# Two motor neuron synergies, invariant across ankle joint angles, activate the triceps surae during plantarflexion

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**Short title:** Invariance of common inputs to motor neurons

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# Abstract

Recent studies have suggested that the nervous system generates movements by controlling groups of motor neurons (synergies) that do not always align with muscle anatomy. In this study, we determined whether these synergies are robust across tasks with different mechanical constraints. We identified motor neuron synergies using principal component analysis (PCA) and cross-correlations between smoothed discharge rates of motor neurons. In Part 1, we used simulations to validate these methods. The results suggested that PCA can accurately identify the number of common inputs and classify motor neurons according to the weights of the common inputs they receive. Moreover, the results confirmed that cross-correlation can separate pairs of motor neurons that receive common inputs from those that do not receive common inputs. In Part 2, sixteen individuals performed plantarflexion at three ankle angles while recording high-density surface electromyography from the gastrocnemius lateralis (GL) and medialis (GM) and the soleus (SOL). PCA revealed two motor neuron synergies, primarily separating motor neurons innervating GL and GM. These motor neuron synergies were relatively stable with no significant differences in the distribution of weights across ankle angles ($p=0.62$). When the cross-correlation was calculated for pairs of motor units tracked across ankle angles, we observed that only 13.0% of pairs of motor units from GL and GM exhibited significant correlations across angles, confirming the low level of common inputs between these muscles. Overall, these results highlight the modularity of movement control at the motor neuron level, suggesting a sensible reduction of computational resources for movement control.


# Key points summary:

- The central nervous system may generate movements by activating clusters of motor neurons with common inputs.
- We show here that two main sources of common inputs drive the motor neurons innervating the triceps surae muscles during isometric ankle plantarflexions.
- We report that the distribution of these common inputs is globally invariant despite changing the mechanical constraints of the tasks, i.e., the ankle angle.
- These results suggest the functional relevance of the modular organization of the central nervous system to control movements.

# Introduction

The neural control of movement relies on the coordinated activity of numerous motor units. A large proportion of these motor units receives common synaptic inputs (Laine *et al.*, 2015; Negro *et al.*, 2016b), which arise from various sources, including descending commands and proprioceptive feedback (Christakos *et al.*, 2006; Lemon, 2008; Heckman & Enoka, 2012; Laine *et al.*, 2014; Glover & Baker, 2022). Simulation studies have shown that the pool of motor neurons tends to linearly transmit these common synaptic inputs to the muscle while it attenuates independent inputs (Farina *et al.*, 2014b). Thus, the time-varying modulation of muscle force during a sustained contraction mainly depends on the modulation of common synaptic inputs, as evidenced by the significant correlation between the low-pass filtered cumulative spike train of motor units and muscle force (De Luca & Erim, 2002; Negro *et al.*, 2009; Thompson *et al.*, 2018).

When considering the coordination of multiple muscles, common synaptic inputs may also be distributed across groups of motor units that share a similar function to generate a force in a desired direction (Tresch & Jarc, 2009; Bizzi & Cheung, 2013). This distribution determines a modular organization with low dimensionality. This concept of synergies (or motor modules) has been mainly demonstrated at the muscle level by combining the recording of multiple muscles with bipolar electromyography and factorization algorithms (Tresch *et al.*, 1999; d'Avella *et al.*, 2003; Ting *et al.*, 2015; Yaron *et al.*, 2020). The recent development of algorithms that identify the discharge activity of active motor units revealed a new dimensionality of movement control, where synergies are considered at the motor neuron level. These motor neuron synergies can cover subsets of motor neurons within each muscle or across portions of different muscles (Hug *et al.*, 2022). For example, it has been shown that motor neurons innervating a single muscle can be separated into several clusters based on their fluctuations of discharge rate, even during tasks of a single degree of freedom (Madarshahian *et al.*, 2021; Del Vecchio *et al.*, 2022).

To date, the identification of clusters of motor neurons, termed ‘motor neuron synergies’, has only been reported for motor tasks with stable mechanical constraints (Madarshahian *et al.*, 2021; Del Vecchio *et al.*, 2022; Hug *et al.*, 2022). It remains unknown whether the distribution of common inputs to groups of motor neurons is robust across motor tasks, as observed for muscle synergies (d'Avella *et al.*, 2003). Addressing this question is fundamental to prove that the observed

clustering of motor neurons is an effective way of simplifying the neural control of movement by modules that are invariant across a repertoire of tasks.

The technical challenge of identifying clusters of motor neurons across tasks is that motor neuron must be tracked across tasks using decompositions of high-density electromyographic (HD-EMG) signals (Farina & Holobar, 2016; Negro *et al.*, 2016a). However, HD-EMG decomposition is currently only applicable during isometric contractions with only slight changes in muscle length since this procedure relies on the stability of muscle geometry to identify the unique spatiotemporal properties of motor unit action potentials as detected by the grid of electrodes (Martinez-Valdes *et al.*, 2017; Vecchio & Farina, 2019; Oliveira & Negro, 2021). For these reasons, we identified and tracked motor neurons innervating the triceps surae muscles (soleus [SOL], gastrocnemius medialis [GM], and gastrocnemius lateralis [GL]) during three isometric contractions performed at different ankle angles. It resulted in differences in muscle length across the contractions, and therefore in differences in muscle force generating capacity. These muscles are of interest as there is evidence of uncoupling of their neural drives during sustained contractions (Hug *et al.*, 2021b; Rossato *et al.*, 2022), despite all being connected to the same distal tendon and being identified within the same motor module during most natural behaviors. Similarly, it was shown that changes in neural drive due to the alteration of force generating capacity during an ankle rotation may differ between GL and GM/SOL (Lacourpaille *et al.*, 2017). This suggests that the central nervous system could control more than one synergy, even during a task with one degree of freedom (Lacourpaille *et al.*, 2017; Rossato *et al.*, 2022).

In this study, we identified motor neuron synergies by estimating the distribution of common synaptic inputs to the group of motor neurons innervating the triceps surae muscles. To this end, we used principal component analysis and cross-correlation of their smoothed discharge rates (Fig. 1A). To validate our approach, we first simulated 100 motor neurons receiving two sources of common synaptic input, and we assessed whether the proposed processing method could identify the low dimensional control of motor neuron behavior. Following this validation, we recorded HD-EMG signals over the triceps surae muscles from sixteen participants to evaluate the robustness of motor neuron synergies across tasks. We hypothesized that 1) despite the single dimension captured by classic electromyographic analysis, the dimensionality of control at the motor neuron level would be greater than one, and 2) the structure of the identified motor neuron synergies would remain invariant across joint angles. The validation of these hypotheses would provide evidence

that the central nervous system controls movements by distributing common inputs to stable groups of motor neurons, ensuring a sensible reduction of dimensions for movement control.

# Methods

## Part 1: Simulations

We first aimed to test the sensitivity of our methods to capture the low dimensional control of motor neurons (Fig. 1B). As previous studies have shown differential changes in neural drive between GL and GM/SOL during constrained isometric plantarflexions (Lacourpaille *et al.*, 2017; Hug *et al.*, 2021b), we hypothesized that two main sources of common inputs may drive the triceps surae muscles during plantarflexion. Thus, we simulated 100 motor neurons receiving common and independent synaptic inputs using the model developed by Elias and Kohn (2013), with one compartment for the soma and one compartment for the dendritic tree. This model is an updated version of a previously published motor neuron model (Cisi & Kohn, 2008) with the addition of a L-type $Ca^{++}$ channel into the dendritic compartment to consider the non-linearity between motor neuron inputs and outputs due to persistent inward currents. Geometric and electronic parameters of the 100 motor neurons were linearly interpolated from the range of values reported in Table 1 of Elias and Kohn (2013) for a S-type motor unit.

The total synaptic input for each motor neuron was the linear summation of common and independent inputs simulated as white Gaussian noise. The common and independent inputs had equal variances, with a bandwidth of 0-2.5Hz for the common inputs, and a bandwidth of 0-50Hz for the independent inputs. The mean and the variance of the total synaptic input were tuned to generate physiologically realistic distributions of interspike intervals (Negro *et al.*, 2016b). To simulate motor neuron synergies, we generated two uncorrelated sources of common synaptic inputs with a range of synaptic weights for the pool of motor neurons. Specifically, 20 motor neurons received 100% of common input (CI) #1, 20 motor neurons received 100% of CI#2, and 60 motor neurons received CI#1 and CI#2 with synaptic weights ranging from 99% CI#1/1% CI#2 to 1% CI#1/99% CI#2. The synaptic weights were shuffled across the 100 motor neurons and fixed for all the simulations.

