



Published in final edited form as:

Exp Brain Res. 2022 September ; 240(9): 2375–2388. doi:10.1007/s00221-022-06422-7.

Quadriceps muscle stimulation evokes heteronymous inhibition onto soleus with limited Ia activation compared to femoral nerve stimulation

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Abstract

Heteronymous excitatory feedback from muscle spindles and inhibitory feedback from Golgi tendon organs and recurrent inhibitory circuits can influence motor coordination. The functional role of inhibitory feedback is difficult to determine because nerve stimulation, the primary method used in humans, cannot evoke inhibition without first activating the largest diameter muscle spindle axons. Here, we tested the hypothesis that quadriceps muscle stimulation could be used to examine heteronymous inhibition more selectively when compared to femoral nerve stimulation by comparing the effects of nerve and muscle stimulation onto ongoing soleus EMG held at 20% of maximal effort. Motor threshold and two higher femoral nerve and quadriceps stimulus intensities matched by twitch evoked torque magnitudes were examined. We found that significantly fewer participants exhibited excitation during quadriceps muscle stimulation when compared to nerve stimulation (14–29% vs 64–71% of participants across stimulation intensities) and the magnitude of heteronymous excitation from muscle stimulation, when present, was much reduced compared to nerve stimulation. Muscle and nerve stimulation resulted in heteronymous inhibition that significantly increased with increasing stimulation evoked torque magnitudes. This study provides novel evidence that muscle stimulation may be used to more selectively examine inhibitory heteronymous feedback between muscles in the human lower limb when compared to nerve stimulation.

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Author contributions

MAL was responsible for study conception and MAL and SLW responsible for design of the research. MAL and CC performed the experiments. MAL and CC performed the analysis. MAL, CC and SLW interpreted the results. MAL drafted the manuscript. MAL, CC and SLW edited the manuscript. All authors approved the final version submitted and are accountable for all aspects of the work.

Declarations

Conflict of interest

The authors declare no conflicts of interest, financial or otherwise.

Keywords

spinal reflex; muscle spindle; recurrent inhibition; Golgi tendon organ; intermuscular

INTRODUCTION

Proprioceptive feedback from muscle spindles (Ia, II) and Golgi tendon organs (GTOs, Ib) facilitate whole limb motor coordination (Rossignol et al. 2006; Nichols et al. 2016). The coordinating function is possible due to heteronymous spinal networks that allow a muscle's length sensitive muscle spindle and force sensitive GTO feedback to influence motor output of other muscles in the limb (Eccles et al. 1957a; Eccles et al. 1957b; Meunier et al. 1993; Wilmink and Nichols 2003; Lyle and Nichols 2018). Indeed, interjoint coordination is severely disrupted when proprioceptive feedback is compromised due to loss of large diameter sensory afferents (Sainburg et al. 1993; Akay et al. 2014), and evidence suggests alterations in heteronymous feedback contribute to coordination deficits after neuropathology, such as stroke and spinal cord injury (Faist et al. 1994; Dyer et al. 2014; Niazi et al. 2020). In addition to muscle spindle and GTO feedback, Renshaw cells, which receive input from motoneuron collaterals, have widespread projections between limb muscle motoneuron pools and consequently may influence limb coordination (Meunier et al. 1994). Thus, heteronymous feedback arising from changes in muscle length, muscle force and motor output can all activate spinal networks that potentially influence limb coordination through provision of neural linkages between limb segments.

Identifying the unique influence of specific heteronymous spinal circuits between muscles of the lower extremity is requisite to clarify both their functional role during movement and influence on motor impairments. However, a current challenge when studying heteronymous circuits (e.g., Ia, Ib, recurrent inhibition) arises from methodologies that bias excitatory responses, particularly in human subjects. For example, although stimulation of peripheral nerves is the most common approach used to study heteronymous sensory feedback in animals and humans (Eccles et al. 1957a; Eccles et al. 1957b; Hultborn et al. 1987; Meunier et al. 1993; Meunier et al. 1994), nerve stimulation activates sensorimotor axons in part due to diameter from largest (Ia) to smallest (Eccles et al. 1957a; Eccles et al. 1957b; Grill and Mortimer 1995), and the stimulation threshold when using pulse widths typically used in humans (0.5 ms) is lower for sensory than motor axons (Panizza et al. 1992) due to longer strength-duration time constants and lower rheobase (Panizza et al. 1998) probably from greater persistent Na⁺ currents (Howells et al. 2012). Thus, heteronymous inhibitory feedback elicited from Ib afferents or recurrent collaterals can only be examined after first eliciting the excitatory effects from the largest diameter muscle spindle axons (Ia). Since most human lower limb muscles have both excitatory Ia and inhibitory heteronymous projections (Meunier et al. 1993; Meunier et al. 1994), alternative approaches that more selectively induce inhibitory heteronymous feedback could provide new opportunities and insight about the unique functional role of inhibitory feedback. In addition, whereas nerve stimulation normally elicits a brief heteronymous excitation attributed to monosynaptic Ia feedback followed by a longer duration inhibition in healthy adults (Meunier et al. 1990; Meunier et al. 1996; Dyer et al. 2014), several studies have reported a marked increase

in heteronymous excitatory magnitude and duration with reduced or absent inhibition in persons after stroke (Dyer et al. 2009; Dyer et al. 2014) Thus, a method capable of more selectively examining heteronymous inhibitory feedback could be beneficial in identifying the fate of inhibitory feedback and its influence on motor impairments after neuropathology.

