Differential behavior of distinct motoneuron pools that innervate the triceps surae

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Abstract

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

New & Noteworthy

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

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

Keywords

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

Introduction

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

Evidence is mounting for differential MU behaviour during standing compared to that observed from non-weight bearing contractions. During quiet standing, the common modulation of MU discharge rates in the SOL is higher compared to voluntary isometric contractions (12) reflecting a stronger common drive during standing than seated contractions. Further, there is greater inhibition of MUs of the MG muscle in response to sural nerve stimulation when individuals are standing compared to lying supine (13). The relative contribution of recruitment or rate coding to ankle plantarflexor force production depends on the posture. That is, when responding to perturbations of increasing intensity during standing, MU recruitment in the gastrocnemius appears to be the main mechanism of force gradation (14), whereas in a sitting position both rate coding and recruitment are used to modulate force gradation in the gastrocnemius (15). Taken together, it appears that the MUs of the plantarflexors behave differently during postural control than during non-weight bearing contractions.

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

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

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

Methods

Participants and Experimental Protocol

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

Board Number: 110471) and conformed to the latest amendment of the Declaration of Helsinki. Participants provided written, informed consent before participating in the study.

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

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

High-Density Surface EMG Electrode Placement

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

HD-sEMG and Forceplate Recordings

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

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

Data Analysis

Motor unit analysis

All EMG data analyses were performed using MATLAB R2020b (The MathWorks, Inc., Natick, MA, USA). Single-differential EMGs between pairs of adjacent electrodes along each column of the matrix were computed from the monopolar signals and visually inspected for quality. Channels presenting contact problems or power line interference were linearly interpolated with the data collected by the surrounding 8 channels. After verifying the quality of all EMG signals, monopolar signals were filtered (Butterworth, 2nd order, 20-350 Hz) and decomposed with the blind source separation method (28) implemented in the DEMUSE tool software (v. 4.9; The University of Maribor, Slovenia). Signals for each leaning direction and LoS condition were decomposed separately, over the central 20s of the 35s period when CoP was maintained at the target location. This decomposition procedure can identify MU discharges over a wide range of contraction intensities and has been extensively validated using experimental and simulated signals (29, 30). After decomposition of the EMG signals, the firing instants of identified MUs were used to trigger and average single differential EMGs over 30ms epochs, providing the surface representation of single MU action potentials (31). The results of the decomposition were inspected visually, according to Power et al. (32), and noisy waveforms were discarded. Examples of retained MUs are shown in Figure 2.

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

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

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

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

Motor unit tracking

A MU tracking analysis was conducted separately for the 60% and 80% LoS conditions. MU action potentials that were common between the different leaning directions were identified by focusing on both the shape and amplitude of the waveform of MU action potentials. For each retained MU, the average rectified value (ARV) was calculated across 30ms epochs centered on individual action potentials. Each retained MU was paired to all other MUs identified during different leaning directions within the same muscle and LoS condition for each participant (e.g., a MU identified in the 0° leaning direction in the 60% LoS condition within the MG is compared to all other units identified in the MG at the 30°, 60°, 90°, and 120° leaning directions of the 60% LoS condition within a single participant). The action potentials for each pair of MUs were aligned in time by maximizing their cross-correlation function. The mean square difference was calculated between the two sets of time-aligned action potentials, averaged across channels, and then normalized with respect to the mean ARV of the two sets of action potentials. This normalization procedure is utilized to reduce the bias of waveform amplitude between two MU pairs (33). Finally, pairs of MU action potentials with a mean square difference smaller than 10% were considered common (19, 20). An expert operator visually confirmed all MUs that were identified as common by the algorithm. This provided us with a group of MUs that will be referred to as *common* units that were present in two leaning conditions or more. When a MU had a mean square difference greater than 10% with all MUs it was paired with, the MU was considered to be a uniquely recruited unit not found in other leaning directions. These MUs will be called *unique* units from this point. An example of a pair of retained *common* MUs is provided in Figure 3.