We generated three simulations with different amplitudes of the two common inputs. In the first simulation, the amplitude of common inputs #1 and #2 were equal (CI#1 = CI#2). In the second (CI#1+ CI#2) and third simulations (CI#1++ CI#2), the amplitude of common input #1 was amplified to generate an averaged increase in discharge rate of 1 pps between each condition for all the motor neurons receiving this input (Fig. 1B). This idea was to mimic the divergent

modulation of two separate sources of common synaptic inputs and to match the discharge rates experimentally observed during the plantarflexions.

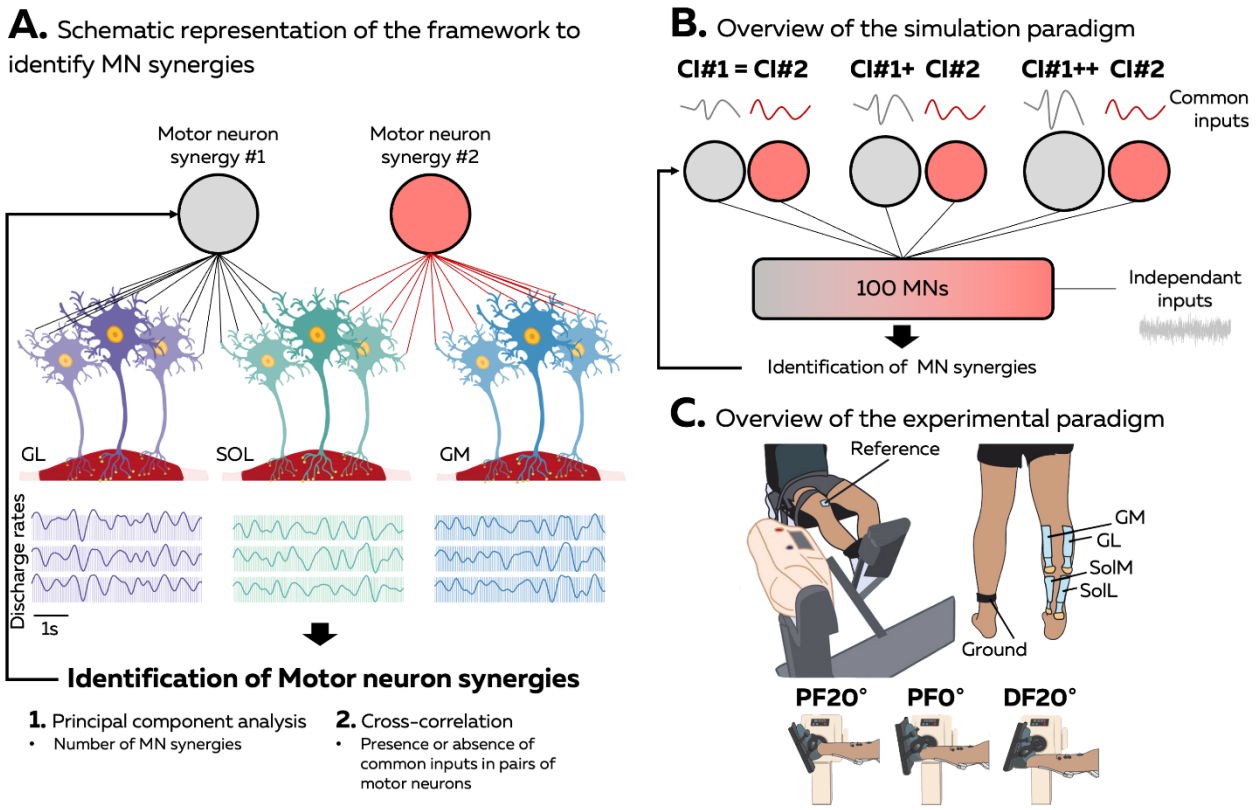


**Figure 1: Overview of the study.** We aimed to identify motor neuron synergies driving the triceps surae muscles during ankle plantarflexion. (A) We hypothesized that two motor neuron synergies would drive the gastrocnemius lateralis (GL), soleus (SOL), and gastrocnemius medialis (GM) muscles. To test this hypothesis, we needed to identify the distribution of common inputs to the pool of motor neurons. Thus, we applied principal component analyses (PCA) and cross-correlation on motor neuron smoothed discharge rates. (B) We first assessed the ability of these methods to identify the distribution of common inputs across motor neurons using 100 simulated motor neurons driven by two sources of common inputs with known synaptic weights. We changed the amplitude of these inputs to mimic the divergent variations in neural drive observed experimentally between GL and GM/SOL muscles. (C) Then, we identified and tracked motor units from high-density electromyographic signals recorded over the GL, GM, and SOL muscles across three ankle angles. As for the simulation, we determined whether the distribution of common inputs to the tracked motor units was robust across the three conditions using PCA and cross-correlation.

## Part 2: Experiments

In the second part of the study, we identified and tracked the motor neuron synergies driving the triceps surae muscles during plantarflexions performed at three ankle joint angles (Fig. 1C).

181

182 ***Participants***

183 A sample of sixteen individuals, with an equal number of males (n = 8; age: 28 ± 4 years, height:
184 178 ± 6 cm, body mass: 77 ± 10 kg) and females (n = 8; age: 29 ± 11 years, height: 162 ± 8 cm,
185 body mass: 60 ± 9 kg), volunteered to participate in this experiment. They had no history of knee
186 nor ankle injury in the last six months before the experiments or lower leg pain that would impact
187 their ability to complete the voluntary contractions. The institutional review board (IRB) of
188 Northwestern University reviewed and approved the protocol of this study (STU00212191).
189 Participants provided their informed written consent before starting the experimental session.

190

191 ***Protocol***

192 Participants sat on a dynamometer (Biodex System 4 Pro, Biodex Medical, Shirley, NY) with their
193 hip flexed at 90º (0º being the neutral position) and their knee fully extended (Fig. 1C). The foot of
194 the dominant leg (i.e., right leg for all the participants) was fixed to a pedal that held the ankle at
195 specified angles (Fig. 1C). Two inextensible straps immobilized their torso, while additional straps
196 around the waist and the thigh of the dominant leg ensured that force generation solely relied on
197 the calf muscles.

198 All experimental tasks consisted of isometric ankle plantarflexion. The participants first performed
199 a standardized warm-up with their ankle positioned at neutral position (0º, i.e., the foot is
200 perpendicular to the shank). Participants were asked to push against the pedal with gradually
201 increasing effort, beginning at about 50% of their subjective maximum capabilities. After three
202 contractions, participants were asked to increase effort until they reached about 80-90% of their
203 subjective maximum capabilities.

204 After the warm-up, experimentation began. The experimental protocol was repeated for three
205 different conditions in a randomized order: the ankle plantarflexed at 20º (PF20°), at 0º (PF0°), and
206 dorsiflexed at 20º (DF20°) (Fig. 1C). For each condition, participants performed two maximal
207 isometric voluntary contractions (MVC) for 3-5 s, with 60 s of rest in between. The peak torque
208 was calculated using a moving average window of 250 ms, and the maximal value was used to set
209 the target of the submaximal isometric contractions at 20% of the MVC. After 60 s of rest,
210 participants performed three trapezoidal contractions, consisting of a 15 s linear ramp-up, 30 s
211 plateau at 20% of the MVC, and 15 s linear ramp-down. These three trapezoidal contractions were

separated by 10 s of rest. Target and torque output feedback were displayed on a monitor in front of the participants. The HD-EMG acquisition system (EMG-Quattrocento; 400-channel EMG amplifier, OT Bioelettronica, Italy) was used to digitize the torque signal at a sampling rate of 2048 Hz. Torque signals were low pass filtered (3$^{rd}$ order Butterworth filter, cutoff frequency: 20 Hz) and baseline corrected to remove the weight of the leg and the pedal. Torque accuracy was calculated as the root mean squared error (RMSE) of the signal over the plateau.

***High-density surface EMG recording***

High-density surface EMG (HD-EMG) signals were recorded from the gastrocnemius medialis (GM), gastrocnemius lateralis (GL), and the lateral and medial regions of the soleus (SOL) using two-dimensional adhesive grids of 64 electrodes (GR08MM1305, 13x5 gold-coated electrodes with one electrode absent on a corner; interelectrode distance: 8mm, OT Bioelettronica, Italy). The grids were aligned in the fascicle direction for the GM and GL. The grids over the soleus were positioned on each side of the Achilles tendon in the muscle shortening direction. Before placing the electrodes, the skin was shaved and cleaned with an abrasive pad and an alcohol wipe. A semi-disposable biadhesive foam layer was used to hold the grids of electrodes to the skin, and conductive paste filled the cavities of the adhesive layers to make skin-electrode contact. Reference electrodes (Ag/AgCl snap electrodes, Noraxon, USA) were placed over the tibia. The EMG signals were recorded in monopolar mode, bandpass filtered at 10-500 Hz, and digitized at a sampling rate of 2048 Hz using the multichannel HD-EMG acquisition system (EMG-Quattrocento, 400 channel EMG amplifier; OT Bioelettronica, Italy).