Several lines of evidence suggest muscle stimulation could be used as an alternative to nerve stimulation to study inhibitory feedback more selectively. Stimulation-evoked muscle contractions are known to mechanically activate GTOs physiologically and reduce spindle discharge due to fascicle shortening (Jansen and Rudjord 1964; Houk and Henneman 1967; McKeon and Burke 1983). In addition, twitch contractions evoked by surface or intramuscular stimulation can elicit heteronymous responses attributed to twitch-contraction mediated Ib feedback without observable effects expected from direct activation of Ia axons (Aguiar and Baker 2018; Lyle and Nichols 2019). These data are consistent with the general finding of minimal H-reflex responses during muscle stimulation even with 1 ms pulse widths (Bergquist et al. 2012; Nakagawa et al. 2020) and preliminary evidence from Meunier, Pierrot-Deseilligny and Simonetta (6) that showed stimulating the vastus lateralis motor point caused a much smaller increase in SOL motor unit firing probability compared to femoral nerve stimulation. Thus, muscle stimulation-evoked twitch contractions may provide an opportunity to bias non-spindle networks in humans (i.e., Ib and recurrent collateral pathways) and thus study their heteronymous effects more selectively than nerve stimulation.

The purpose of the current study was to test the hypothesis that muscle stimulation more selectively evokes heteronymous inhibition when compared to nerve stimulation in the human lower limb. Specifically, this study compared the influence of femoral nerve (FN) and quadriceps (Q) muscle stimulation onto ongoing soleus (SOL) EMG. Many studies have shown that FN stimulation typically evokes short latency monosynaptic spindle (Ia) excitation followed by inhibition onto SOL (Meunier et al. 1990; Meunier et al. 1996; Dyer et al. 2014). Here, we examined the heteronymous effects using 3 stimulation intensities for both FN and Q stimulation. The FN and Q intensities were matched by the peak torque produced thus attempting to control for the mechanical effect on muscle receptors. We expect that FN but not Q stimulation will elicit excitatory feedback onto SOL. Second, we expect that the magnitude and duration of heteronymous inhibition will be similar for FN and Q stimulation and will increase with increasing magnitude of twitch contractions (i.e., stimulation intensity).

A unique aspect of this work is evaluating muscle stimulation as a more physiological stimulus to selectively study heteronymous inhibitory feedback when compared to nerve stimulation. This advance is an important first step toward examining the functional role of inhibitory feedback during movement and potentially identifying the extent to which impairments in inhibitory feedback after neuropathology contribute to motor discoordination. In addition, new considerations about the origin of inhibitory feedback are discussed by carefully considering the differential effects of nerve and muscle stimulation on axons and the mechanical effects on GTO receptors caused by muscle and nerve stimulation.

METHODS

Participants

Fourteen healthy participants (7 males and 7 females, 26.2 ± 2.9 years old) with no recent history of lower limb injury or neuromotor disorder participated in this study. Participants provided written informed consent in accordance with procedures approved by the Emory University Institutional Review Board (IRB00114765) and the study was performed in accordance with the ethical standards per the 1964 Declaration of Helsinki and its later amendment. Per institutional guidelines, participants tested after March 2020 wore a face shield and mask during sessions.

EMG, torque and estimate of muscle twitch onset

All EMG, torque and accelerometer were acquired at 2000 Hz using an MP150 acquisition unit (Biopac Systems Inc, Goleta, CA, USA). Muscle activity was recorded from soleus (SOL), vastus lateralis (VL), and vastus medialis (VM) muscles using self-adhesive Ag-AgCl electrodes (2.2×3.5 cm, Vermed, A10041, Nissha Medical Technologies, Buffalo, NY) and SignaGel (Parker Laboratories, INC. Fairfield, NJ). Skin was gently abraded and cleaned with isopropyl alcohol. SOL electrodes were positioned in the midline of the posterior aspect of the shank just distal to the gastrocnemius. The VL and VM electrode pairs were located on the distal third of the respective muscle belly and aligned parallel to the direction of their muscle fibers. The reference electrode was placed at the proximal medial tibia. Surface EMG signals were differentially amplified and band-pass filtered (10–1000 Hz, AMT-8, Bortec Biomedical, Calgary, AB, Canada).

