>p-value</i>
Direction				
<i>Unique & Common</i>	0°-30°	1.65	1.19-2.12	<0.0001
	0°-60°	1.52	1.10-2.67	<0.0001
	0°-90°	1.27	0.98-2.67	0.003
	120°-30°	2.32	1.05-3.92	<0.0001
	120°-60°	2.15	1.76-3.52	<0.0001
	120°-90°	2.09	1.65-3.52	<0.0001
Muscle				
<i>Unique & Common</i>	MG-SOL	1.44	0.98-2.05	<0.0001
	LG-SOL	1.39	0.98-2.50	<0.0001
MU Subgroup				
	Unique-Common	3.48	1.46-7.40	<0.0001

Estimated difference = Mean estimated difference between comparisons in Hz.

CI = confidence intervals. MU = Motor unit. P-values are adjusted using Bonferroni corrections. No interactions were observed; therefore, the motor unit subgroup marginal means are given equal weighting.

Table 3: Post hoc results from the linear mixed model using coefficient of variation of the interspike interval (CoV_{ISI}) as the dependent variable

<i>Predictors</i>	Comparison	Estimated difference (a.u.)	95% CI	<i>p-value</i>
Direction				
<i>Unique</i>	0°-60°	0.02	0.01-0.04	0.002
	120°-30°	0.04	0.02-0.06	<0.0001
	120°-60°	0.05	0.03-0.07	<0.0001
	120°-90°	0.04	0.02-0.06	<0.0001
Muscle				
<i>Unique</i>	MG-SOL	0.03	0.02-0.05	<0.0001
	LG-SOL	0.04	0.02-0.06	<0.0001
<i>Common</i>	MG-SOL	0.06	0.03-0.08	<0.0001
	LG-SOL	0.04	0.02-0.06	0.003
MU Subgroup				
	Unique-Common	0.071	0.21-0.164	<0.0001
Direction x MU Subgroup				
<i>Unique-Common</i>	0°-60°	0.015	0.01-0.03	0.04
	120°-60°	0.04	0.02-0.06	0.009
LoS				
	60%-80%	0.02	0.01-0.04	<0.0001

Estimated difference = Mean estimated difference between conditions. a.u. = arbitrary units. CI = confidence interval. MU = motor unit. LoS = limit of stability. P-values are adjusted using a Bonferroni correction.

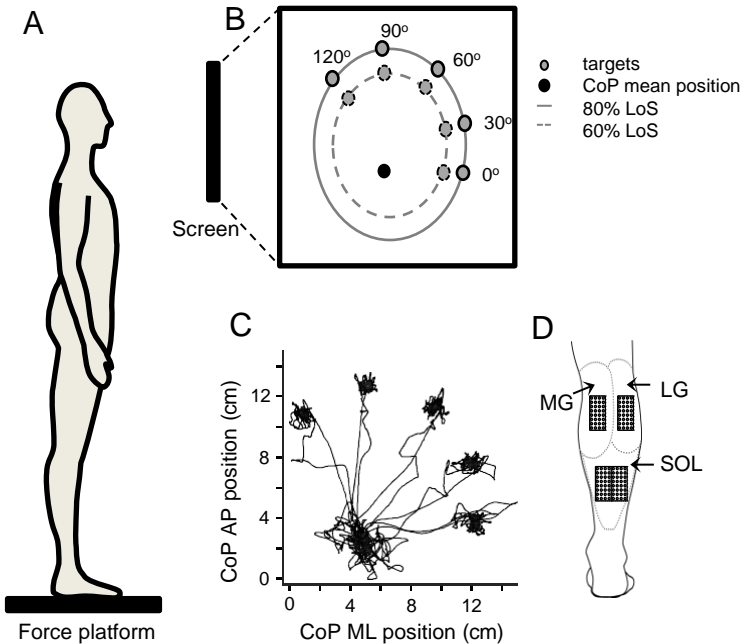
Table 4: Post hoc results from the linear mixed model using degree of intermittency as a dependent variable.