Statistical Analysis

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

Motor Unit Behaviour

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

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

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

Balance Performance

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

Results

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

Average Firing Rate

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

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

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

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

Coefficient of Variation of the Interspike Interval

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

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

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

Degree of Intermittency

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

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

Discussion

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

Differential behaviour of motor unit subgroups

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

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

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

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

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

Shifts in the distribution of the AFR

The distribution of AFRs in unique units showed peaks at lower frequency during the 0° and 120° leaning directions and peaks at higher frequency during the 60°-90° (Figure 4). The common units displayed lower frequency peaks for all leaning directions (Figure 4). To our knowledge, this is the first study to demonstrate different AFR distributions for separate sub-populations of MUs within the same muscle in humans. Previous studies have investigated similar characteristics in animal models that may help explain our findings. Tansey and Botterman (59) assessed MU firing rate behaviours during muscle contractions evoked by stimulation of the mesencephalic locomotor region in cats. The distribution of instantaneous firing rates between slow-twitch and fast-twitch units was different. Both types of MUs displayed unimodal distribution patterns, but at opposite ends of the distribution pattern; slow-twitch units had distribution peaks near 20 Hz, whereas fast-twitch had distribution peaks near 40 Hz. In our study, it is possible that the common units were comprised of mostly slow-twitch units, whereas the unique units were both slow and fast-twitch units. Given that slow-twitch MUs tend to fire more tonically, this explanation fits well with the idea that the common units are first recruited to create baseline, stable torque (see above).

Coefficient of Variation of the Interspike Interval and Degree of Intermittency

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

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

The degree of intermittency of both MU subgroups had a similar response to the directions, however the unique units were more intermittent compared to the common units. It has been reported that an intermittent control pattern allows for precise control (65) while minimizing the neural cost compared to a constant signal (64). This efficiency allows the CNS to reduce the degrees of freedom of standing balance (64). While these models were derived during quiet standing, our results provide more evidence that intermittent control could be used to reduce the degrees of freedom during more demanding tasks.