## Data Analysis

Data were analyzed using MATLAB custom-written scripts (R2021a; The MathWorks, Natick, MA).

***HD-EMG decomposition***

The monopolar EMG signals were bandpass filtered between 20 and 500 Hz with a second-order Butterworth filter. After visual inspection, channels with low signal-to-noise ratio or artifacts were discarded. The HD-EMG signals were then decomposed into motor unit spike trains. We applied a

convolutive blind-source separation (Negro *et al.*, 2016a). In short, the EMG signals were first extended and whitened. Thereafter, a fixed-point algorithm that maximized the sparsity was applied to identify the sources of the EMG signals, i.e., the motor unit spike trains. The spikes were separated from the noise using K-means classification and a second algorithm refined the estimation of the discharge times by minimizing the coefficient of variation of the inter-spike intervals while iteratively updating motor unit filters. This decomposition procedure has been validated using experimental and simulated signals (Negro *et al.*, 2016a). After the automatic identification of the motor units, all of the motor unit spike trains were visually checked for false positives and false negatives, and manually corrected when necessary (Del Vecchio *et al.*, 2020; Hug *et al.*, 2021a). Motor units with a pulse-to-noise ratio > 30 dB were retained for further analysis (Holobar *et al.*, 2014).

***Crosstalk and duplicates between motor units***

We carefully checked all identified motor units for potential crosstalk units or duplicates, thus ensuring the uniqueness of each motor unit within the dataset (Hug *et al.*, 2021b). To this end, we used the discharge times as a trigger to segment and average the HD-EMG signal over a window of 50 ms and identify the motor unit action potentials over the 64 electrodes of each grid. Then, we calculated the average peak-to-peak amplitude of the action potentials for each grid. We considered that a motor unit was falsely associated with a muscle due to crosstalk when the peak-to-peak amplitude was larger in a neighboring muscle than in the muscle of origin. On average, 2.6 ± 2.5 MUs were discarded from the analysis per participant. In addition, we verified that motor units identified from the grid located over the lateral and the medial parts of the SOL were not the same, i.e., duplicates. We considered duplicates as motor units that shared at least 30% of their discharge times with a tolerance of one data point (i.e., 0.5 ms; (Holobar *et al.*, 2014)). At the end of these analyses, motor units identified over the lateral and medial parts of the SOL were pooled, with on average 0.7 ± 1.4 MUs discarded per participant.

***Motor unit tracking***

To assess whether a change in muscle length impacts the distribution of common synaptic inputs and motor unit behavior, we tracked the same motor units across conditions. Thus, we matched

motor units using the unique spatiotemporal properties of motor unit action potentials within the grids of electrodes (Martinez-Valdes *et al.*, 2017; Vecchio & Farina, 2019; Oliveira & Negro, 2021). Specifically, EMG signals were first differentiated in the column direction to obtain 59 single-differential EMG signals. The motor unit action potential shapes were identified by spike-triggered averaging each of the 59 EMG signals. As the position of the action potentials within the window may differ between motor units, we considered the local maxima, i.e., the action potential with the highest peak-to-peak amplitude within the grid, as the center of the 50 ms window. Finally, we concatenated the motor unit action potentials from the 59 channels, and we performed a 2D cross-correlation between motor units. Pairs with a correlation coefficient higher than 0.75 were considered as matches (Martinez-Valdes *et al.*, 2017; Vecchio & Farina, 2019). The matches were visually checked to guarantee the similarity of the motor unit action potential shapes. Motor units were separately tracked between PF20° and PF0° and between DF20° and PF0°. Only motor units tracked across the three conditions were considered for the analyses. Average discharge rate over the torque plateau was estimated for all tracked motor units.

***Motor neuron synergies***

We identified the common dynamics of motor unit smoothed discharge rates in Parts 1 and 2 using principal component analysis (PCA). The motivation behind this analysis was first to assess the ability of PCA to accurately identify the number of common inputs, and then to estimate the relative distribution of these common inputs, or motor neuron synergies, across motor units. All motor units spike trains were converted into binary vectors where discharge times were equal to one. These binary signals were then convoluted with a 400-ms Hanning window, such that the correlation was calculated from the oscillations of the signal related to the fluctuation of force (i.e., < 2.5 Hz). It is noteworthy that focusing on the lowest bandwidth also limits the effect of the nonlinear relationship between the synaptic input and the output signal (Negro & Farina, 2012). These signals were high pass filtered to remove offsets and trends using a Butterworth filter $2^{nd}$ order with a cut-off frequency of 0.75 Hz. Finally, we normalized the smoothed discharge rates between 0 and 1 to avoid any bias of the principal component analysis output due to differences in discharge rates between motor units and conditions.

Normalized smoothed discharge rates were then concatenated in a $n \times T$ matrix, where n is the number of tracked motor units and T the number of time samples. We applied PCA to this matrix

303 and identified n components ranked with the level of variance explained by each component. To
304 select the number of components of interest (i.e., motor neuron synergies), we fitted portions of the
305 curve of the variance explained with straight lines by iteratively decreasing the number of points
306 considered in the analysis. The number of components of interest was considered as the first data
307 point where the mean squared error of the fit falls under $5e^{-4}$ (Cheung *et al.*, 2005; d'Avella *et al.*,
308 2011). In our study, this threshold was reached with two components for both the simulated and
309 experimental data. Then, we displayed two-dimensional biplots where x and y coordinates were
310 the weights of each motor unit in the two first components, and we calculated the angle between
311 vectors. We considered that the higher the angle between two vectors, i.e., two motor units, the
312 lower the proportion of inputs shared between these units. Therefore, we described the changes in
313 the distribution of common synaptic inputs to motor units by comparing the angles between
314 conditions.
315
316 ***Pairs of motor units receiving common synaptic inputs***
317 For both Part 1 and Part 2, we estimated for each pair of motor units whether they received a
318 significant level of common synaptic input. To this end, we calculated the cross-correlation
319 between their smoothed discharge rates over a 10 s window that maximized the number of motor
320 units continuously firing during the plateaus of the submaximal contractions. The presence of
321 common synaptic input for each pair of motor units was estimated through the maximal cross-
322 correlation coefficient with a maximal lag of ± 100 ms (De Luca & Erim, 2002). We considered
323 that a pair of motor units received common synaptic input when the cross-correlation coefficient
324 was statistically significant (Rodriguez-Falces *et al.*, 2017). Specifically, we defined a significance
325 threshold as the $99^{th}$ percentile of the cross-correlation coefficient distribution generated with
326 resampled versions of all motor unit spike trains per participant and condition. To this end, we
327 generated random spike trains for each motor unit by bootstrapping the interspike intervals (random
328 sampling with replacement). This random spike train had the same number of spikes and the same
329 discharge rate (mean and standard deviation) as the original motor unit spike train. We repeated
330 this step four times per pair of motor units.
331

## Statistics

All statistical analyses were performed with RStudio (USA). First, quantile-quantile plots and histograms were displayed to check the normality of the data distribution. If the data were determined not to be normal, they were transformed to remove the skew. All statistical analyses were performed using linear mixed effect models implemented in the R package *lmerTest* with the Kenward-Roger method to estimate the denominator degrees of freedom and the *p*-values. This method considers the dependence of data points (i.e., individual motor units) within each participant due to, e.g., common synaptic inputs. When necessary, multiple comparisons were performed using the R package *emmeans*, which adjusts the *p*-value using the Tukey method. The significance level was set at 0.05. Values are reported as mean ± standard deviation.

In Part 1, we assessed the ability of our methods to identify the number of synergies and classify the motor neurons according to the weights of the common inputs they receive. After verifying that the PCA accurately predicts the number of motor neuron synergies, we assessed the correlation between the angles calculated between vectors from the biplots and the level of common inputs. Then, we measured the level of common inputs between motor neurons having significant and non-significant cross correlation coefficients between their smoothed discharge rates across simulations. In this way, we aimed to quantify the minimal level of common input needed to reach the level of significance in the three conditions, and assess the maximal level of common input, if any, needed to have non-significant correlations in the three conditions.

In Part 2, we first compared MVC and torque RMSE using a linear mixed effects model with Condition (PF20°, PF0°, DF20°) as a fixed effect and Participant as a random effect. The averaged discharge rates of matched motor units were compared using a linear mixed effects model with Condition (PF20°, PF0°, DF20°) and Muscle (GL, GM, SOL) as fixed effects and Participant as a random effect.