Knee extensor torque was measured using a dynamometer (Humac Norm, CSMI, Inc, Worcester, MA). Participants were positioned with the knee joint aligned with the dynamometer axis of rotation. Stimulation evoked twitch onset was estimated with uniaxial piezoelectric accelerometers (Model A352C65; Gain $\times 10$, Model 482C16, PCB Piezotronics, Inc Depew, NY, USA) placed on the muscle bellies of VM and VL. Twitch onset was used to aid in determining stimulation motor threshold and as an estimate of the first mechanical deformation of muscle fibers (Bichler 2000; Sasaki et al. 2011) that could influence muscle mechanoreceptors such as Golgi tendon organs. Torque and accelerometer analogue outputs were bandpass filtered with a zero-lag, fourth-order Butterworth filter (0.1–100 Hz).

General Procedures

The experimental procedures consisted of three parts: I. SOL maximal voluntary isometric contraction (MVIC), II. motor threshold and stimulation-torque recruitment curves when stimulating FN or Q muscles and III. evaluating the effect of FN and Q stimulation (3 intensities) onto ongoing SOL EMG.

I. MVIC—To record maximal SOL EMG, participants completed a maximal voluntary isometric plantarflexion contraction while seated on a dynamometer (Humac Norm, CSMI, Inc, Worcester, MA) with the hip at 70 degrees of flexion, the knee at 60 degrees of flexion, and the ankle in a neutral position (i.e., absolute angle of 90 degrees) (Figure 1). The

ankle was rigidly strapped to the dynamometer ankle device fixed to the floor (Figure 1A). A dynamometer pad was firmly positioned against the anterior distal tibia to block shank motion. Participants were asked to maximally plantarflex focusing on the ankle to minimize knee extension. Participants completed three maximal effort trials of 5 s duration. Verbal encouragement and raw SOL EMG feedback were provided to facilitate best effort. The SOL EMG was bandpass filtered 10–500 Hz and a 400 ms RMS window was used to identify the peak SOL value for each trial. The mean of the three trials determined maximal SOL EMG.

Three maximal voluntary isometric knee extension torque trials for 5 seconds were recorded with the knee at 90 degrees in 12 of 14 participants. Verbal and visual feedback of torque was provided to facilitate maximal effort. The average of the 3 peak torque values were used to calculate the relative magnitude of the stimulation evoked twitch contractions used to examine heteronymous feedback from the quadriceps.

II. Nerve and Muscle stimulation: motor threshold and stimulation-torque

recruitment—For motor threshold and stimulus-torque recruitment procedures, the knee was positioned at 90° of flexion and rigidly strapped to the dynamometer pad. FN and Q muscles were stimulated with STM100C stimulators (Biopac System Inc, CA, USA).

The FN was stimulated with cathode in the femoral triangle (1 ms pulse width, circular 2.5 cm or 2.2 × 3.5 cm electrodes) just medial to the sartorius and approximately 1 cm distal to the inguinal ligament. A motor point pen electrode (Compex Medical SA, Switzerland) was used to identify the best stimulation location characterized by the lowest intensity that produced primary knee extension with palpable VL and VM contraction. The anode was positioned on the posterolateral buttock (7.5 × 13 cm, ValuTrode, Axelgaard Co., Ltd, CA, USA). Motor threshold (MT) for FN stimulation was based on the minimal current intensity that produced: 1) a visible M-wave in VL and/or VM EMG and 2) deflection of accelerometers over the VL and VM with onset 20 ms from stimulation. The rationale for selecting 20 ms is that the Q H-reflex onset is typically 18–20 ms (Larsen and Voigt 2006), and thus accelerometer deflection prior to 20 ms indicates mechanical activation from a M-wave.

Quadriceps muscles stimulation was in the form of a doublet (two unipolar pulses, 50 μs pulse width, 200 Hz) to take advantage of the catchlike property (Burke et al. 1970; Binder-Macleod and Kesar 2005) and short pulse width to reduce likelihood of activating sensory axons (Panizza et al. 1992; Pratt 1995; Lyle and Nichols 2019). Stimulation was completed with four 5×5 cm electrodes positioned on motor points identified with a pen electrode (Botter et al. 2011) at the distal VM and mid rectus femoris, and distal and proximal VL. Motor threshold was determined as the minimal current intensity that produced: 1) palpable contraction in the proximal and distal portions of the VL and VM, 2) with visible motion of the shank after stimulation. In relation to the timing of FN stimulation, the first Q stimulus pulse was 2.5 ms before and the second pulse 2.5 ms after when the FN stimuli were applied.

After determining motor threshold (MT) for FN and Q stimulation, participants were asked to relax the lower extremity while stimulations were sequentially applied at 1.0xMT, 1.25xMT, 1.5xMT, 2.0xMT, 2.5xMT and 3xMT. Each stimulation intensity was repeated at least two times. The peak knee extension torque values were averaged to characterize a stimulation-torque relation.

