

Reciprocal inhibition between motor neurons of the tibialis anterior and triceps surae in humans

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Abstract

Motor neurons innervating antagonist muscles receive reciprocal inhibitory afferent inputs in order to facilitate the joint movement in the two directions. The present study investigates the mutual transmission of reciprocal inhibitory afferent inputs between the tibialis anterior (TA) and triceps surae (soleus and medial gastrocnemius) motor units. We assessed this mutual mechanism in large populations of motor units for building a statistical distribution of the inhibition amplitudes during standardized input to the motor neuron pools in order to minimize the effect of modulatory pathways. Single motor unit activities were identified using high-density surface electromyography (HDsEMG) recorded from the TA, soleus (Sol) and medial gastrocnemius (GM) muscles during isometric dorsi- and plantar-flexion. Reciprocal inhibition on the antagonist muscle was elicited by electrical stimulation of the tibial (TN) or common peroneal nerves (CPN). The probability density distributions of reflex strength for each muscle were estimated in order to examine the strength of mutual transmission of reciprocal inhibitory input. The results showed that the strength of reciprocal inhibition in the TA motor units was a 4-fold greater than for the GM and the Sol motor units. This suggests an asymmetric transmission of reciprocal inhibition between ankle extensor and flexor muscles. This asymmetry cannot be explained by differences in motor unit type composition between the investigated muscles since we sampled low-threshold motor units in all cases. Therefore, the differences observed for the strength of inhibition are presumably due to a differential reciprocal spindle afferent input and the relative contribution of non-reciprocal inhibitory pathways.

New & Noteworthy

We investigated the mutual transmission of reciprocal inhibition in large samples of motor units using a standardized input (electrical stimulation) to the motor neurons. The results demonstrated that the disynaptic reciprocal inhibition exerted between ankle flexor and extensor muscles is asymmetrical. The functional implication of asymmetric transmission may be associated with the neural strategies of postural control.

Keywords: reciprocal inhibition, single motor unit, high density EMG, synaptic distribution

Introduction

The proprioceptive muscle spindle system is a fundamental component of the sensory–motor organization in human motor control. When a muscle fiber stretches, muscle spindle afferents (Ia) produce monosynaptic activation of the alpha motor neurons of the homonymous muscle. The excitatory activity requires a corresponding relaxation of the antagonist muscle for function optimization (Sherrington, 1913). Although this simple model of muscle coordination cannot always be explained by a functional need, it has been generally accepted that alpha motor neurons are reciprocally inhibited via Ia afferents in voluntary movement (Knikou, 2008). The reciprocal inhibitory pathway has been described in detail through intracellular recordings in animal (Eccles et al., 1956; Eccles and Lundberg, 1958) and intramuscular recordings in human studies (Kudina, 1980). Reciprocal Ia afferent inhibits antagonist motor neurons through at least one inhibitory interneuron (Crone et al., 1987; Katz et al., 1991). Reciprocal inhibition contributes to postural and movement control by means of a task dependent amplitude modulation. The functional role of this mutual inhibitory pathway in different motor tasks, especially during bipedal walking (Lavoie et al., 1997; Petersen et al., 1999) and posture (Kasai

et al., 1998), has also been extensively investigated. However, these results focused on the amplitude modulation of reciprocal inhibition in a specific motor task and not on the mutual distribution of reciprocal inhibitory input.

The distribution of synaptic inputs and the intrinsic properties of alpha motor neurons determine the excitability of the motor neuron pool (Burke, 1968, 1970; Buller et al., 1980; Miles and Türker, 1986; Semmler and Türker, 1994; Heckman et al., 2009). Therefore, we hypothesized an asymmetric reciprocal inhibition between ankle extensor and flexor muscles due to differences between motor neuron pools. This asymmetry was indeed observed in early studies using global surface EMG and single motor unit analysis in humans (Crone et al., 1987; Nielsen and Kagamiharat, 1992), and cats (Eccles and Lundberg, 1958; Nichols, 1989). However, in these studies, it was not possible to establish whether the asymmetric reciprocal inhibition was due to the different mutual distribution of reciprocal inhibitory input, mainly because of methodological limitations. The only way to directly investigate the effect of reciprocal inhibition on the output of the motoneuron pool is to measure the influence of standardized input on the timings of discharge of individual motor units and sampling a large number of motor units for building statistical distributions (Nielsen and Kagamiharat, 1992; Yavuz et al., 2015). In this study, we utilized this approach to examine the distribution of Ia reciprocal inhibition between motor units of the tibialis anterior (TA) and triceps surae (soleus and medial gastrocnemius) muscles.

Methods

Seven healthy men (age: 35 ± 4 years) took part in the experiment. The experimental protocol was approved by the Human Ethics Committee of the University Medical Center, Georg-August-University of Göttingen (approval date: 1/10/12), and was conducted in accordance with the Declaration of Helsinki. Each subject provided informed written consent prior to the experiments.

Experimental protocol

Subjects were seated on the chair of a Biodex System 3 (Biodex Medical Systems Inc., NY, USA) with the right leg and foot firmly fastened. Three 64-channel high-density surface EMG (HDsEMG) grids (5×13 electrode grid, 8-mm interelectrode distance, ELSCH064NM2, OT Bioelettronica, Torino, Italy) were placed on the belly of the TA, soleus (Sol) and medial gastrocnemius (GM) muscles. The ankle joint was fixed in the anatomical resting position. The knee joint angle was at 120° when inhibition was recorded from TA and Sol muscles. For the recordings on the GM muscle, the leg was extended at 170° of knee angle, where the amplitude of EMG signal from GM was sufficient for the reliable decomposition of the EMG. An ankle strap electrode was used as the reference (WS2, OT Bioelettronica, Torino, Italy). The tibial (TN) and the deep branch of common peroneal (CPN) nerves were stimulated individually in order to elicit reciprocal inhibition of the TA and triceps surae (GM and Sol) motor units, respectively (Figure 1). The metal pin anode and cathode (8-mm diameter, 3-cm interelectrode distance) were placed on the skin distal to the caput fibulae for stimulating the CPN and on the popliteal fossa for stimulating the TN. The cavities of the metal pins were filled with conductive gel. The optimal position was found moving the stimulation electrodes in small steps to ensure that only the nerve of the homonymous muscle was stimulated.