Then, we used PCA to report the number of motor neuron synergies at each ankle angle for each participant. We calculated the angles between all the motor units, and we used a linear mixed effects model to compare these angles with Muscle (GL, GM, SOL, GL-GM, GL-SOL, GM-SOL) and Condition (PF20°, PF0°, DF20°) as fixed effects and Participant as a random effect. Finally, we assessed the level of common synaptic input to pairs of motor neurons from the same and different muscles using the cross-correlation coefficients between smoothed discharge rates. We

used a linear mixed effects model to compare correlation coefficients with Muscle (GL, GM, SOL, GL-GM, GL-SOL, GM-SOL) and Condition (PF20°, PF0°, DF20°) as fixed effects and Participant as a random effect. The ratio of pairs of motor units with correlation coefficients above the significance threshold at each ankle angle, as well as the ratio of pairs of motor units with significant or non-significant correlations across all angles were also reported.

# Results

## Part 1: Simulations

We first investigated the ability of our approach to capture the low dimensional control of motor neurons with PCA. The cumulative sum of the variance explained reached a plateau with two components for all simulations (Fig. 2D). The value of variance explained with two components was 68.8, 70.9, and 72.1% for conditions CI#1 = CI#2, CI#1+ CI#2, and CI#1++ CI#2, respectively. The angles between motor neurons ranged between 0° and 135° across the three conditions (Fig. 2F). We computed correlations between the angles and the difference in ratio of common inputs, calculated as the differences in weights of CI#1 between the two motor neurons. For example, two motor neurons receiving 100% CI#1 / 0% CI#2 and 0% CI#1 / 100% CI#2 have a difference in ratio equal to 1, Adjusted $R^2$ values reached 0.91, 0.91, and 0.92 for conditions CI#1 = CI#2, CI#1+ CI#2, and CI#1++ CI#2, respectively. It demonstrates that the angle between two motor neurons in the biplots is largely explained by level of common inputs they receive (Fig. 2F).

We then performed cross-correlation between smoothed discharge rates of individual motor neurons with a significance threshold fixed at 0.29 (Fig. 2E). Correlation coefficients are displayed in Figure 2E according to the difference in ratio of common inputs. Motor neurons with a difference in ratio of common inputs of 0.29 ± 0.23, 0.30 ± 0.23, and 0.30 ± 0.23 had a significant correlation between their smoothed discharge rates in conditions CI#1 = CI#2, CI#1+ CI#2, and CI#1++ CI#2, respectively. Conversely, motor neurons with a difference in ratio of common inputs of 0.88 ± 0.13, had non-significant correlation between their smoothed discharge rates (Fig. 2E). 68.5% and 20.1% of the pairs of motor neurons had respectively significant or non-significant correlations between their smoothed discharge rates across the three simulations. Pairs of motor neurons with consistent non-significant correlations across simulations mostly received different inputs, with a difference in ratio of common inputs of 0.93 ± 0.09 (Fig. 2G). Pairs of motor neurons with consistent significant correlations across simulations mostly received the same inputs, with a difference in ratio of common inputs of 0.27 ± 0.21 (Fig. 2G). Pairs of motor neurons with changes in significance across simulated conditions had differences in ratio of common inputs of 0.71 ± 0.12 (Fig. 2G). Together, these results showed that changes in angles within the biplots after PCA or changes in level of correlation in the cross-correlation analysis can be explained by the modulation of the amplitude of common synaptic inputs, despite fixed weights between simulations. It is

noteworthy that motor neurons receiving low or high levels of the same sources of common inputs had the same level of correlation between the simulations.

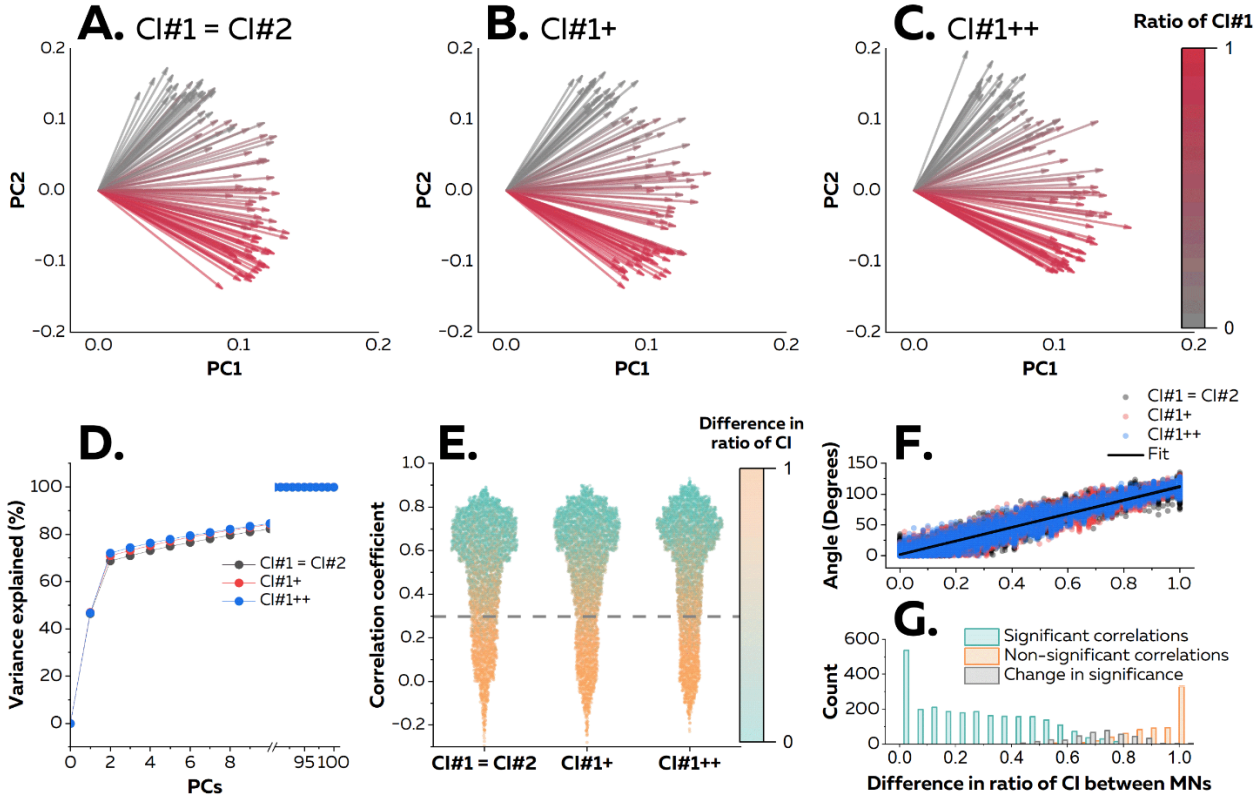


**Figure 2: Simulation of a pool of motor neurons with two sources of common synaptic inputs.** We simulated 100 motor neurons using an open-source model (Elias & Kohn, 2013). Each motor neuron received either 100% Common input #1 (CI#1, 20 motor neurons), 100% Common input #2 (CI#2, 20 motor neurons), or a ratio of CI#1 and CI#2 from 99% CI#1/ 1% CI#2 to 1% CI#1/ 99% CI#2 (60 motor neurons). We generated three simulations with different amplitudes of the common input #1 to mimic the divergent modulation of two separate sources of common synaptic inputs and to match the discharge rates experimentally observed during the plantarflexions. (A, B, C) We applied principal component analysis (PCA) and displayed the two-dimensional biplots based on the two main components. (D) Indeed, the cumulative sum of the variance explained reached a plateau with two components. Each vector represents an individual motor neuron with the color depending on the ratio of CI#1 received by the motor neuron. (E) Then, we estimated the level of common synaptic input for all pairs of motor neurons using cross-correlation analyses between individual motor neuron smoothed discharge rates. All correlations above 0.29 (dash grey line) were significant. The color of scatters depends on the difference in the ratio of common input #1 between motor neurons in each pair. For example, two motor neurons receiving 100% CI#1 / 0% CI#2 and 0% CI#1 / 100% CI#2 have a difference in ratio equal to 1. (F) Angles between motor neurons were largely explained by differences in ratio of CI#1.

(G) When we modulated the amplitude of CI#1 and CI#2 across simulated conditions, a portion (albeit small) of the pairs exhibited variable levels of correlation. Distribution of difference in ratio for CI#1 for each pair of motor neurons with significant, non-significant, or different level of significance across simulations.