III. Effect of femoral nerve and quadriceps stimulation onto ongoing SOL

EMG—Participants were positioned as described above for SOL MVIC testing on the dynamometer with hip 110 degrees, the knee 60 degrees, and the ankle at 90 degrees (Figure 1A). The ankle was rigidly strapped to the dynamometer ankle device which was secured to the floor. A dynamometer pad was firmly positioned against the anterior distal tibia to block shank motion. Participants were required to perform isometric plantarflexion at 20% SOL MVIC. Ongoing feedback of their SOL EMG was provided on a computer monitor with the goal to target 20% of SOL MVIC with boundaries set $\pm 5\%$ (See figure 1A). They performed practice trials until comfortably achieving the magnitude while staying within the boundary lines. Participants were instructed to focus on rotating only the ankle while keeping the knee muscles relaxed. Participants were reminded to keep the Q relaxed during the experiment if we observed visible contraction and increased Q EMG.

To examine the influence of heteronymous proprioceptive feedback from Q onto SOL, the FN was stimulated while participants targeted 20% SOL MVIC, typically during periods lasting 30–60 s. The FN stimuli were randomly elicited during the 30–60 s periods with a minimum of 5 s between stimuli. Rest was provided for a minimum of 1 minute between each 30–60 s testing period or longer if needed. A total of 20–35 stimulus repetitions were recorded for FN at each of three stimulation intensities. The FN stimulation intensities chosen, identified from the stimulus-recruitment curves, were motor threshold (18.9 ± 8.9 mA) and 2 intensities above MT (24.4 ± 9.9 and 32.2 ± 11.9 mA) to represent 3 approximately linearly increasing levels of twitch evoked torques (i.e., 3 levels of force sensitive Golgi tendon organ activation). After the 3 FN intensities were recorded, the same protocol was completed while stimulating the Q muscles. The Q stimulation intensities were motor threshold (12.8 ± 5.3 mA) and 2 intensities above motor threshold (27.5 ± 11.7 and 36.9 ± 13.4 mA) that were matched to the FN intensity twitch torque recordings. Generally, we observed that FN stimulation evoked greater torque than Q stimulation for a given motor threshold stimulus with some participants exhibiting steep FN stimulus-torque relation. Thus, we chose to limit the FN stimulation 3 intensity so that the evoked peak torque was no greater than 30 Nm. This decision ensured we could identify tolerable Q stimulation intensities. The stimulation intensities in terms of motor threshold (MT) for intensity 2 was 1.3 ± 0.19 vs 2.2 ± 0.61 MT and for intensity 3 was 1.7 ± 0.32 vs 3.0 ± 0.69 MT for FN and Q, respectively.

Data Analysis

Stimulation evoked torque and estimate of twitch onset—All data were processed using routines written in MATLAB R2019a (Mathworks, Natick, MA). The peak knee extensor torque and time to peak torque in response to FN and Q stimulation were extracted from time-torque traces for each of the stimulation intensities. Twitch onset was estimated

from the accelerometers located on the VL and VM muscles. Based on pilot data, twitch onset was defined as the time at which the accelerometer trace exceeded $1.5 \text{ m}\cdot\text{s}^{-2}$ after electrical stimulation of the FN or Q. Since each stimulation intensity was repeated at least 2 times during stimulation-torque recruitment procedures, the average peak torque and twitch onset values were used to characterize the Q muscle responses to stimulation across stimulation condition and intensities.

Heteronymous feedback—Excitatory and inhibitory effects from FN and Q muscle stimulation onto ongoing SOL EMG were measured after bandpass filtering (10–1000 Hz, zero phase shift) and rectification. The analysis is shown graphically in Figure 1C. The rectified SOL EMG was normalized to each participants' MVIC value and averaged across trials (20–35 trials) for each stimulation condition and intensity. The mean SOL EMG and SD over a 400 ms period prior to stimulation were calculated. To more clearly express heteronymous effects, the mean pre-stimulation SOL EMG was subtracted from the SOL EMG trace, thus delineating the pre-stimulation mean as 0% MVIC and post-stimulation values above and below 0% MVIC excitatory and inhibitory feedback, respectively.

The pre-stimulus SOL EMG mean and SD for each condition were used to identify excitation and inhibition onset, duration, and magnitudes (Fig 1C). Similar in principle to Horslen et al. (2017), excitation onset was determined as the time point when the SOL EMG trace exceeded 1SD above the mean for ≥ 2 ms. The end of excitation was determined as the time at which the SOL EMG trace returned below the 1 SD line for a period of ≥ 2 ms. Only excitatory traces with onset ≥ 23 ms were considered as arising from muscle spindle axons (Hultborn et al. 1987; Meunier et al. 1993). Inhibition onset and termination were determined as the SOL EMG moving 1SD below the mean background SOL EMG for a period of ≥ 2 ms and returning above the 1 SD line for ≥ 2 ms. Inhibitory responses were considered in analysis only if the onset was < 45 ms since the fastest transcortical effects could manifest soon thereafter (Barbeau et al. 2000). The durations of excitation and inhibition were calculated as the difference between effect onset and termination. The excitation and inhibition magnitudes were calculated as the area relative to background SOL EMG using trapezoidal numerical integration (trapz in Matlab). The inhibitory magnitude was additionally calculated as the mean SOL EMG during a fixed window from 35–73 ms after stimulation (Dyer et al. 2014; Maupas et al. 2017).