Figure 1

The HDsEMG, force and stimulation trigger signals were recorded at 10240 sample/s with resolution of 12 bit, using a multichannel EMG data acquisition system (EMG-USB2, OT Bioelettronica, Torino, Italy). The EMG data were band-pass filtered (20-500 Hz) and amplified by a factor of 2000 in all sessions. The stimulation currents were delivered using a constant current stimulator (Digitimer DS-5) that was controlled by a custom-made Matlab (The

MathWorks, Inc.) script. The software provided a force feedback to the subject and the online trigger-averaged surface EMG in the interval -20ms +100ms for online monitoring of the H-reflex elicited in the agonist muscle.

The subjects were asked to perform three isometric maximum voluntary ankle dorsi- and plantar-flexion contractions, with 2 min of rest between contractions. The maximum forces of the three contractions for each task were defined as maximum voluntary contraction (MVC) forces. The subject then performed dorsiflexion or plantarflexion contractions linearly increasing the force with a speed of 2% MVC/s to reach 20% or 40% MVC. The force was then decreased and maintained to the target force levels 10 or 20% MVC while electrical stimuli were delivered to the TN during plantar-flexion or to the CPN during dorsi-flexion. A minimum of 150 stimuli were delivered with 1-2 s random inter-pulse interval, corresponding to an average duration of the sustained contractions of 250 s. The six contractions (10 and 20% MVC for TA, Sol and GM) were performed in random order. To identify the stimulation intensity, the Hoffmann reflex (H-reflex) and maximum direct motor response (maximum M-wave) were recorded from the homonymous muscle before each trial. The intensity of the stimulation (300 – 500 μ s pulse duration) was set to a value that elicited an H-reflex with peak to peak amplitude of 10% of the maximum M-wave of the agonist muscle (Figure 2A), where the stimulation intensity was on the ascending part of the H-reflex recruitment curve and the M-wave was absent or of negligible amplitude. The H-reflex and M-wave were monitored by the operator during the experiment to verify limited variability in H-reflex amplitude and negligible M-wave amplitude.

Analysis

Single motor units were identified from the HDsEMG signals using a blind source separation technique (Holobar and Zazula, 2007; Negro et al., 2016) that provides fully automatic

decomposition. Only units with a silhouette value (SIL) greater than 0.9 were used (Negro et al., 2016). The accuracy of HDsEMG decomposition in conditions similar to the current study has been previously assessed (Yavuz et al., 2015). The EMG signals were digitally band-pass filtered with 20-500 Hz cut-off frequencies, down-sampled at 2048 Hz, and divided into intervals of 60 s with 10 s overlap that were decomposed individually. The duration of the intervals for decomposition was chosen empirically to assure signal stationarity. The decomposition results from the overlapping 60-s intervals were merged by identifying the common motor units from the cross-correlation of their discharge timings in the 10-s intervals of overlap, as previously described (Yavuz et al., 2015). Reciprocal inhibition in single motor units was quantified with the peri-stimulus frequencygram (PSF). The PSF represents the instantaneous discharge rate of a single motor unit against the time instant of the stimulus (Awiszus et al., 1991; Türker and Cheng, 1994). The cumulative sum (CUSUM) of the PSF was calculated in a 400-ms window (200 ms before and after stimulation) in order to magnify the subtle reductions in motor unit discharges during the inhibition. The CUSUM was estimated according to the following formula:

$$CUSUM(t_{pre}) = 0, CUSUM(t) = \sum_{t_{pre}}^{t_{post}} (x(t) - M) \quad t | t_{pre} < t < t_{post} \quad (1)$$

where $x(t)$ is the instantaneous discharge rate at time t , M is the mean pre-stimulus discharge rate of the PSF (equivalent to the number of occurrences of the peri-stimulus time histogram (PSTH)), and t_{pre} , t_{post} are the time instants that define the interval of analysis (Yavuz et. al., 2015). The strength of the inhibitory response was quantified using the PSF-CUSUM graph. The significance threshold was determined as twice the maximum of the PSF-CUSUM deviation in the pre-stimulus time interval. Only those values that exceeded the significance threshold were considered genuine inhibitory responses (Brinkworth and Türker, 2003; Turker and Powers,

2003). The onset was determined visually as the first deflection point of monotonically descending PSF-CUSUM during inhibition. The difference between the onset value and the minimum value of the PSF-CUSUM was used as a measure of the strength of reciprocal inhibition (Figure 2). This amplitude value was divided by the number of stimulations. This normalized value provides an estimation of the extra discharge reduction per stimulation (Yavuz et al., 2014). The onset of the inhibition was estimated from the PSTH (Türker and Powers, 1999).

The mean discharge rate of each motor unit was calculated in the pre-stimulus time interval of 500ms. The motor unit recruitment thresholds were estimated from the low-pass filtered surface EMG that was normalized to the average value of the EMG recorded during MVC. A 4th order Butterworth filter with 1 Hz cutoff frequency was used for this purpose. The normalized EMG value corresponding to the first motor unit discharge was defined as the recruitment threshold.