## Part 2: Experiments

### *Torque*

As expected, the MVC torque was significantly different between conditions ($F = 36.94$, $p < 0.001$), with MVC torque at PF20° (383.4 ± 225.4 N.m) being significantly lower than that at PF0° (715.7 ± 354.8 N.m, $p < 0.001$) and DF20° (790.0 ± 448.2 N.m, $p < 0.001$). There was no difference between PF0° and DF20° ($p = 0.892$). The RMSE was not significantly different between conditions ($F = 2.73$, $p = 0.082$), meaning that the target torque was matched with similar accuracy across conditions.

### *Motor unit discharge characteristics*

A total of 595 (37 ± 22 per participant; range: 5-79), 613 (38 ± 27 per participant; range: 3-96), and 471 (29 ± 22 per participant; range: 3-76) motor units were identified at PF20°, PF0°, and DF20°, respectively. They had an average pulse-to-noise ratio of 33.3 ± 2.6 dB. 96 motor units (17.2%) were tracked over the three conditions, with 17, 45, and 34 motor units for the GL, GM, and SOL, respectively. It is noteworthy that six participants were excluded from the analyses, as no motor units could be tracked across conditions, and thus data are reported for the 10 remaining participants. We first compared the discharge characteristics of these tracked motor units (Fig. 3). When considering the average discharge rates, significant effects of Condition ($F = 18.89$, $p < 0.001$), Muscle ($F = 3.41$, $p = 0.034$), and an interaction Condition × Muscle ($F = 2.56$, $p = 0.039$) were observed. The average discharge rate of motor units from the GL was significantly lower at DF20° (8.1 ± 1.8 pps) than at PF20° (10.5 ± 2.5 pps). Similarly, the average discharge rate of motor units from the SOL was significantly lower at DF20° (7.8 ± 2.3 pps) than at PF20° (9.1 ± 2.7 pps). No significant differences were observed for other angles or muscles (all $p > 0.109$).

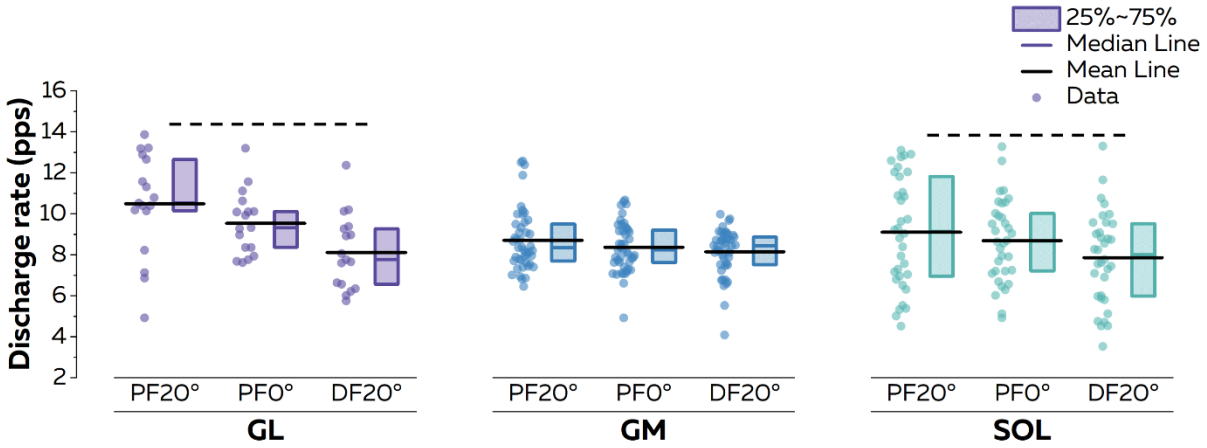


**Figure 3: Motor unit discharge rate.** We compared the discharge rate of all tracked motor units. The average discharge rate was estimated after smoothing instantaneous discharge rates with a 1s Hanning window over the plateau. Each scatter represents an individual motor unit. The horizontal dash lines on top of the plots depict the significant statistical differences with a $p < 0.05$. For the sake of clarity, we only reported the differences within muscles. GL: Gastrocnemius lateralis; GM: Gastrocnemius medialis; SOL: Soleus. PF, Plantarflexion; DF: Dorsiflexion.

### *Motor neuron synergies*

The motor neuron synergy analysis was performed for participants with motor units identified from all muscles in the three conditions (n = 12; 34 ± 17 MUs per participant). PCA was performed on the normalized smoothed discharge rates. We first intended to identify the number of common inputs, or motor neuron synergies. To this end, we used mean-square error to determine the number of components where the cumulative sum of the variance explained reached a plateau (Cheung *et al.*, 2005; d'Avella *et al.*, 2011). We found on average two motor neuron synergies for the three conditions. The two-dimensional biplots with the weight of each motor unit in the two motor neuron synergies are displayed in Fig. 4.

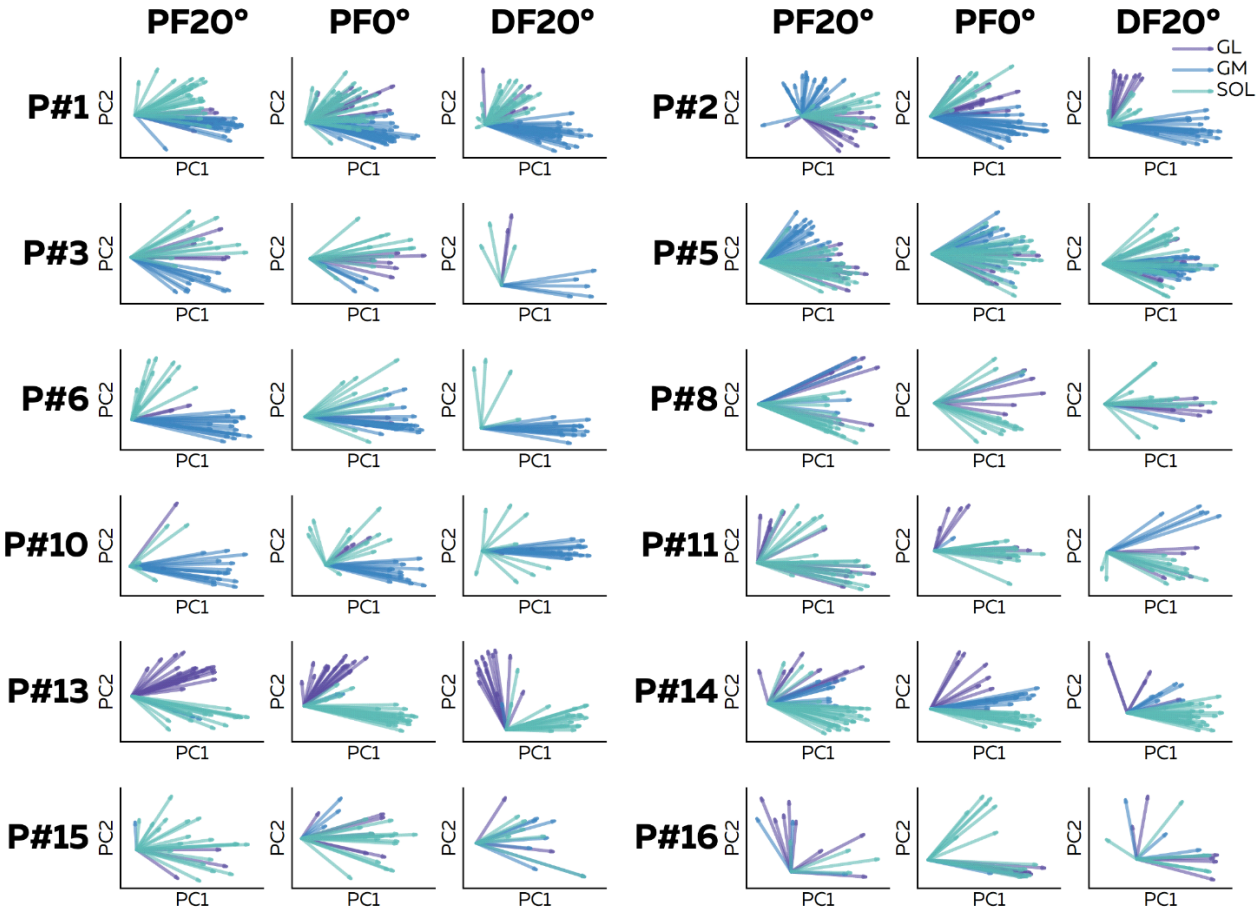


**Figure 4: Biplots of motor unit weights from the two motor neuron synergies.** For each participant (n=12), we applied principal component analysis on normalized smoothed discharge rates concatenated in a matrix. The identified components were ranked based on the variance they explained, with two components sufficient such that the variance explained reached a plateau. Then, we displayed two dimensional biplots where coordinates of each motor unit are the weights of the two motor neuron synergies. Each vector represents an individual motor unit. The color of the vector depends on the muscle. GL: Gastrocnemius lateralis; GM: Gastrocnemius medialis; SOL: Soleus.