Statistical analysis

All statistical analyses were performed with R-Studio, version 1.3.1073 (© 2009–2020 RStudio, PBC) and SAS 9.4 (The SAS Institute, Cary, NC). Descriptive statistics are reported in the text and figures as mean \pm 1 standard deviation. The normality assumption was first inspected with the quantile-quartile plots for each variable and condition separately and evaluated with Shapiro-Wilks test. To test the influence of stimulation intensity and condition on the dependent variables, the MIXED procedure was used to complete repeated-measures ANOVAs. Subject was treated as a random factor and the stimulation condition (i.e., FN and Q muscle stimulation) and intensity (stimulation intensities 1, 2 and 3) were fixed factors. The *F*-values were computed using a compound symmetry variance-covariance structure and the Kenward-Roger method. Pairwise comparisons were completed with

Bonferroni correction. McNemar's tests were used to determine whether the frequency of excitation occurrences across participants (i.e., presence or absence of excitation) differed between femoral nerve and quadriceps stimulation for each stimulation intensity.

RESULTS

Stimulation evoked torques across conditions and intensities

Three stimulation intensities of increasing magnitude were used with the goal to achieve equivalent torques at stimulation intensities 2 and 3 for both FN and Q stimulation. A significant main effect of torque magnitude was found for stimulation condition ($F_{(1,65)} = 9.60, P=0.003$) and stimulation intensity ($F_{(2,65)} = 232.22, P<0.001$) with no interaction (Figure 2). Pairwise comparisons between conditions revealed that the evoked torque from stimulation intensity 1 (motor threshold stimulation) was significantly larger for FN compared to Q stimulation (mean: 5.1 ± 5.5 vs. 2.3 ± 1.1 Nm, $P=0.0095$), but there were no differences for the other intensities (13.0 ± 5.0 vs 11.8 ± 4.0 Nm; 20.6 ± 5.0 vs 19.0 ± 3.6 Nm). The larger FN evoked torque at motor threshold (stimulation intensity 1) can be attributed to prominent H-reflex responses in several participants (e.g., participant 12) which were absent using Q stimulation.

The maximal voluntary isometric knee extension peak torque was 168.4 ± 58.5 Nm (from 12 of 14 participants). The stimulation intensity 3 torque values for these 12 participants for FN and Q muscle stimulation were, on average, 13.8 ± 4.7 % (range: 5–21%) and 12.4 ± 4.1 % (range: 5–17%) of peak voluntary torque. Thus, the highest stimulation evoked peak torques were a relatively low percentage of knee extension torque capacity.

In addition to torque magnitude, the stimulation evoked torque temporal profiles were similar between FN and Q stimulation as determined from twitch onset and time to peak torque (Figure 1B). The time to peak torque and twitch onset times across conditions and intensities are shown in Table 1

Background EMG across stimulation conditions and intensities

Participants consistently achieved the goal of maintaining 20% MVIC SOL EMG during trials (grand mean: 20.01 ± 1.24 %). The background SOL EMG was not different across stimulation conditions ($F_{(1,65)} = 0.02, P=0.88$) or the three stimulation intensities ($F_{(2,65)} = 1.3, P=0.28$). In addition, the standard deviation of the background SOL EMG (grand mean: 3.55 ± 0.73 %) was the same across conditions ($F_{(1,65)} = 0.00, P=0.99$) and intensity ($F_{(2,65)} = 0.66, P=0.52$).

Heteronymous Excitation

FN stimulation resulted in more frequent excitatory responses onto SOL compared to Q stimulation across intensities in the 14 participants (Intensity 1: 9 vs 2, McNemar's chi-squared = 4, df = 1, $P=0.045$; Intensity 2: 10 vs 3, McNemar's chi-squared = 4, df = 1, $P=0.045$; Intensity 3: 10 vs 4, McNemar's chi-squared = 4.1667, df = 1, $P=0.041$, Figure 3). On average, the time of excitation onset across stimulation intensities was 30.5 ± 3.4 ms for FN stimulation and 29.3 ± 2.8 ms for Q stimulation. The mean duration of excitation

across stimulation intensities ranged from 4 to 6 ms for FN stimulation and 2 to 3.6 ms for Q stimulation. The magnitude of excitatory responses expressed as EMG area are shown in Figure 3 with individual participants represented as numbers. Excitatory responses in SOL evoked from FN stimulation were greater than the Q evoked responses, when present, with one exception in which one participant exhibited excitation that was similar across conditions (see #2, Figure 3). Due to the few excitatory response observations for the Q stimulation condition, an ANOVA evaluating a main effect of intensity was only evaluated for FN stimulation. A significant main effect of FN stimulation intensity was found for excitation magnitude ($F_{(2,9)} = 15.37, P=0.0013$). FN stimulation intensities 2 and 3 resulted in significantly greater SOL EMG excitatory responses than intensity 1, with both $P < 0.01$ (Intensity 1: 58.2 ± 38.8 ; Intensity 2: 89.7 ± 69.1 ; Intensity 3: $79.9 \pm 61.8, \%MVIC \cdot ms$).