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

Possible Limitations

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

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

Conclusion

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

Conflict of interest

The authors have no conflicts of interest to declare

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada

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Figure Legends

Figure 1

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

Figure 2

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

Figure 3

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

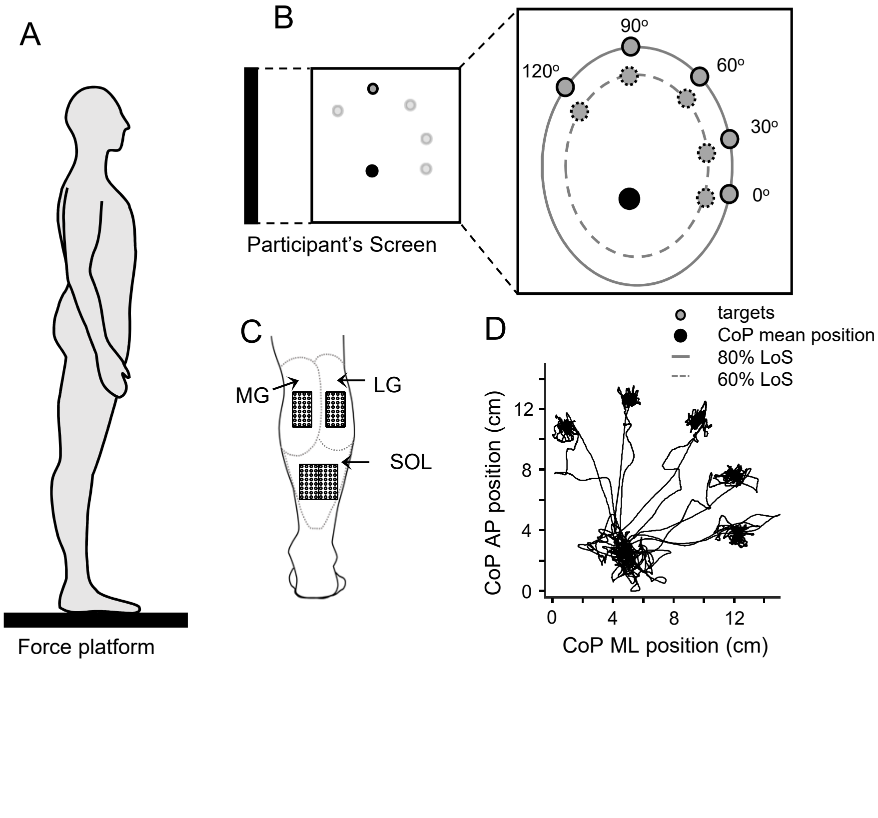
Figure 4

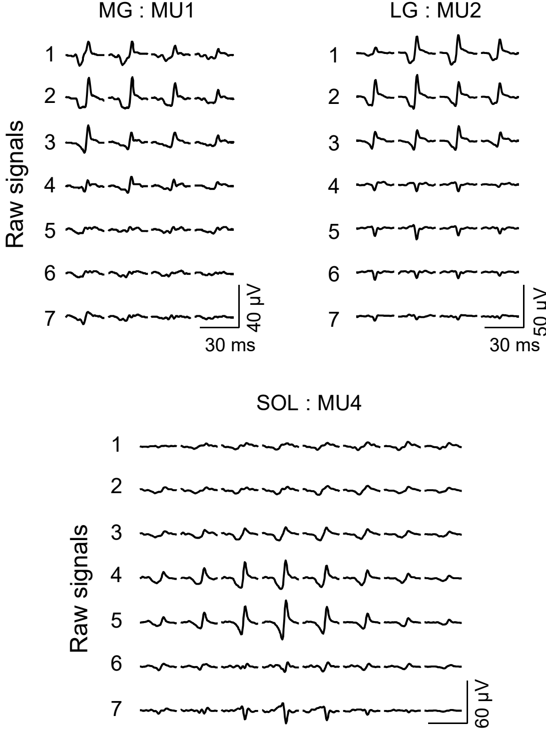
Violin plots for each leaning direction of the average firing rate (AFR) distribution of the motor units (MUs) for all participants combined, identified during both 60% and 80% limits of stability conditions overlaid by the MU AFR for the unique (left column; A, B) and common (right column; C, D) units in the medial and lateral gastrocnemius (MG: white circles, LG: black circles; A, C) and soleus (SOL: white circles; B, D). The number of MUs for each direction are indicated under each violin plot. A small amount of swarm was added to the x-axis for clarity of display. The horizontal location of the individual MU AFR does not have an effect on the distributions of the violin plots. There is a shift toward higher frequency in the AFR distribution for the unique units during the 30°-90° leaning directions.

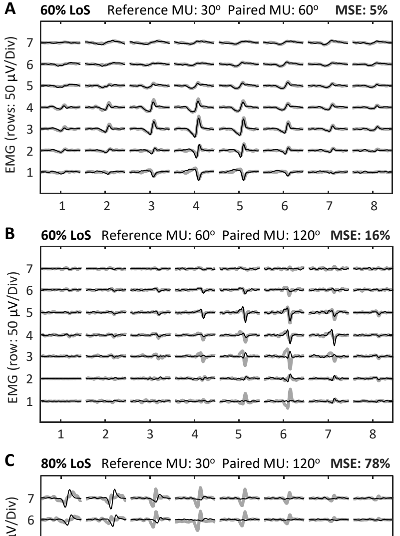
Figure 5
Average firing rates (AFR) for unique (circles) and common (triangles) motor units during 60% (grey) and 80% (black) limits of stability (LoS) reported for the medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL). The data are estimated means and 95% confidence intervals. The estimates are calculated from the linear mixed model, with subjects as random intercepts, and fixed effects of leaning direction, muscle, limit of stability, and motor unit subgroup. Main effects are reported, no interactions were observed. The AFR was significantly higher for both unique and common units in medial and lateral gastrocnemius as compared to soleus (see Table 2). * Indicates significantly different from the 0° and 120° leaning directions for both 60% and 80% LoS ($p < 0.001$). † Indicates significantly different between unique and common units ($p < 0.001$).