Figure 2

Statistical Analysis

The test H-reflex amplitudes normalized to the maximum M-wave were compared between subjects and between muscles using t-tests since the data were normally distributed. These tests verified whether the amount of reflex input was similar across subjects. The motor units with low decomposition accuracy ($SIL < 0.9$) and coefficient of variation (CoV) of the inter-spike interval greater than 35% were excluded from the statistical analyses. The differences between reciprocal inhibition strengths and between recruitment thresholds across motor units were tested using the Mann-Whitney U test since these data were not normally distributed. The probability density distribution of single motor unit responses to stimuli was estimated using the data from all trials

(10% and 20% of MVC at dorsi- and plantar-flexion). The Freedman-Diaconis' rule, which is based on the interquartile range, was used to estimate the bin size of the probability density histogram. For each muscle and contraction force, the best fitting distribution function was selected among normal, gamma and exponential, according to the highest goodness of fit (Breeze et al., 1988). The median, interquartile (IQR), and the absolute range of the selected distribution were compared between muscles. The association between the motor unit recruitment threshold and the reciprocal inhibition amplitude was examined using the bivariate Pearson correlation analysis. One-way ANOVA was used to investigate whether the portion of responsive motor units was different between muscles since these data were normally distributed. In all tests, the significance level was $p < 0.05$.

Results

The normalized test H-reflex amplitudes were not significantly different between subjects ($p = 0.853$) and between muscles ($p = 0.624$) at the same force level. The mean stimulation intensities were 10.3 ± 1.9 mA and 8.8 ± 2.9 mA for eliciting the test H-reflex in the TA and Sol, respectively. The absolute peak to peak amplitude of test H-reflex were 1.95 ± 0.55 mV in the Sol and 1.40 ± 0.49 mV in the TA. Trials with a strong direct motor response (M-wave) were excluded from the analysis. The CoV of the surface EMG amplitude was estimated at a time interval from stimulation time to the onset of inhibition for each muscle. The average CoVs were not different between muscles ($12.4 \pm 3.5\%$, $14.2 \pm 4.1\%$ and $10.6 \pm 1.8\%$ at 10% MVC and $11.7 \pm 2.5\%$, $12.7 \pm 3.8\%$ and $9.7 \pm 1.4\%$ at 20% MVC for GM, Sol and TA muscles respectively. one-way ANOVA; $p = 0.242$ for 10% MVC and $p = 0.085$ for 20% of MVC).

In total, 788 motor units were identified from the three muscles of the seven subjects at the two contraction forces. This number includes potential common motor units that were identified at

both contraction forces. The reciprocal inhibition amplitude was significant for 386 of the identified motor units. These motor units will be referred to as “responsive motor units” in the following. The average proportion of responsive motor units across subjects was significantly greater for the TA ($83\pm7\%$ and $74\pm14\%$ for 10% and 20% MVC) than for both $37\pm9\%$ and $38\pm18\%$ the Sol ($37\pm9\%$ and $38\pm18\%$) and the GM ($30\pm15\%$ and $23\pm9\%$) ($p < 0.001$).

Figure 3 depicts the empirical probability density histogram and estimated fitting curves for individual muscles at the two contraction levels. The gamma distribution function was the best fitting model to the empirical data (goodness of fit values: 0.75 and 0.82 for TA, 0.83 and 0.88 for Sol, 0.77 and 0.80 for GM, at 10% and 20% MVC, respectively). The absolute range of probability density histograms was approximately four times greater for TA (0.13 - 2.33 Hz/Stimulus and 0.09 - 2.75 Hz/Stimulus, at 10% and 20% MVC, respectively) than Sol (0.03 - 0.59 Hz/Stimulus, 0.03 - 0.28 Hz/Stimulus) and GM (0.06 - 0.48 Hz/Stimulus, 0.4 - 0.38 Hz/Stimulus) (Figure 3). Figure 4 shows the cumulative probability curve for the three muscles. The Mann-Whitney U test revealed that the median and the range of reciprocal inhibition amplitude distribution were greater for the TA than for the other muscles at both contraction levels ($p<0.001$, Figure 4), with no differences between contraction levels ($p=0.424$ for TA, $p=0.345$ for GM, $p=0.324$ for Sol). The amplitude of the inhibitory responses measured from the CUSUM of the EMG was greater for TA ($2.98\pm0.82\text{mV/Stim}$ and $4.51\pm2.35\text{ mV/Stim}$ at 10 and 20% of MVC) than for the other muscles (for GM: $0.90\pm0.52\text{ mV/Stim}$ and $1.71\pm0.97\text{ mV/Stim}$; for Sol: $0.51\pm0.19\text{ mV/Stim}$ and $0.72\pm0.48\text{ mV/Stim}$ at 10 and 20% of MVC, respectively).

Figure 3

Figure 4

The average motor unit recruitment threshold was similar across muscles at 10% MVC ($6.3 \pm 1.6\%$ MVC for GM, $7.6 \pm 1.1\%$ MVC for Sol and $7.1 \pm 2.1\%$ MVC for TA, $p=0.237$), but TA motor units had greater thresholds than the other muscles for 20% MVC ($13.5 \pm 4.4\%$ MVC for GM, $14.9 \pm 1.1\%$ MVC for Sol and $18.8 \pm 7.2\%$ MVC for TA, $p=0.031$). The distribution of reflex amplitude was calculated as a function of the recruitment threshold (Figure 5). The bivariate Pearson correlation test revealed that the reflex amplitude was negatively correlated with recruitment threshold for TA motor units only for the contractions at 20% MVC ($r = -0.355$, $p < 0.001$). There was no significant association between recruitment thresholds and reflex amplitude for the TA at 10% MVC or for the other muscles ($r = 0.136$, $p = 0.299$ for GM; $r = 0.088$, $p = 0.365$ for Sol; $r = -0.085$, $p = 0.359$ for TA at 10% MVC, $r = 0.056$, $p = 0.747$ for GM, $r = -0.111$, $p = 0.280$ for Sol at 20% MVC).