Heteronymous Inhibition

FN and Q stimulation resulted in a similar frequency of inhibitory responses onto SOL across intensities in the 14 participants (Intensity 1: 10 vs 11, Intensities 2 and 3: 14 vs 14, Figure 4). The onset latency of heteronymous inhibition caused by FN and Q stimulation was not significantly different ($F_{(1,53)} = 1.29, P=0.26$). Stimulation intensity 3 resulted in a small but significantly earlier inhibitory onset of 1.1 ms compared to stimulation intensity 1 (Figure 4A, 36.2 and 37.3, respectively; $P=0.04$).

Inhibitory magnitude evaluated as EMG area revealed a significant main effect for condition ($F_{(1,53)} = 36.45, P<0.0001$) and intensity ($F_{(2,53)} = 25.35, P<0.0001$) with no interaction (Figure 4B). The overall mean inhibition due to FN stimulation was $571.1 \pm 265.1 \%MVIC \cdot ms$ and for Q stimulation was $353.5 \pm 237.7 \%MVIC \cdot ms$. The pairwise comparisons for condition showed that FN stimulation resulted in significantly greater inhibition compared to Q stimulation for intensity 1 ($P=0.0007$) and intensity 3 ($P=0.009$), but no difference when comparing stimulation intensity 2 ($P=0.517$). For the main effect of intensity, the overall mean inhibition for stimulation 1, 2 and 3 were $254.0 \pm 230.7, 449.0 \pm 191.7, \text{ and } 627.9 \pm 266.6 \%MVIC \cdot ms$, respectively. The inhibitory magnitude for FN stimulation intensity 3 was significantly greater than stimulation intensity 2 and 1 ($P=0.024$ and $P=0.005$), but no difference between stimulation intensity 1 and 2 ($P=1.0$). The inhibitory magnitude for Q stimulation intensities 3 and 2 were significantly greater than stimulation intensity 1 ($P<0.0001$ and $P=0.0006$; pairwise comparison of intensity 2 and 3 $P=0.332$).

The mean inhibitory magnitude evaluated during a fixed 35–73 ms window after stimulation showed a significant main effect for condition ($F_{(1,49)} = 21.8, P<0.0001$) and intensity ($F_{(2,49)} = 21.38, P<0.0001$) with significant interaction ($F_{(2,49)} = 4.93, P=0.011$, Figure 4C). FN stimulation caused significantly greater inhibition compared to Q for stimulation intensity 1 (-7.9 ± 2.9 vs. -3.9 ± 1.2 SOL $\%MVIC$), $P=0.001$), but there was no difference between conditions for stimulation intensities 2 or 3 ($P=1.0$ for both). The inhibitory magnitudes were not statistically different across FN stimulation intensities (1 and 2, $P=1$; 1 and 3 $P=0.246$; 2 and 3 $P=0.301$). The inhibitory magnitude for Q stimulation intensities 3 and 2 were significantly greater than stimulation intensity 1 ($P<0.0001$ and $P<0.0001$; 2 and 3, $P=0.846$).

The inhibitory duration showed a significant main effect for stimulation condition ($F_{(1,53)} = 31.1$, $P < 0.0001$) and intensity ($F_{(2,53)} = 14.18$, $P < 0.0001$) with interaction ($F_{(2,53)} = 5.96$, $P = 0.0046$, Figure 4D). The duration of inhibition increased with increasing stimulation intensity (37.5 vs 13 ms, 47 vs 46, 63 vs 55 ms). The inhibitory duration from FN stimulation was significantly greater than for Q stimulation for intensity 1 ($P = 0.0001$) but not for intensity 2 ($P = 1.0$) or stimulation intensity 3 ($P = 0.11$). There were no significant differences in inhibitory duration across FN stimulation intensities (all $P > 0.103$). The inhibitory duration for Q stimulation intensity 1 was significantly shorter than intensities 2 and 3 (both $P < 0.0001$), whereas no difference was found between intensities 2 and 3 ($P = 1.0$).

Discussion

The actions of heteronymous sensory circuits in humans are typically examined by quantifying the effect of stimulating a peripheral nerve on the motor output in another muscle (e.g., EMG or H-reflex) (Meunier et al. 1990; Meunier et al. 1996; Barbeau et al. 2000; Dyer et al. 2014). Because electrical stimulation of a nerve excites axons in part due to diameter and sensory axons have a lower stimulation threshold compared to motor axons with the most commonly used pulse widths (0.5 to 1 ms) (Panizza et al. 1992; Grill and Mortimer 1995; Panizza et al. 1998), inhibitory feedback from Ib or recurrent pathways from antidromic motor axon volleys cannot be examined without also evoking effects from the largest diameter muscle spindle axons. This study tested the hypothesis that transcutaneous muscular stimulation with a short pulse width doublet could more selectively elicit heteronymous inhibitory feedback and thus overcome a drawback of using nerve stimulation. This hypothesis was tested by comparing the influence of FN and Q muscle stimulation onto ongoing SOL EMG. As noted below, the novel findings from this study support the hypothesis that muscle stimulation may be used to bias inhibitory heteronymous feedback more selectively when compared to nerve stimulation.