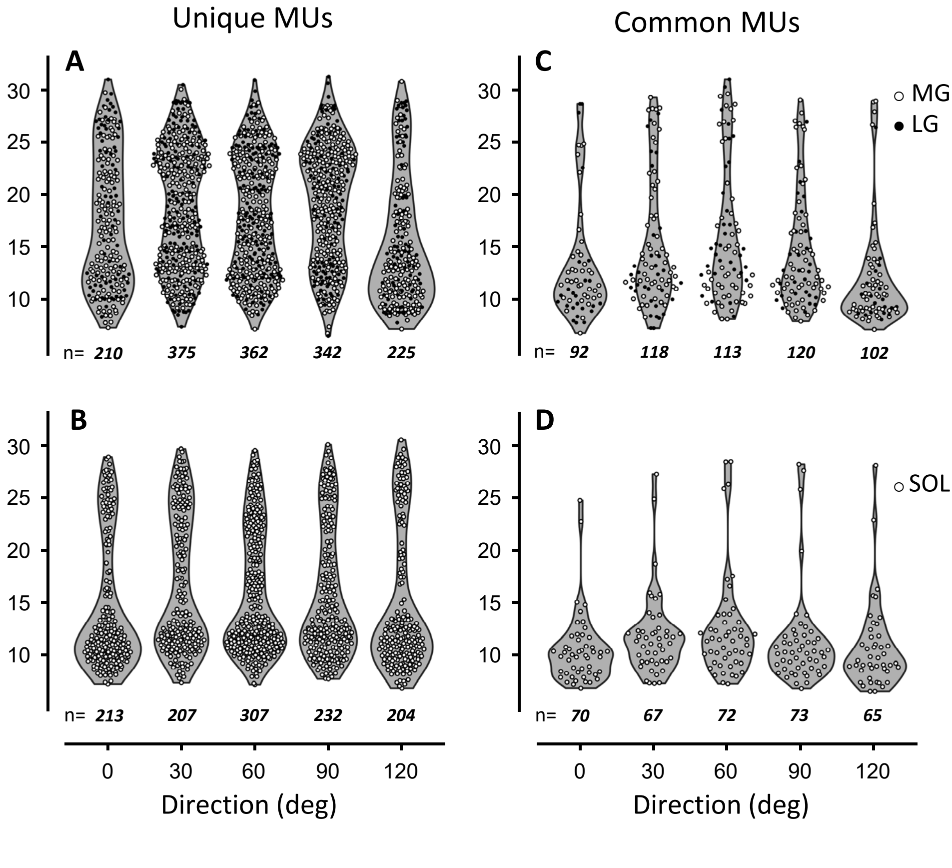
Figure 6
Interspike intervals coefficient of variation (CoV_{ISI}) for unique (circles) and common (triangles) motor units during 60% (grey) and 80% (black) limits of stability (LoS) reported for the medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL). The data are estimated means and 95% confidence intervals. The estimates are calculated from the linear mixed models, with a participant as a random intercept, and fixed effects of leaning direction, muscle, limit of stability and motor unit subgroup. The CoV_{ISI} was significantly higher for both unique and common units in the medial and lateral gastrocnemius as compared to soleus (see Table 3). $^{\times}$ and * indicate significantly different from the 0° and 120° leaning direction, respectively, for both 60% and 80% LoS. † Indicates significantly different CoV_{ISI} between unique and common units. ‡ Indicates significantly different CoV_{ISI} between 60% and 80% LoS for both unique and common units.