Figure 5

We further compared the strength of reciprocal inhibition for motor units with similar baseline discharge rates (in the range 5-11 Hz at 10% MVC and 5-13 Hz at 20% MVC) for the three muscles ($n=100$ motor units for TA, $n=68$ for Sol, and $n=37$ for GM at 10% MVC; $n=66$ for TA, $n=48$ for Sol, and $n=22$ for GM at 20% MVC) (Figure 6). These comparisons were tested by the Mann-Whitney U test since the normality test failed. When matched by discharge rates, the TA motor units showed a greater inhibition (0.45 ± 0.24 and 0.49 ± 0.31 Hz/Stimulus at 10 and 20% MVC) than GM (0.16 ± 0.25 and 0.14 ± 0.08 Hz/Stimulus at 10 and 20% MVC) and Sol (0.12 ± 0.09 and 0.12 ± 0.05 Hz/Stimulus at 10 and 20% MVC) ($p < 0.001$).

Figure 6

Discussion

The reciprocal inhibition from the triceps surae muscles to the TA motor units was greater than that from the TA muscle to the triceps surae motor units (Figures 3 and 4). The reciprocal interaction between ankle extensor and flexor muscles is a crucial neural mechanism in humans for adjusting the ankle joint torque during gait as well as for maintaining an upright posture. It has been shown that the strength of reciprocal inhibition is modulated in different phases of walking in the cat (Pratt and Jordan, 1987) and human (Capaday et al., 1990; Lavoie et al., 1997; Petersen et al., 1999). Moreover, the predominant transmission of reciprocal inhibition changes direction between plantarflexor and dorsiflexor muscles during the stance and swing phases of the walking cycle (Petersen et al., 1999). These studies revealed the state dependency of reciprocal inhibition during voluntary movement where the modulatory effects, including supraspinal and spinal segmental interneuronal circuits, cannot be excluded (Nielsen, 2016). However, it is still not clear how the anatomical distribution of the reciprocal Ia input influences the strength of inhibition of antagonist muscles.

This study examined the mutual transmission of reciprocal inhibition between antagonist lower limb muscles during isometric contraction. The main result was that the Ia reciprocal inhibition between triceps surae and TA is not symmetric. The GM and the Sol motor units have less pronounced reciprocal inhibition responses compared to the TA motor units. We observed an amplitude of reciprocal inhibition of the TA motor units 4-fold greater than in the Sol and GM motor units. Three potential factors may influence the gain of reciprocal inhibition between these antagonist muscles: 1) the relative input from the reciprocal group I afferents, 2) the distribution of these input currents to motor neurons of different sizes, and 3) the distribution of motor unit types in the muscles.

The number of muscle spindles in the triceps surae and TA muscles is ~550 (~150 in GM and ~400 in Sol) and ~280, respectively (Voss, 1937; Banks, 2006; De Luca and Kline, 2012). The greater number of muscle spindles in the triceps surae may partly explain the greater reciprocal inhibition from triceps surae to TA. However, the difference between the mutual inhibitions in this study was very large, thus presumably there were other factors of influence. One of these factors may have been the heterogenic reflexes that serve mutual excitation between synergistic muscles (Mao et al., 1984; De Luca and Kline, 2012). For example, stretching the Sol enhances a stretch response in the GM, and vice versa, so that these two muscles work as synergists (Nichols, 1989, Smilde et al., 2016). The Ia facilitation between Sol and GM may suppress the reciprocal inhibition from TA.

Other factors of influence are non-reciprocal group Ib inhibition from Golgi tendon organs and recurrent inhibition (Renshaw cells). Reciprocal Ia and homonymous Ib afferents co-excite inhibitory interneurons in the cat (Jankowska and McCrea, 1983). On the other hand, recurrent inhibition mediated by Renshaw cells inhibits their corresponding Ia inhibitory interneurons (Hultborn et al., 1971; Katz et al., 1991; Baret et al., 2003) and consequently depresses the reciprocal inhibition. This implies that tonically activated homonymous Ib and Renshaw cells inputs have opposite effects. Therefore, it is difficult to estimate their net effect on the results of this study.

Previous studies showed that synaptic currents generated by most of the sensory and descending inputs are non-uniformly distributed among motor neurons (Heckman and Binder, 1988; Semmler and Türker, 1994; Powers and Binder, 2001). Smaller, slow-twitch motor units receive larger excitatory (Burke, 1967; Lüscher et al., 1979) and inhibitory (Henneman et al., 1965; Burke et al., 1970; Dum and Kennedy, 1980) postsynaptic potentials (EPSP and IPSP).

Therefore, the different motor unit composition of the TA, Sol and GM muscles may have influenced the results. Sol specifically contains predominantly slow-twitch motor units (Gollnick et al., 1974; Edgerton et al., 1975; Garnett et al., 1979). Since motor unit recruitment threshold is associated to motor neuron size (Henneman, 1957; Heckman and Enoka, 2012), we examined the correlation between recruitment thresholds and reflex response. This correlation was not significant, with the exception of the TA at 20% MVC (Figure 5). Despite these differences in motor unit composition, it is unlikely that the motor units sampled at 10% and 20% MVC from the three muscles were substantially different since these contraction levels correspond to recruitment of exclusively slow-twitch motor units. Accordingly, the results of asymmetric inhibition were confirmed when we limited the analysis to motor units with the same baseline discharge rate for the three muscles. This result also excluded recurrent inhibition as a contributing factor since the activity of Renshaw cells depends linearly on the discharge rate of motor units (Moore et al., 2015).

From the above discussion, it can be concluded that the observed asymmetric reciprocal inhibition is presumably due to differences in input from muscle spindles between ankle extensor and flexor muscles.

Reciprocal inhibition between ankle flexors and extensors has been reported in some previous animal and human studies that also showed asymmetry in the strength of inhibition. For example, Nichols (Nichols, 1989) reported in the decerebrate cat that the inhibition from Sol to TA was weak or absent during the cross extension reflex while a heterogenic inhibition was consistently observed from TA to Sol, which is opposite to our observations. This disagreement may be due to the initial conditions. On the other hand, the same study showed that the GM inhibits the TA substantially more than the inhibition from TA to GM, which is in accordance with our findings.