We then calculated the angles for each pair of vectors (i.e., each pair of motor units) in the biplot. Simulations demonstrated that these angles provide a good estimate of the level of common inputs between two motor units (Fig. 2F). Angles from the 26589 pairs of motor units displayed in Figure 5 were averaged per participant to compare angles between muscles and conditions (Fig. 5B). We found a significant main effect of Muscle ($F = 22.28$; $p < 0.001$), but neither a significant main effect of Condition ($F = 0.47$; $p = 0.62$) nor a significant interaction ($F = 1.30$; $p = 0.23$). Specifically, angles were significantly lower between motor units within the GL ($33.0 \pm 22.3°$) and

within the GM (23.6 ± 18.2°) than between pairs of motor units within the SOL (50.7 ± 26.6°; $p = 0.005$ and $p < 0.001$, respectively; Fig. 5B). Moreover, the angles between motor units within the GL and within the GM were also significantly lower than the angles between motor units from different muscles, i.e., GL-GM, GL-SOL, and GM-SOL (all $p < 0.001$; Fig. 5B). Finally, the angles between motor units within the SOL were lower than the angles between motor units of GM-SOL (68.7 ± 15.9°; $p = 0.006$). The differences between pairs of muscles are displayed on Figure 5. Taken together, these results suggest that motor units within the GL or within the GM receive the highest proportion of common input.

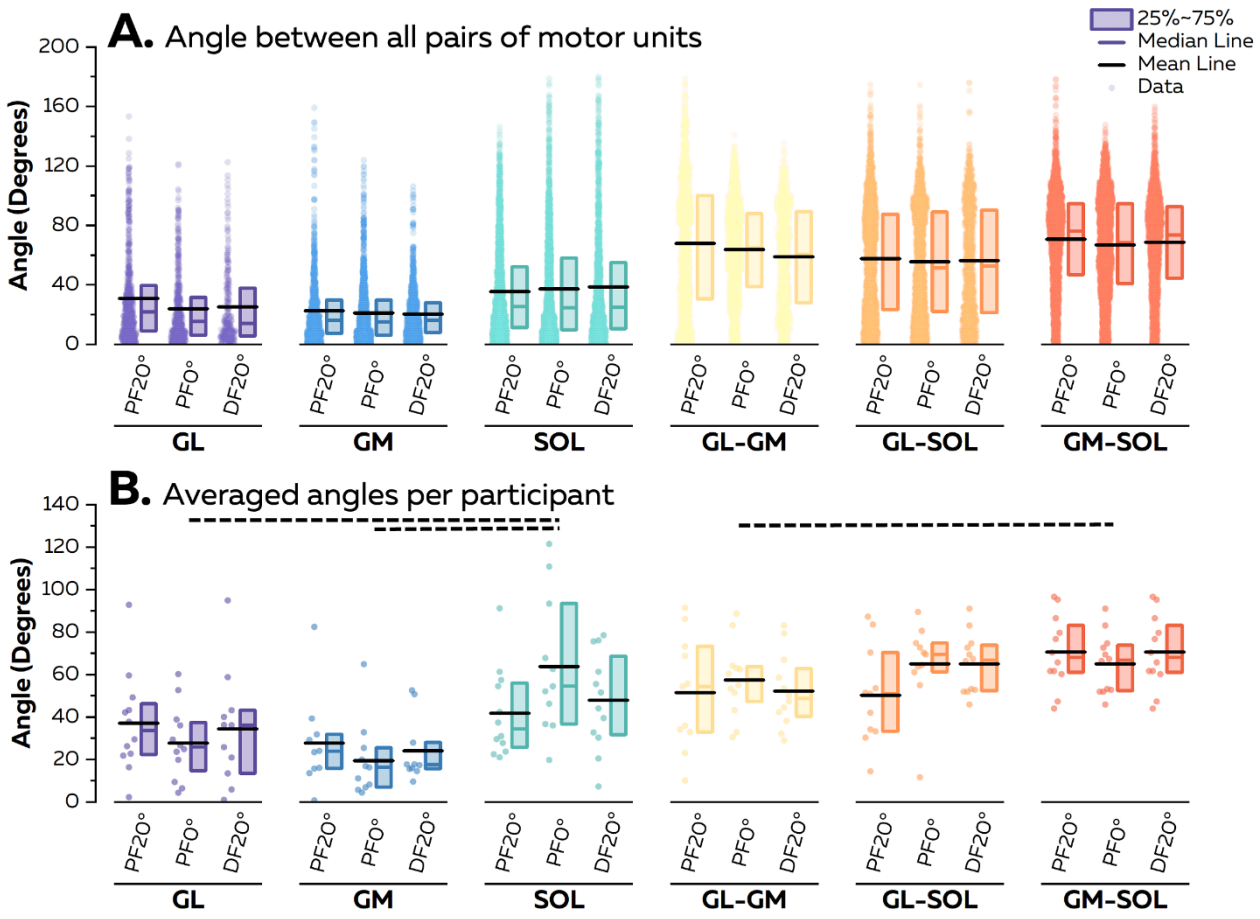


**Figure 5: Angles between vectors representing motor units in biplots.** After applying principal component analysis, we displayed two dimensional biplots where coordinates of each motor unit were the weights of that motor unit in each of the two motor neuron synergies. We then calculated the angles between these vectors to estimate the strength of common inputs received by each pair of motor unit. (A) We report the angle values for all the pairs of motor units. Each scatter represents an individual pair of motor units. (B) We then averaged these values per participant and performed the statistical analyses to compare these values between muscles and conditions. Each scatter represents an

individual. The horizontal dash lines on top of the plot depict the significant statistical differences with a $p < 0.05$. For the sake of clarity, note that we did not display the significant difference between GL and GM with all of the pairs of muscles. GL: Gastrocnemius lateralis; GM: Gastrocnemius medialis; SOL: Soleus; PF, plantarflexion; DF: dorsiflexion.

***Pairs of motor units receiving common synaptic inputs***

We analyzed the correlation for 416 pairs of matched motor unit activities. Specifically, we had 34, 147, and 92 pairs of motor units for GL, GM, and SOL, respectively, and 46, 29, and 68 pairs of motor units for GL-GM, GL-SOL, and GM-SOL, respectively. The coefficients of correlation were significantly different between Conditions ($F = 43.65$, $p < 0.001$) and Muscles ($F = 141.46$, $p < 0.001$) with a significant interaction ($F = 15.14$, $p < 0.001$). Specifically, the coefficients of correlation for pairs of motor units within the GL and GM were significantly higher for PF0° ($p < 0.001$) and DF20° ($p < 0.001$) compared to PF20°. The coefficients for the SOL were not significantly different across ankle angles. The coefficients of correlation calculated between motor units of GL-GM, GL-SOL and GM-SOL were significantly higher for PF0° than for DF20° ($p = 0.012$, $p < 0.001$, and $p < 0.001$, respectively). The coefficients of correlation calculated between motor units of GL-SOL and GM-SOL were also significantly higher for PF0° than for PF20° ($p < 0.001$, and $p < 0.001$, respectively). The ratio of pairs of motor units with significant correlations reached on average 98.0%, 96.1%, and 98.2% for GL, GM, and SOL, respectively (Fig. 6B). The ratio of pairs of motor units with significant correlations reached on average 36.9%, 78.2%, and 90.2% for GL-GM, GL-SOL, and GM-SOL, respectively.

We then compared the distribution of common inputs across ankle angles by calculating the ratio of pairs of motor units with the same level of correlation (either significant or non-significant correlations) across the three angles (Fig. 6C). The rest of the motor units switched between significant and non-significant correlations across ankle angles. None of the pairs of motor units had a non-significant level of correlation across all three ankle angles when considering motor units from GL, GM, SOL, GL-SOL, and GM-SOL; but 26.1% of pairs of motor units from GL-GM had a non-significant level of correlation across all conditions. 94.1%, 88.4%, and 94.6% of the pairs of motor units had a significant level of correlation across all three ankle angles for GL, GM, and SOL, respectively (Fig. 6C). When considering pairs of motor units from different muscles, 13.0% (GL-GM), 48.3% (GL-SOL), and 73.5% (GM-SOL) of pairs of motor units had a consistent

significant level of correlation across angles (Fig. 6C). It is noteworthy that we failed to identify a significant level of correlation in a large percentage of pairs of motor units between GL and GM. Conversely, some pairs of motor units from GL-SOL and GM-SOL exhibited significant correlations at all three angles, supporting the findings from the PCA of two common inputs.