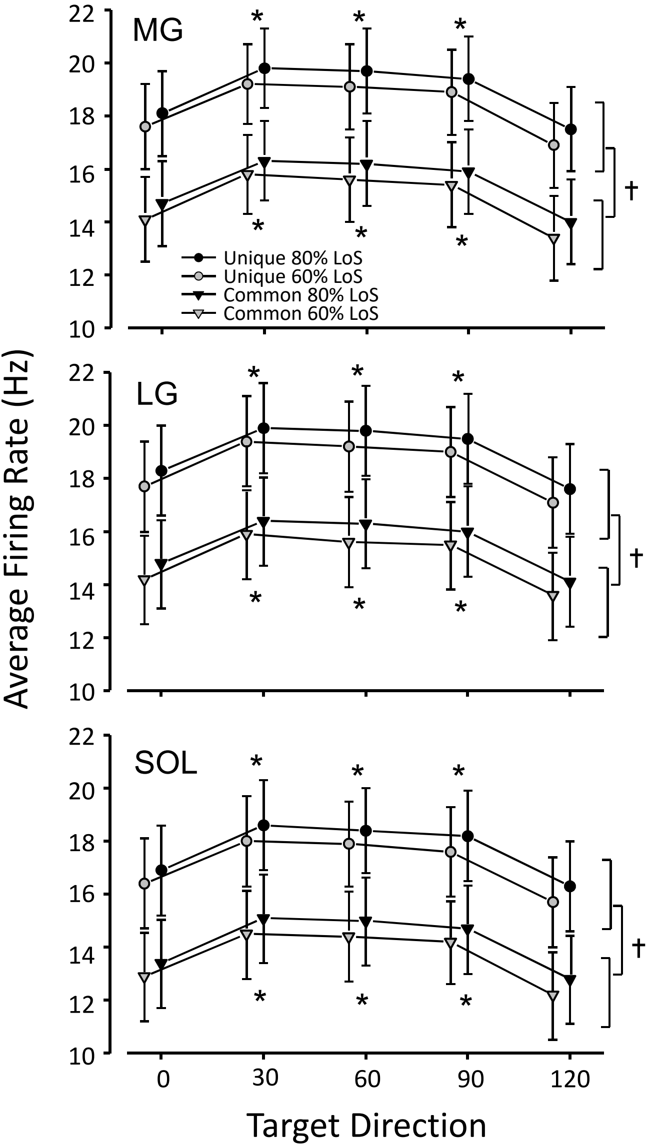
Figure 7
Degree of intermittency for unique (circles) and common (triangles) motor units. Only the 60% (grey) limits of stability (LoS) are reported for the medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL). The 80% LoS had near identical values, and has been omitted for clarity. The data are estimated means and 95% confidence intervals for the log transformed values. The estimates are calculated from the linear mixed model with participants as a random intercept, and fixed effects of leaning direction, muscle, limit of stability and motor unit subgroup. * Indicates significantly different from the 0° and 120° leaning directions for all muscles ($p < 0.001$). † Indicates significantly different degree of intermittency between unique and common units. The degree of intermittency was significantly higher for the common units in the medial gastrocnemius as compared to soleus (see Table 4).


A
Force platform
B
Participant's Screen
90°
120°
60°
30°
0°
C
MG
LG
SOL
D
targets
CoP mean position
80% LoS
60% LoS
CoP AP position (cm)
CoP ML position (cm)
0
4
8
12