Frequency and magnitude of heteronymous excitation was reduced with muscle stimulation

Excitatory feedback, attributed to monosynaptic spindle afferents, is widely distributed between muscles that cross different joints in the human lower limb (Meunier et al. 1993). In the present study, the similar magnitude and frequency of participants exhibiting FN evoked excitation for stimulation intensities 2 and 3 suggest no significant additional recruitment of spindle afferents. The frequency of excitation observed among participants and the 29 ms onset latency is comparable to data from previous reports (Hultborn et al. 1987; Meunier et al. 1993; Meunier et al. 1996; Dyer et al. 2009). A novel finding of this study was that transcutaneous stimulation of the Q muscles caused an excitatory SOL EMG response in less than a third (14–29%) of participants when compared to FN stimulation (64–71%). Importantly, 3 of the 4 participants who demonstrated an excitatory SOL response from Q stimulation had an excitatory magnitude that was less than half of that observed for FN stimulation, and the other participant had similar excitation for both FN and Q. Meunier et al. (1990), using post-stimulus histogram analysis, also found that FN stimulation resulted in a much higher increase in SOL motor unit firing probability compared to VL motor

point stimulation. Other studies have shown no evidence of heteronymous excitation from Ia afferents when using intramuscular stimulation in the cat hindlimb (Lyle and Nichols 2019) or baboon forelimb muscles (Aguiar and Baker 2018), or when using transcutaneous muscle stimulation in human forearm muscles (Aguiar and Baker 2018). The data from the current study indicate muscle spindle axons were activated to a much-reduced extent (i.e., minimal excitatory responses) with muscle stimulation over Q motor points compared to FN stimulation (Figure 5A vs B).

The reduced frequency and magnitude of excitatory responses from muscle stimulation in this study may be due to several factors. First, the short pulse width used for muscle stimulation in this study has been shown to bias to some extent the motor axons compared to spindle axons (Panizza et al. 1992; Bergquist et al. 2011b). Nonetheless, even stimulating motor points with pulse widths that favor activation of spindle axons (i.e., 1 ms) cause very small H-reflex responses (Bergquist et al. 2012; Nakagawa et al. 2020). Another reason for reduced heteronymous excitation with muscle stimulation could be due to temporal dispersion of any activated spindle sensory afferents compared to that elicited with nerve stimulation (Bergquist et al. 2011b). A potential additional consideration is the fact that reduced spindle firing should correspond with active shortening from the twitch contraction (Jansen and Rudjord 1964; McKeon and Burke 1983). The reason that 4 participants exhibited excitatory responses with muscle stimulation is unknown but is consistent with interindividual differences previously observed by (Panizza et al. 1992) and the possibility that spindle axons were more concentrated superficially in these individuals (Bergquist et al. 2011b). Collectively, the findings from this and other studies suggest muscle stimulation can be used to examine heteronymous feedback with reduced influence from spindle afferents compared to nerve stimulation.

Heteronymous inhibition was similar for nerve and muscle stimulation

Inhibitory feedback is widespread between muscles in the cat hindlimb (Eccles et al. 1957b; Lyle and Nichols 2018) and human lower limb (Meunier et al. 1994). To date, heteronymous inhibition evoked by stimulating peripheral nerves above motor threshold has been consistently observed in humans (Hultborn et al. 1987; Meunier et al. 1994; Meunier et al. 1996; Dyer et al. 2014). An important finding of the present study was that FN and Q muscle stimulation evoked similar inhibitory effects onto ongoing SOL EMG. The inhibitory onset occurring 36–37 ms after stimulation was not different between stimulation conditions or intensities and is consistent with prior studies (Meunier et al. 1994; Meunier et al. 1996; Dyer et al. 2014).

A unique aspect of the present study was comparing heteronymous inhibition between femoral nerve and Q stimulation while controlling for the mechanical effects of stimulation evoked twitch contractions. The evoked torques from the three stimulation intensities for FN and Q stimulation were matched as best as possible from dynamometer recordings. The twitch onset identified from the accelerometer recordings provides an estimate of when muscle force sensitive GTO receptors are expected to begin firing and the time to peak torque an estimate of when the GTO firing will likely start decreasing as the muscle begins to relax (Houk and Henneman 1967; McKeon and Burke 1983; Bichler 2000; Sasaki et

al. 2011). The onset of twitch contractions for FN and Q muscle stimulation decreased in a similar fashion with increasing stimulation intensity with a small latency delay for FN as expected due to the more proximal stimulation location. Consistent with prior work (Rodriguez-Falces and Place 2013), the time to peak torque slightly decreased with increasing stimulation intensity for FN but not Q muscle stimulation. As such, other than a slightly earlier twitch onset for Q muscle stimulation, the twitch contraction temporal profiles suggest mechanoreceptors were exposed to similar mechanical conditions during FN and Q stimulation.