Our results are also in agreement with a previous human study that investigated a conditioning reflex protocol (Crone et al., 1985, 1987). The authors reported that the TA conditioning H-reflexes were inhibited to a greater extent from the posterior TN, than the Sol H-reflex. With respect to this approach, we estimated the reflex response amplitude in single motor units which reveals the net post synaptic potential (Türker and Powers, 2005). To the best of our knowledge, the only motor unit study mutually comparing the reciprocal inhibition of lower limb antagonist muscles is by Nielsen and Kagamihara (1992). This previous study focused on the presynaptic modulation on disynaptic reciprocal inhibition during various initial conditions (i.e., co-contraction, rest and standing), but not on the distribution of reciprocal inhibitory input. Nonetheless, several other mechanisms can change the transmission gain of reciprocal inhibition during co-contraction tasks. One of the possible mechanisms can be the modulatory presynaptic inhibition effects of Ia inputs to inhibitory interneurons (Nielsen and Kagamihara, 1993). Another factor of influence is that Renshaw cells provide recurrent inhibition to spinal motoneurons and inhibit their corresponding Ia inhibitory interneurons (Hultborn et al., 1971). There is evidence that Renshaw cells are facilitated during co-contraction of antagonists (Nielsen and Pierrot-Deseilligny, 1996). In the present study, we used a standard input provided by electrical stimulation. The intensity of the stimulation was small as to selectively excite the Ia axons of the antagonist muscle. Thus, we directly elicited inhibition on the discharge of antagonist muscle motor units, instead of estimating depression in conditioning H-reflex, which may bias the strength of inhibition based on the different strength of the conditioning H-reflex of TA and Sol. Moreover, we tested isometric contractions to minimize the co-activation of the antagonist muscles. Finally, we were able to identify large samples of motor units (a total of 788 motor units) based on a novel methodology (Yavuz et al., 2015) that provides a stronger statistical basis for estimating probability densities.

An asymmetric strength in inhibitory pathways between TA and the triceps surae is presumably associated to the functional interplay between these muscles. During quiet standing, the triceps surae muscles counteract the micro-falls forward of the human body and stabilize posture (Loram et al., 2005). Whereas the Sol is mainly tonically active, the GM shows bursts of activations corresponding to each micro-fall (Vieira et al., 2012). During these activations, a strong inhibitory pathway to the TA may be needed in order to maximize the effect of the bursting activity, as in ballistic tasks. Indeed, motor unit recruitment in the GM during these bursts of activations is extremely fast. Conversely, the TA has very limited activation during quite standing since the forward sway is determined by gravity and by the position of the center of pressure ahead of the feet.

In conclusion, we demonstrated that the strength of reciprocal inhibition between TA and triceps surae muscle is asymmetric, with a substantially stronger inhibition of the TA than of the triceps surae. This mechanism was observed in large samples of motor units and in standardized synaptic input to the motor neurons. This disparity in the mutual inhibition may be associated with the specific neural control strategies of posture.

Limitations of the study

This study cannot fully confirm whether the motor unit composition of muscles is a factor that enhances the reciprocal inhibition asymmetry between ankle extensors and flexors due to the limited range of analysed contraction forces. The motor units sampled at 10% and 20% MVC from the three muscles were presumably of the same type. On the other hand, performing experiments at higher contraction forces is challenged by muscle fatigue. The methods we used for estimating reciprocal inhibition (PSF and PSTH) indeed require long recording sessions (a

minimum of 150 stimuli is necessary for assessing PSF and PSTH (Brinkworth and Türker, 2003).

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Figure Captions:

Figure 1: Experimental setup. Three high density surface EMG electrodes (HDsEMG) were placed on the tibialis anterior (TA), soleus (Sol) and medial gastrocnemius (GM). The metal pin anode and cathode (8-mm diameter, 3-cm interelectrode distance) were placed on the skin distal to the caput fibulae for stimulating the CPN (blue spot) and on the popliteal fossa for stimulating the TN (red spot). A dynamometer was used to measure ankle torque during plantar- and dorsi-flexion. The torque was displayed on the feedback screen.

Figure 2: Sequence of signal processing from data acquisition to the offline estimation of single motor unit reflex response amplitude. A) The test H-reflex of the homonymous muscle was monitored during the experiment in order to tune the stimulation intensity as to elicit an H-reflex with size equal to 10% of the maximum M-wave. B) The high-density surface EMG (HDsEMG) was recorded from the antagonistic muscle. C) Single motor units were decomposed from the

