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

HAL Id: hal-04395083

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

Submitted on 16 Jan 2024

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

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Running head: Control of the gastrocnemii during their two main functions

Abstract

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

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

New & Noteworthy

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

Introduction

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

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

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

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

Methods

Participants

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

Experimental design

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

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

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

Surface electromyography recordings

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

Data analysis

Global surface electromyography

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

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

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

EMG decomposition

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

Assessment of crosstalk

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

Assessment of motor unit discharge rate and spatial location

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

Coherence analysis

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

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

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

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

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

Motor unit matching

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

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

Statistical analysis

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

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

Results

Global surface EMG

EMG amplitude

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

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

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

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

Regional activation

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

Motor unit discharge characteristics

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

Average discharge rate

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

Spatial localization of identified motor units

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

Within- and between-muscle coherence

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

514 **Acknowledgements**

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

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529

530 **Data availability**

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

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Figures legends

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

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

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

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

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

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

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

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

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

Y axis displays both the EMG amplitude (% of

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

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

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

References

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

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

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

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