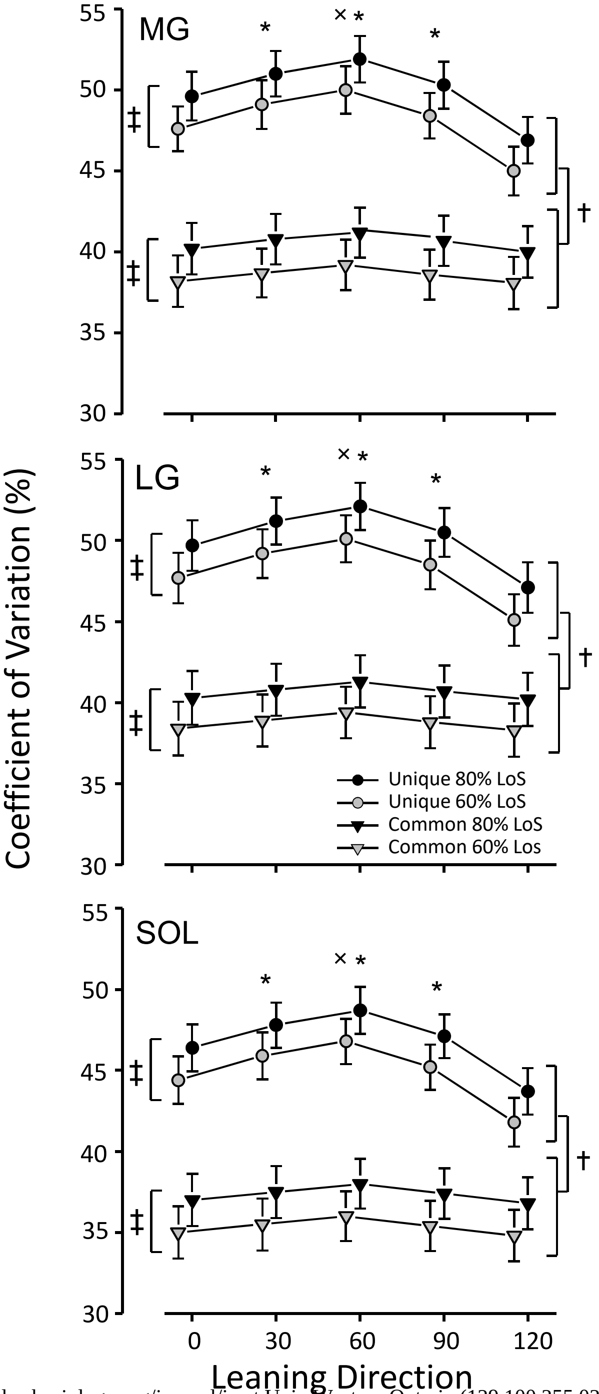

MG : MU1
LG : MU2
Raw signals
1
2
3
4
5
6
7
40 µV
30 ms
50 µV
30 ms
SOL : MU4
Raw signals
1
2
3
4
5
6
7
60 µV


A
60% LoS Reference MU: 30° Paired MU: 60° MSE: 5%
EMG (rows: 50 µV/Div)
B
60% LoS Reference MU: 60° Paired MU: 120° MSE: 16%
EMG (row: 50 µV/Div)
C
80% LoS Reference MU: 30° Paired MU: 120° MSE: 78%
EMG (rows: 50 µV/Div)
1 2 3 4 5 6 7
1 2 3 4 5 6 7 8
Time (columns: 40 ms/Div)


Unique MUs
Common MUs
A
B
C
D
MG
LG
SOL
30
25
20
15
10
n= 210 375 362 342 225
n= 92 118 113 120 102
n= 213 207 307 232 204
n= 70 67 72 73 65
0 30 60 90 120
Direction (deg)



Average Firing Rate (Hz)
MG
LG
SOL
Unique 80% LoS
Unique 60% LoS
Common 80% LoS
Common 60% LoS
22
20
18
16
14
12
10
0
30
60
90
120
Target Direction
*
†



MG
LG
SOL
Coefficient of Variation (%)
55
50
45
40
35
30
Unique 80% LoS
Unique 60% LoS
Common 80% LoS
Common 60% Los
0
30
60
90
120
Leaning Direction