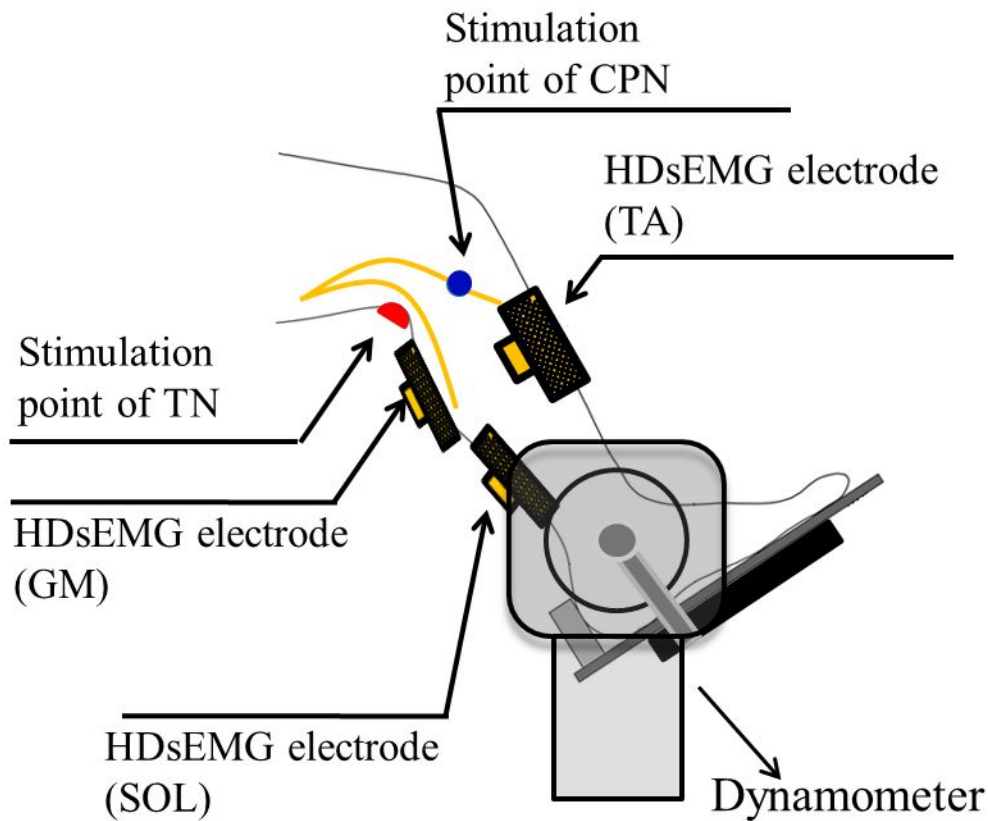
507 HDsEMG signals. Each row of vertical lines refers to the discharge spike trains of a single motor
508 unit. D) From bottom to top, the graphs depict peri-stimulus frequencygram (PSF), cumulative
509 summation of PSF (PSF_{cusum}), peri-stimulus time histogram (PSTH), and PSTH_{cusum},
510 respectively. The horizontal red lines show the significance thresholds. The vertical dashed lines
511 indicate the stimulation time (time zero) and the onset of the inhibition. The arrow shows the
512 distance between minimum and the onset values where the inhibition amplitude was measured.

513 Figure 3: The probability density distribution of reflex amplitudes for tibialis anterior (TA),
514 medial gastrocnemius (GM), and soleus (Sol) at each contraction level (10% and 20% of MVC).
515 The histograms show the probability density of empirical data. Solid curves depict the estimated
516 best fitting functions.

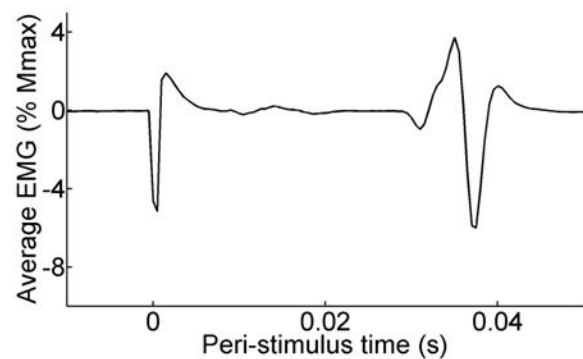
517 Figure 4: Cumulative probability (upper row) and significance between distributions through the
518 median and range values (lower row). The dashed lines in the cumulative probability graphs
519 (upper row) and the vertical red lines in the box plots show the median reflex amplitude values.
520 Asterisks (*) indicate significant differences between groups ($P < 0.05$).

521 Figure 5: Ia reciprocal inhibition strength as a function of recruitment threshold. The dashed lines
522 depict the linear regressions.

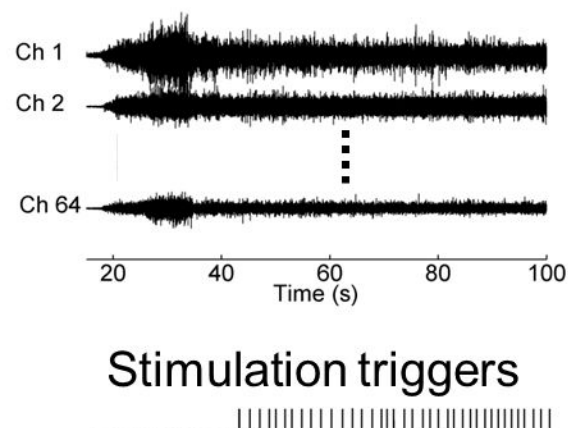
523 Figure 6: The reflex amplitude for motor units of the GM ($n=37$ for 10%MVC and $n=22$ for
524 20%MVC), Sol ($n=68$ for 10%MVC and $n=48$ for 20%MVC) and TA ($n=100$ for 10%MVC and
525 $n=66$ for 20%MVC), with similar baseline discharge rate (5-11 Hz for 10% MVC and 5-13Hz for
526 20%MVC). The asterisk indicates that TA had a significantly greater reflex amplitude ($P < 0.05$).
527 The red horizontal lines indicate the median reflex amplitudes. The cross (+) indicates the
528 average reflex amplitudes.