<i>Predictors</i>	Comparison	Estimated difference (log(IO/s))	95% CI	<i>p-value</i>
Direction				
<i>Unique</i>	0°-30°	0.16	0.027-0.29	0.003
	0°-60°	0.13	0.007-0.26	0.006
	0°-90°	0.18	0.05-0.33	<0.0001
	120°-30°	0.21	0.06-0.32	<0.0001
	120°-60°	0.18	0.06-0.32	<0.0001
	120°-90°	0.21	0.11-0.37	<0.0001
MU Subgroup				
<i>Unique-Common</i>	0°	0.13	0.05-0.26	0.0024
	120°	0.28	0.13-0.35	<0.0001
Muscle x MU Subgroup				
<i>Common</i>	MG-SOL	0.07	0.02-0.38	0.04

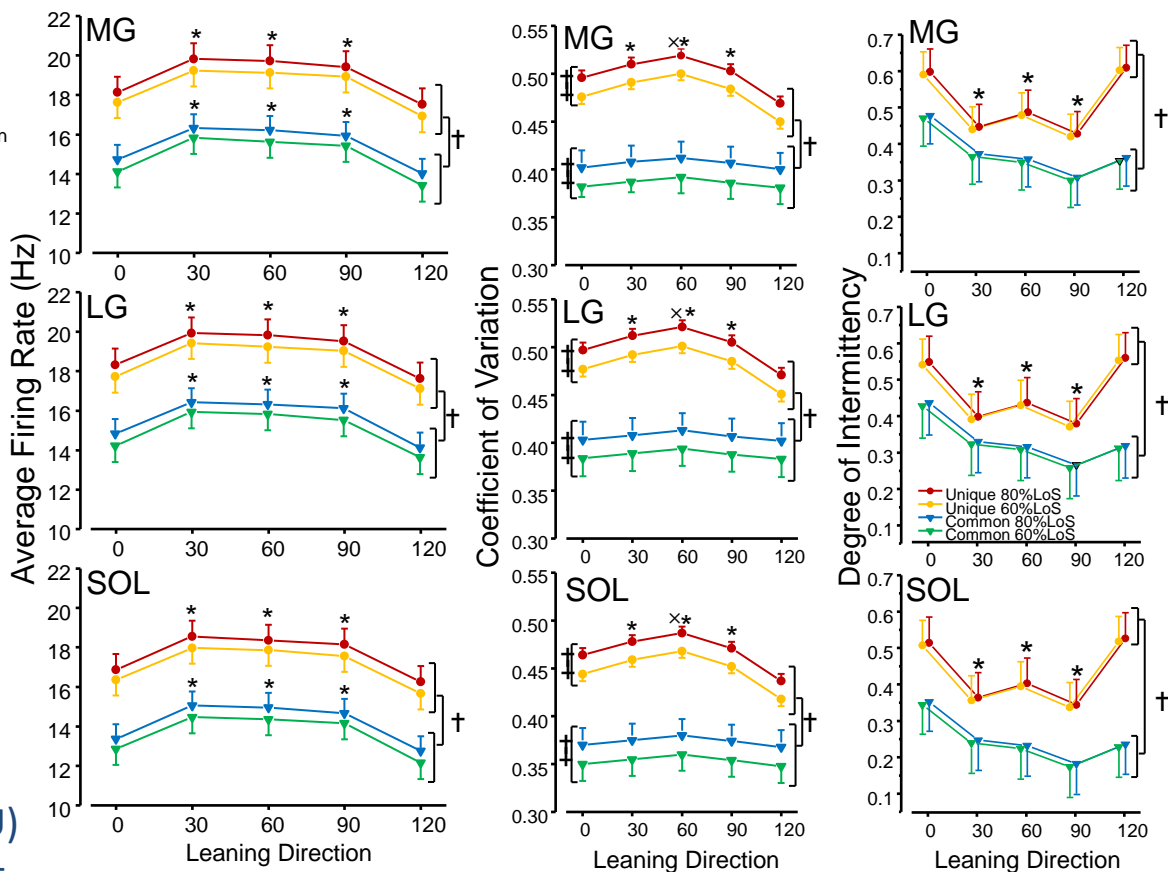
Estimated difference = Mean estimated difference between conditions. IO/s = intermittent occurrences/second. CI = confidence interval. LoS = limit of stability. P-values are adjusted using a Bonferroni correction.

Differential control of distinct motoneuron pools in the ankle plantarflexors

METHODS



OUTCOME



CONCLUSION

There is differential behavior of distinct motoneuron pools within the triceps surae utilized during postural control

Purpose: To evaluate the motor unit (MU) behaviour (average firing rate, coefficient of variation, intermittent behaviour) for MU active across multiple directions (common) and in one direction (unique)