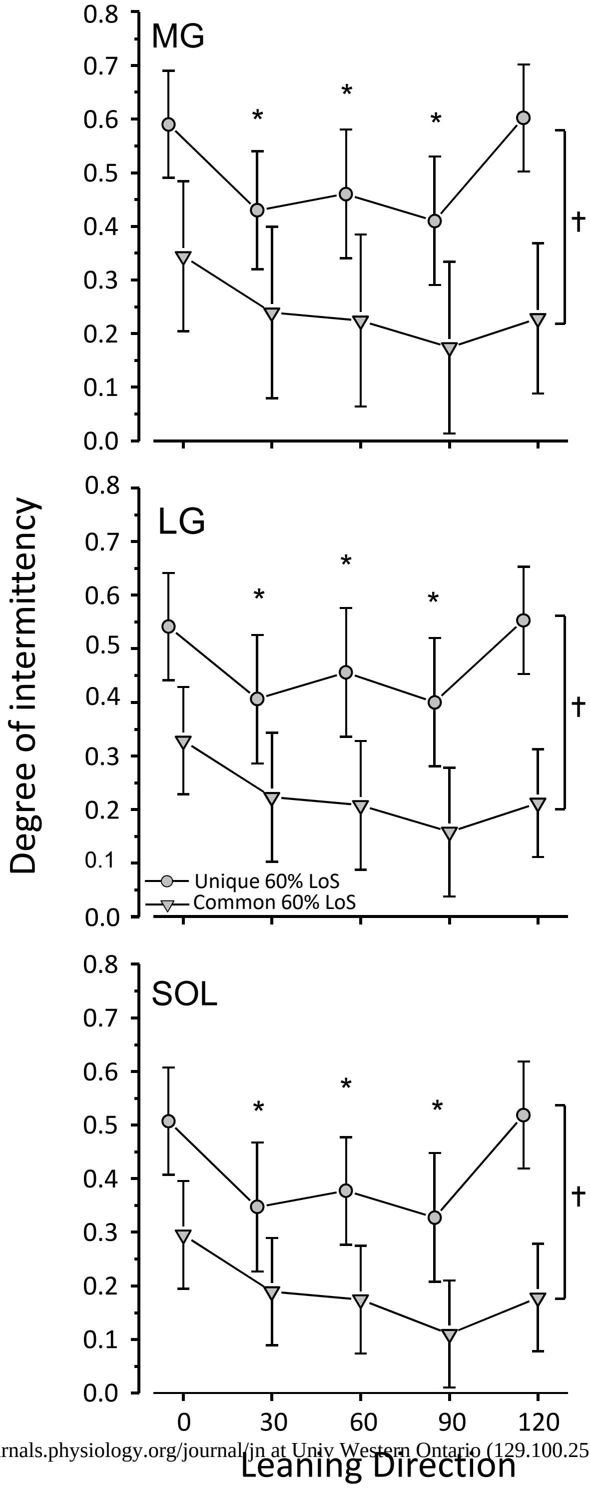

MG
LG
SOL
Degree of intermittency
Leaning Direction
Unique 60% LoS
Common 60% LoS
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
30
60
90
120
*
†

Table 1. Number of identified motor units

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

Predictors	Comparison	Estimated difference (Hz)	95% CI	*p-value*
Direction				
Unique & Common	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				
Unique & Common	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

Predictors	Comparison	Estimated difference (a.u.)	95% CI	*p-value*
Direction				
Unique	0^o-60^o	0.02	0.01-0.04	0.002
	120^o-30^o	0.04	0.02-0.06	<0.0001
	120^o-60^o	0.05	0.03-0.07	<0.0001
	120^o-90^o	0.04	0.02-0.06	<0.0001
Muscle				
Unique	MG-SOL	0.03	0.02-0.05	<0.0001
	LG-SOL	0.04	0.02-0.06	<0.0001
Common	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				
Unique-Common	0^o-60^o	0.015	0.01-0.03	0.04
	120^o-60^o	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.

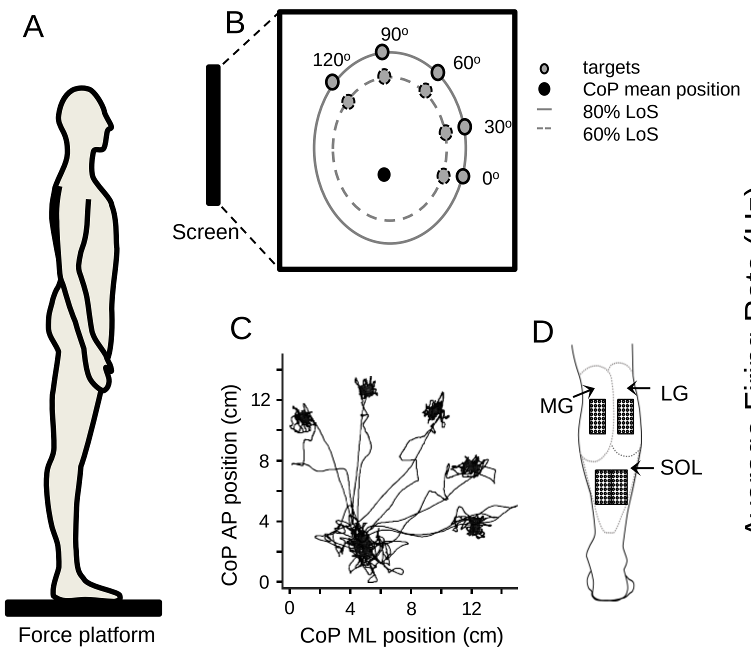
Table 4: Post hoc results from the linear mixed model using degree of intermittency as a dependent variable.