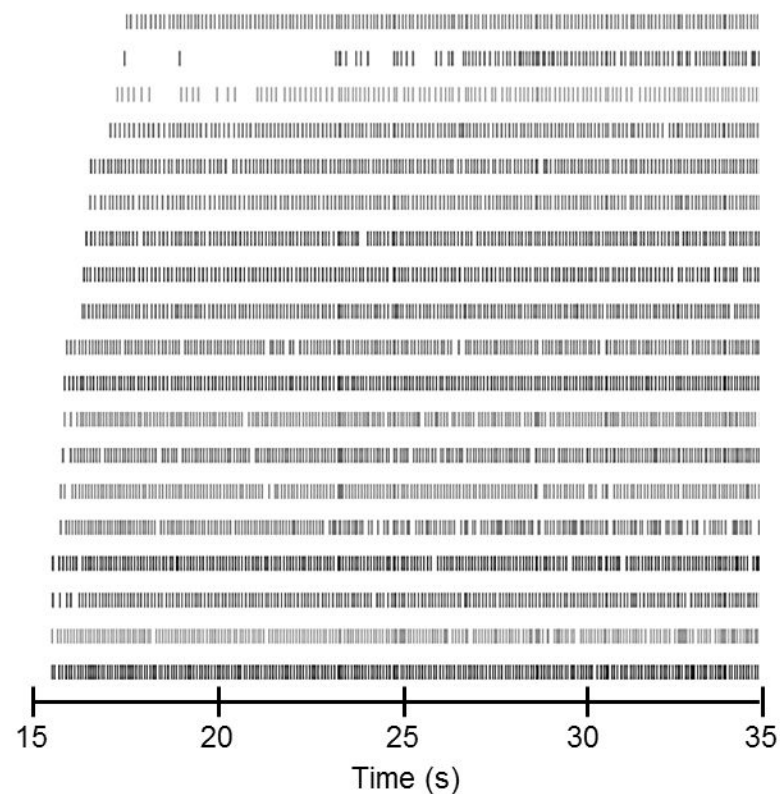
A) H-reflex on homonymous muscle



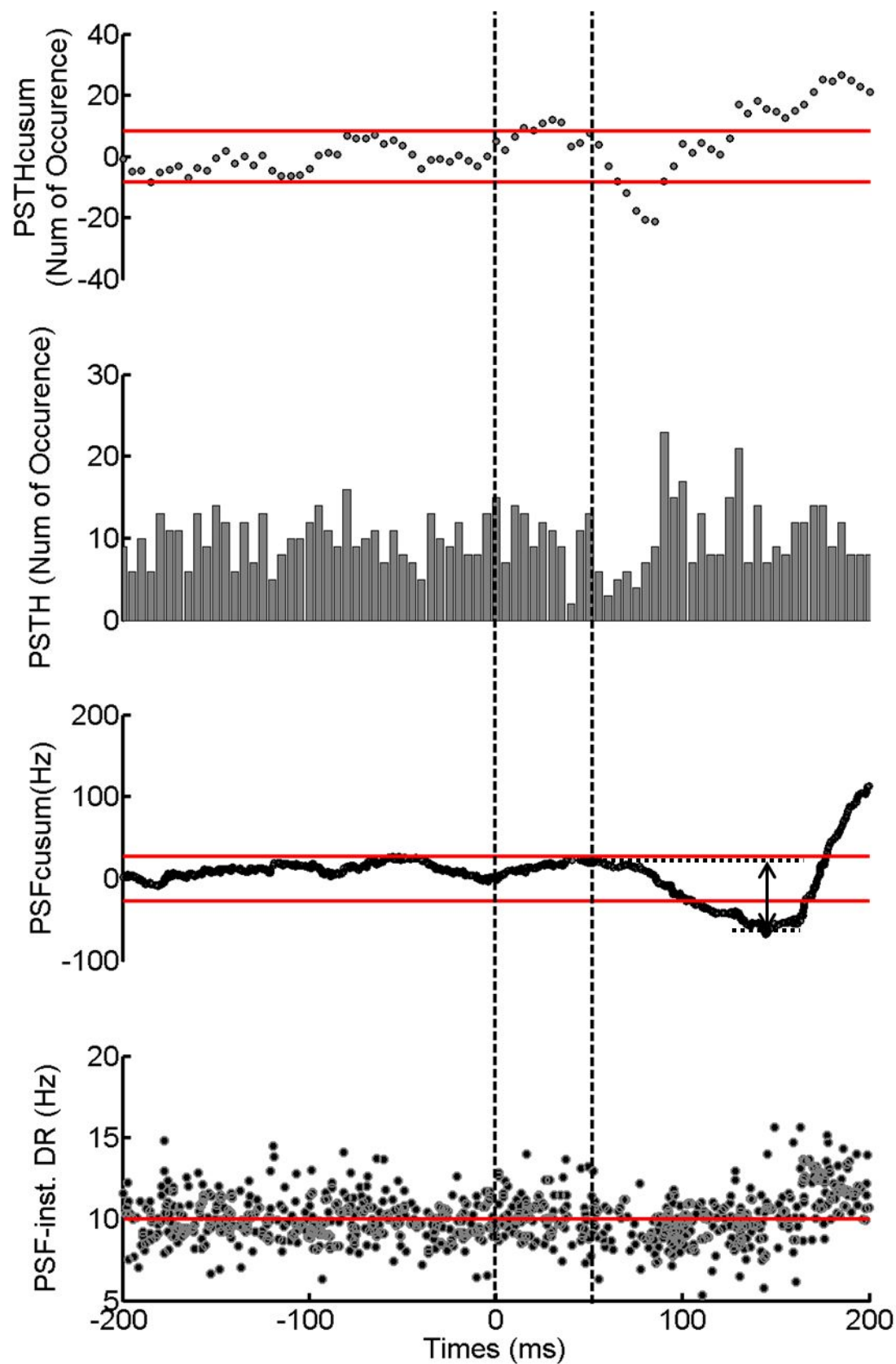
B) HDsEMG of Antagonist Muscle

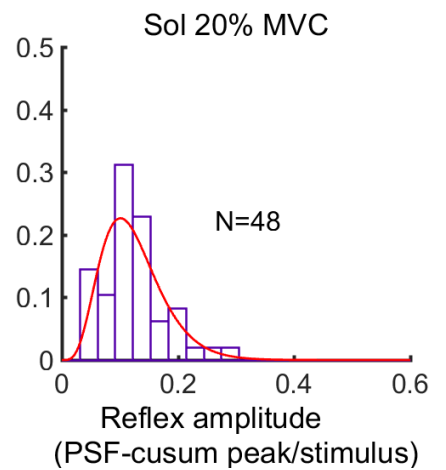
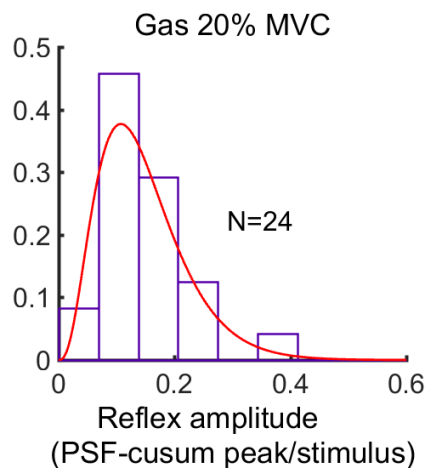
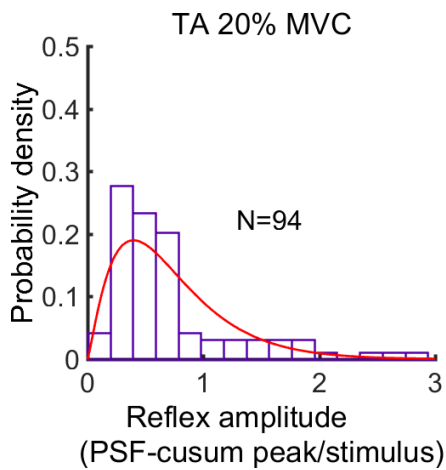