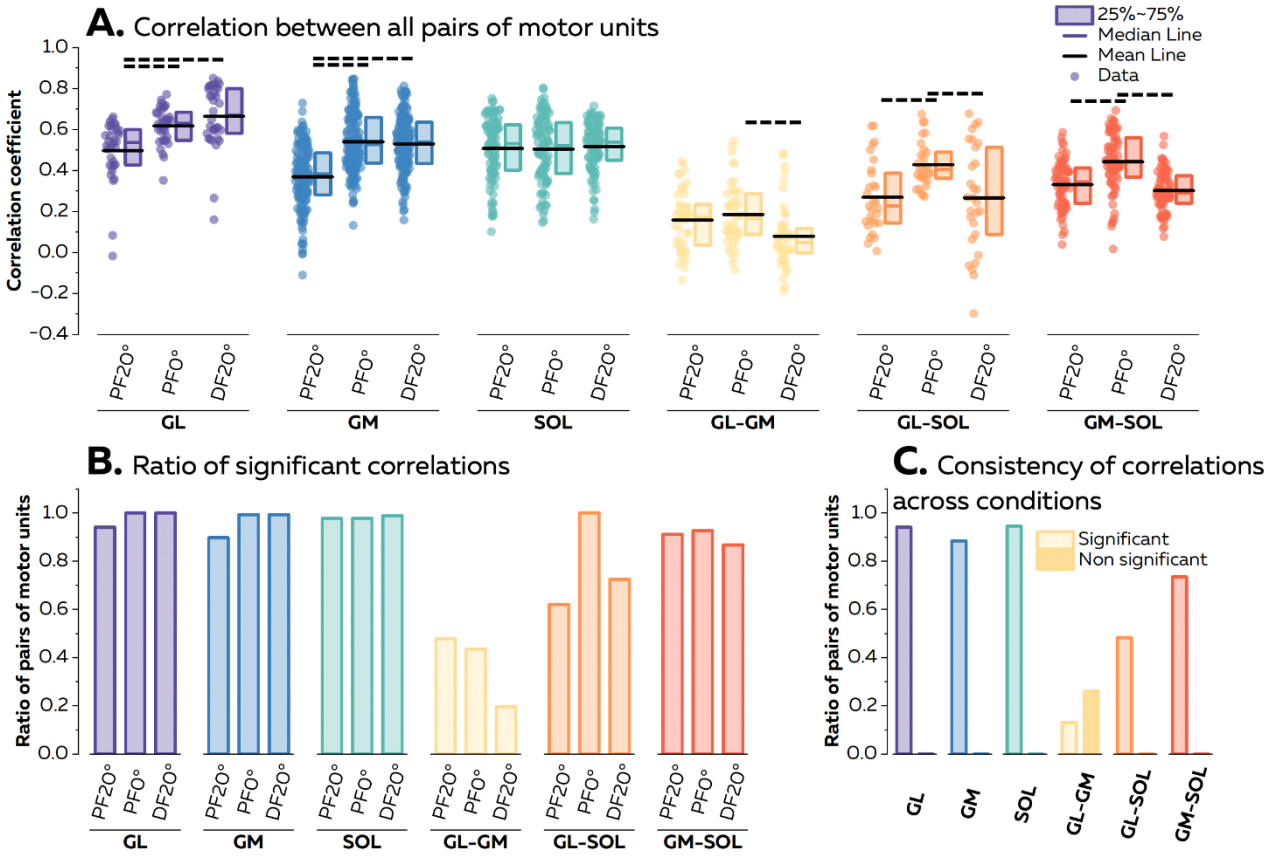


**Figure 6: Common inputs to pairs of motor units.** We estimated the level of common synaptic input for all pairs of motor units from the same and different muscles using cross-correlation analyses between individual motor units smoothed discharge rates. (A) We first compared the level of correlation for each individual muscle and pairs of muscles. Each scatter represents a pair of motor units. The horizontal dash lines on top of the plots depict the significant statistical differences with a $p < 0.05$. For the sake of clarity, we only reported the differences for the interaction. (B) We calculated the ratio of pairs of motor units with significant correlations for each individual muscle and pairs of muscles. (C) To compare the changes in common synaptic input to pairs of motor units across conditions, we calculated the ratio of pairs of motor units which exhibited consistent behavior across ankle angles, i.e., either non-significant or significant level of correlation. GL: Gastrocnemius lateralis; GM: Gastrocnemius medialis; SOL: Soleus.

# Discussion

This study aimed to test the robustness of motor neuron synergies across isometric plantarflexion tasks performed with different mechanical constraints. In the first part, we used simulated signals to estimate the sensitivity of our methods (i.e., principal component analysis and correlation of smoothed discharge rates) to identify the number of common inputs and their distribution across motor neurons with various amplitudes of synaptic inputs. We found that PCA can accurately classify motor neurons according to the common inputs they receive. Moreover, cross-correlations can accurately identify pairs of motor neurons receiving or not receiving a significant level of common inputs. This approach was then applied to experimental data. We identified two main common inputs driving motor neurons innervating the GL, GM, and SOL, with one common input mainly distributed between GL and SOL motor neurons and another common input mainly distributed between GM and SOL motor neurons. We did not find a significant systematic change in the distribution of weights across ankle angles. Moreover, the cross-correlation analysis revealed that 94.1%, 88.4%, and 94.6% of the pairs of tracked motor units for GL, GM, and SOL, respectively, kept the same level of correlation across ankle angles. Overall, these results highlight a modular organization of the control of motor neurons innervating the triceps surae muscles with a reduced dimensionality.

**Methodological considerations**

Our experiment is a first step toward the demonstration of the modular organization of the neural control of motor neurons across tasks with different mechanical constraints. As previously noted, the assessment of motor neuron synergies across tasks required the tracking of the same motor units. To this end, we matched the unique spatiotemporal properties of the motor unit action potential shapes over the grids of electrodes between trials (Martinez-Valdes *et al.*, 2017; Vecchio & Farina, 2019; Oliveira & Negro, 2021). As changes in ankle position inevitably impacted the geometry of the volume conductor, the shapes of action potentials over the skin slightly varied across conditions. Although we successfully tracked a significant number of motor units (96), there was a significant reduction in the motor unit sample that could be analyzed across tasks. The recent development of intramuscular thin-film arrays with a high density of electrodes (Muceli *et al.*, 2022) may open new perspectives to accomplish this goal as these electrodes may be less affected

571 by the changes in muscle geometry. Additionally, Oliveira and Negro (2021) have demonstrated
572 the ability to decompose HD-EMG signals during slow dynamic contractions, which may provide
573 opportunities to show the robustness of motor neuron synergies during more natural behaviors.

574 Despite the above-mentioned methodological limitations and perspectives, studying the robustness
575 of the distribution of common inputs driving the triceps surae muscles during plantarflexions
576 performed at multiple angles has revealed very relevant results. Previous works highlighted a
577 differential change in neural drive between the three heads of the triceps surae during sustained
578 contractions and in response to the alteration of force generating capacity during an ankle rotation
579 (Lacourpaille *et al.*, 2017; Hug *et al.*, 2021b; Rossato *et al.*, 2022). Additionally, Marshall et al.
580 (2022) have recently shown that the covariation of motor unit discharge rates and their recruitment
581 order may change between short and long muscle lengths. Both results suggested that the central
582 nervous system could drive these muscles with more than one common input, even during a task
583 with one degree of freedom. Our results supported this hypothesis, with the identification of two
584 sources of common inputs driving the triceps surae muscles. However, whether the distribution of
585 common inputs to motor units is robust across muscle lengths was unknown. In this study we
586 further described the distribution of these common inputs within and between these muscles,
587 showing a flexible combination of these inputs across motor units.