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

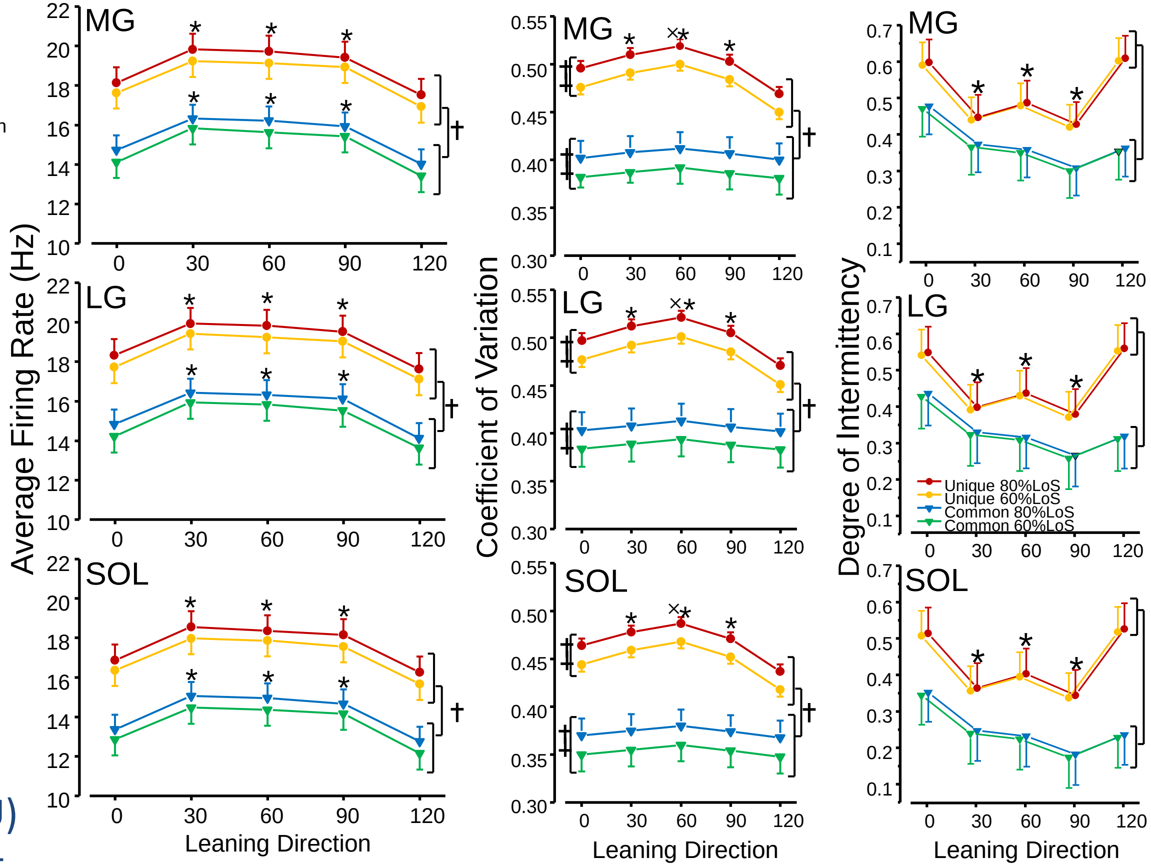
Estimated difference = Mean estimated difference between conditions. IO/s = intermittent occurrences/second. CI = confidence interval. LoS = limit of stability. P-values are adjusted using a Bonferroni correction.

Differential control of distinct motoneuron pools in the ankle plantarflexors

METHODS



OUTCOME



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)

CONCLUSION

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