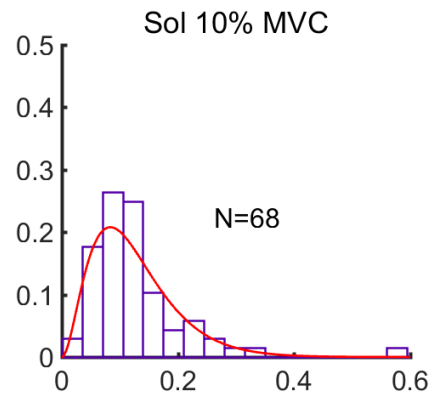
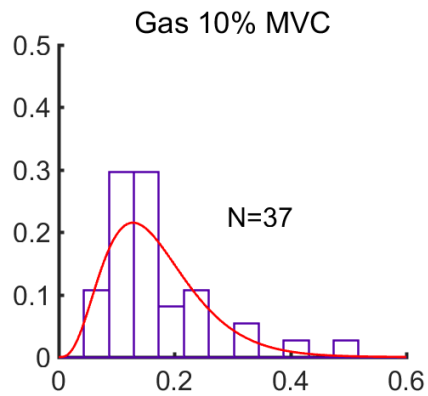
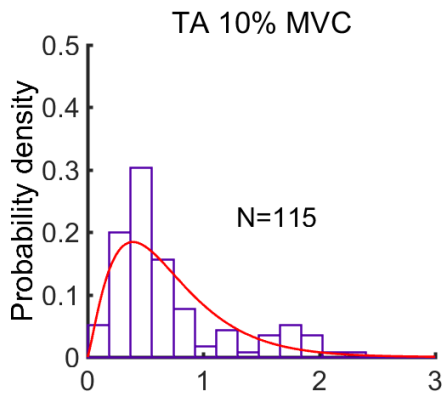


C) Decomposed MU spike trains

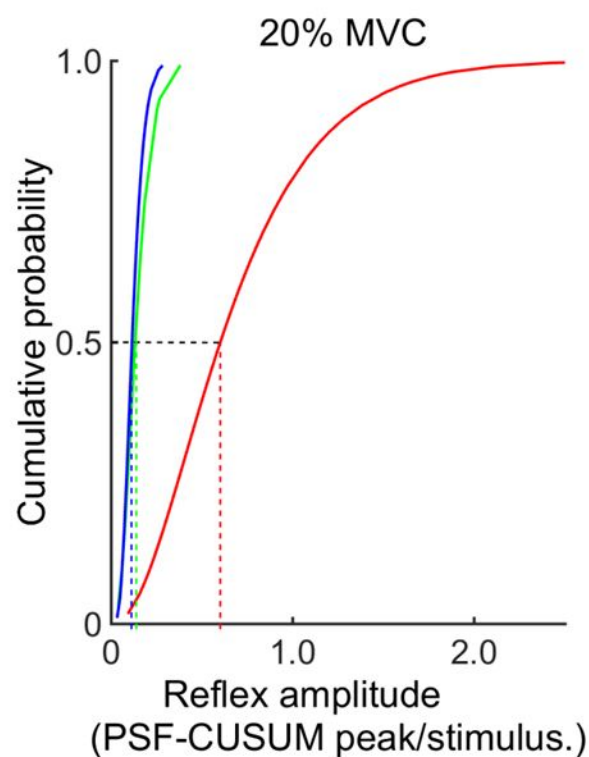
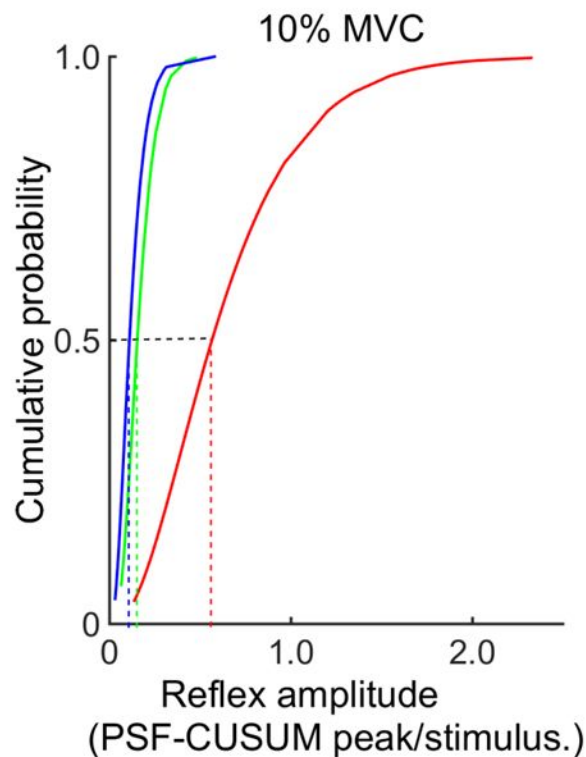


D) Reflex estimation





A)



Sol
GM
TA

B)