588

589 **Distribution of common inputs to modulate muscle force**

590 Numerous works have shown that most of the motor units from the same muscle display common
591 variations of their discharge rate, which is necessary to modulate force during isometric
592 contractions (Farina *et al.*, 2014b; Farina & Negro, 2015; Enoka & Farina, 2021). These common
593 fluctuations depend on the modulation of correlated synaptic inputs, or common inputs, received
594 by all of these motor units (Negro *et al.*, 2009; Farina *et al.*, 2014a; Farina *et al.*, 2014b; Farina &
595 Negro, 2015; Feeney *et al.*, 2018). In line with these studies, we observed that none of the 273 pairs
596 of motor units identified from GL, GM or SOL had a non-significant level of correlation for all
597 three ankle angles (Fig. 6C). The level of common input to motor neurons from either GL or GM
598 was particularly high, with a ratio of pairs with significant correlation for all three ankle angles
599 reaching 94.1% and 88.4% for GL and GM, respectively, and angles between vectors representing
600 motor units within either GL or GM lower than the angles measured for the SOL and all pairs of

601 muscles (Fig. 5). On the contrary, the degree of common input between motor units from GL and
602 GM was lower than for the other pairs of muscles, with 13% of pairs of motor units with significant
603 correlation across all three ankle angles. This is in agreement with previous works that used
604 methods at the muscle level, such as intramuscular coherence (Hug *et al.*, 2021b; Rossato *et al.*,
605 2022) or PCA (Mazzo *et al.*, 2022), to assess the common fluctuations of motor unit discharge rates
606 in the triceps surae muscles. Motor units from the SOL appear to have lower correlated fluctuations
607 in discharge rates than GL and GM. This may be a result of the geometry and compartmentalization
608 of this muscle (Bolsterlee *et al.*, 2018), requiring multiple sources of common input to drive
609 multiple functions (Segal *et al.*, 1991; English *et al.*, 1993). Such results have been observed in
610 muscles from the forearm with multiple compartments and tendons (Keen & Fuglevand, 2004;
611 Hockensmith *et al.*, 2005; McIsaac & Fuglevand, 2007). Alternatively, one may consider the
612 absence of correlation as a result of different non-linear inputs/outputs functions for each motor
613 neuron due to the activation of persistent inward currents (Heckman *et al.*, 2008; Binder *et al.*,
614 2020). However, we focused our analysis on oscillations with frequencies below the averaged
615 motor unit discharge rates, which are less affected by these non-linear transformations of synaptic
616 inputs (Negro & Farina, 2012). Therefore, we are confident that this absence of significant
617 correlation identifies motor units receiving independent sources of common input, as suggested for
618 motor units from muscles of the upper (Madarshahian *et al.*, 2021; Marshall *et al.*, 2022) and lower
619 limbs (Del Vecchio *et al.*, 2022; Hug *et al.*, 2022).

620

621 **From muscle to motor neuron synergies**

622 The fact that the central nervous system may group motor units from synergistic muscles to reduce
623 the dimensionality of the neural control of movements has been supported for decades by the theory
624 of muscle synergies (Tresch & Jarc, 2009; Dominici *et al.*, 2011; Bizzi & Cheung, 2013; Ting *et*
625 *al.*, 2015; Takei *et al.*, 2017). Of note, these synergies have been mostly described at the muscle
626 level. However, several studies have highlighted similar levels of correlation between the firing
627 activities of motor units originating from two synergistic muscles as from the same muscle (Del
628 Vecchio *et al.*, 2022; Hug *et al.*, 2022). Thus, recent studies have proposed to take advantage of
629 the large sampling of motor unit activity from decomposed HD-EMG signals to extend the synergy
630 concept to the motor neuron level (Madarshahian *et al.*, 2021; Tanzarella *et al.*, 2021; Del Vecchio

*et al.*, 2022; Hug *et al.*, 2022; Hug *et al.*, 2023). Here, we found two main motor neuron synergies through PCA, mostly grouping motor units from GL/SOL and GM/SOL. The relative lower ratio of pairs of motor units from GL and GM receiving common inputs is consistent with previous works highlighting non-significant coherence between these two muscles (Hug *et al.*, 2021b; Rossato *et al.*, 2022). Conversely, Gibbs et al. (1995) reported that motor units from the gastrocnemius and the soleus muscles did receive common inputs, consistent with our results.

The results of our study supported three of the hypotheses proposed by Hug *et al.* (2023) within the motor neuron synergies framework, namely that during movement 1) motor neurons are grouped into functional groups (clusters) based on the common inputs they receive; 2) clusters may significantly differ from the classical definition of motor neuron pools, such that clusters of motor neurons may span across muscles (i.e., GL and SOL, GM and SOL) and/or involve only a portion of a muscle (i.e., SOL); and 3) clusters represent functional modules used by the central nervous system to reduce the dimensionality of control. The latter observation is the main novelty of our study. Previous studies identified motor neuron synergies during isometric contractions performed at a single joint angle (Madarshahian *et al.*, 2021; Del Vecchio *et al.*, 2022; Hug *et al.*, 2022) and, therefore, did not demonstrate their invariance across tasks, which is needed to reduce the computational burden of movement control (d'Avella *et al.*, 2003; d'Avella & Bizzi, 2005). To this end, we tested the robustness of motor neuron synergies across ankle angles, with no systematic changes in the distribution of principal component weights within and between muscles (Fig. 5), and a large proportion of pairs of motor units consistently having significant and non-significant correlations between their smoothed discharge rates (Fig. 6C). While a few pairs of motor units switched between significant and non-significant level of correlations across ankle angles, our simulation showed that these motor units may receive the two sources of common inputs with relatively balanced weights (i.e., 34%/66% to 49%/51 in the simulation). The different changes in the amplitude of each source of common input may then impact the level of covariation between motor unit discharge rates across angles. To support this idea, divergent variations in neural drive to the triceps surae muscles have been indirectly observed while the position of the ankle changed during isometric contractions (Huang *et al.*, 2016; Cibulka *et al.*, 2017; Lacourpaille *et al.*, 2017; Hug *et al.*, 2021b). Overall, we suggest that the distribution of common inputs across motor units is indeed robust across tasks with some changes in level of correlation due to divergent changes in the amplitude of common inputs to motor neurons receiving the two inputs.

662 The concept of combining motor neuron synergies to generate movements may bridge the gap
663 between studies supporting modular control and flexible control of motor units. Proponents of
664 flexible control of motor units suggest that motor units from the same muscle may receive
665 independent inputs instead of one common input, explaining changes in recruitment order between
666 the same motor units across multiple tasks (Basmajian, 1963; Formento *et al.*, 2021; Marshall *et*
667 *al.*, 2022). We show here that most of the motor units from the triceps surae muscles may receive
668 two sources of common inputs. The combination of these two independent inputs may explain why
669 previous works reported changes in recruitment strategies between motor units from the same
670 muscle while changing the mechanical constraints of the task (Desnedt & Gidaux, 1981; ter Haar
671 Romeny *et al.*, 1984; Herrmann & Flanders, 1998; Marshall *et al.*, 2022).

672

673 **Neural structures that shape the modular control of calf muscles**

674 These results suggest that the neural control of movements may be hierarchically organized, where
675 motor neuron synergies are controlled instead of individual motor neurons (Loeb *et al.*, 1999).
676 Thus, spinal circuits involving premotor neurons and interneurons may span across motor nuclei
677 to transmit these correlated inputs to synergist muscles (Levine *et al.*, 2014; Takei *et al.*, 2017;
678 Ronzano *et al.*, 2021). However, as motor neurons are the final common pathways where inputs
679 from supraspinal centers and afferent fibers converge, we cannot make any inference about the
680 origin of the correlated inputs though several candidates exist. For example, previous works found
681 that the stimulation of the motor cortex (Overduin *et al.*, 2012) or premotor neurons in the spinal
682 cord (Takei *et al.*, 2017) evoked synergies comparable to those observed during natural behaviors.
683 Additionally, Cheung et al. (2005) observed similar synergies in animals with intact and
684 deafferented nervous systems, suggesting that the activation of synergies would mostly be a
685 feedforward process. Alternatively, others proposed that the correlated activity between synergistic
686 muscles may aim to regulate mechanical features such as internal joint stress, thus emphasizing the
687 key role of sensory feedback (Alessandro *et al.*, 2020). This could also be the case in our study as
688 the variation of ankle angles undoubtedly impacts the behavior of motor neurons during
689 plantarflexions (Kennedy & Cresswell, 2001; Trypidakis *et al.*, 2022). Additionally, our two
690 synergies may allow the central nervous system to tune the behavior of the lateral and medial
691 portions of the calf muscles to stabilize the foot producing plantarflexion in the frontal plane (Lee

& Piazza, 2008), thus limiting the strain and the stress of the ankle joint. Overall, future work is needed to identify the neural bases of correlated inputs to motor neurons during voluntary movements (Cheung & Seki, 2021).

# Conclusion

Our results support the concept that the central nervous system may flexibly combine modular structures, i.e., motor neuron synergies, to produce movements. Specifically, we identified two motor neuron synergies grouping motor units from GL/SOL and GM/SOL during plantarflexion performed at multiple ankle angles. Importantly, we found that the structure of these motor synergies is invariant across conditions despite the variation of mechanical constraints with some level of flexibility that may be accounted for by modulated magnitude of common inputs. This provides a novel observation that significantly contributes to demonstrate the functional relevance of such modular structures to generate movements.

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# Data Availability Declaration

Data and codes will be made available upon request to the authors.

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