Inhibitory magnitude and duration increased with increasing stimulation evoked twitch contractions for both conditioning stimuli. On average, the FN stimulation resulted in greater inhibition compared to Q stimulation. The greater inhibition from FN stimulation could be the result of differences in relative activation of sensory axons (see below) and the fact that FN stimulation presumably activates feedback from all quadriceps muscles whereas Q stimulation does not (e.g., no activation of vastus intermedius). Statistically, the greater inhibition from FN was primarily due to the difference from stimulation intensity 1 for which the torque was also higher. These data suggest estimates of muscle contraction magnitude evoked by stimulation was related to heteronymous inhibition. Moreover, these data illustrate that the relative inhibitory influence from stimulation intensities 2 and 3 was substantial for most participants with ongoing SOL EMG decreasing by 50% or more (i.e., a drop from 20% MVIC to 10% SOL EMG, see Figure 4C mean inhibition). Importantly, the evoked peak torques from FN and Q stimulation intensities 2 and 3 were about 12 and 20 Nm on average and accounted for less than 14% of participants voluntary torque capacity. The torques for stimulation intensities 2 and 3 in terms of motor threshold (MT) were achieved for FN using, on average, 1.3 to 1.7 MT and for Q using 2.2 to 3.0 MT, respectively. Together, these data suggest consistent inhibitory responses of similar magnitude when using muscle stimulation evoked twitch contractions equivalent to that evoked by nerve stimulation.

Potential origin of heteronymous inhibition

The similar inhibitory effects from FN and Q stimulation suggest inhibition was mediated by the same circuitry with subtle differences in circuit activation between conditions discussed below and represented graphically in Figure 5. Prior work in the cat hindlimb and human lower limb have attributed heteronymous inhibitory feedback to GTO afferents and recurrent inhibition (Eccles et al. 1957b; Meunier et al. 1990; Bonasera and Nichols 1994; Meunier et al. 1994). Heteronymous inhibition due to GTO feedback can occur experimentally by directly activating Ib axons via nerve stimulation (Eccles et al. 1957b) or by muscle contraction mechanically activating GTOs (Jansen and Rudjord 1964; Houk and Henneman 1967; McKeon and Burke 1983; Inglis et al. 1995). Heteronymous inhibition attributed to recurrent inhibition can occur experimentally from antidromic motor axon projections acting via recurrent collaterals onto Renshaw cells when stimulating above motor threshold or from motoneuron firing (e.g., from stretch or H-reflex). Thus, heteronymous inhibitory effects due to FN stimulation in this study could be attributed primarily to direct activation of Ib sensory afferents and recurrent inhibition, as well as from afferent feedback from the twitch contraction mechanically activating GTOs. Heteronymous inhibitory effects due to Q muscle

stimulation could be attributed to recurrent inhibition and physiological activation of Ib afferent feedback from the twitch contraction with comparatively less direct activation of spindle or GTO sensory axons compared to FN stimulation (Figure 5B).

While the relative contribution of specific sensorimotor circuits to heteronymous inhibition cannot be determined from this study, our findings and those from prior studies support direct activation of Ib axons, recurrent inhibition from antidromic motor axon projections, and GTO receptor firing as plausible factors. We acknowledge that cutaneous afferents could contribute given the surface stimulation used but presume a minimal influence based on prior work (e.g., Van de Crommert et al. 2003). Prior studies in humans have favored recurrent inhibition as the primary source of heteronymous inhibition (Meunier et al. 1990; Meunier et al. 1994) based on the short latency and the observation that inhibition has been observed with tendon taps which can provide synchronous firing of motoneurons that could cause recurrent inhibition without activating GTOs (Meunier et al. 1990). In the present study, estimates of the mechanical activation onset of GTO receptors from the twitch contractions differed by stimulation condition and intensity (Table 1). Thus, the same inhibitory onset across conditions and intensities suggests direct activation of Ib axons or antidromic volleys are responsible for inhibitory onset during FN stimulation. The most parsimonious explanation for inhibitory onset for Q stimulation is antidromic volleys since direct activation of sensory axons, although possible (Pierrot-Deseilligny et al. 1979; Horslen et al. 2017), appear less likely with the Q stimulation methods used in the present study (See Figure 3; Bergquist et al. 2012; Nakagawa et al. 2020). As previously shown by Meunier et al. (1990), the more distal stimulation location for Q would be expected to cause a small delay (i.e., 2–3 ms) in inhibitory onset when compared to nerve stimulation. The reason for the same inhibitory onset for FN and Q stimulation in the present study could be because, when compared to the trials examining FN stimulation, the first of the 2 quadriceps pulses was applied 2.5 ms prior to when the FN stimulus was applied (i.e., FN applied at time 0 and Q pulse 1 at –2.5 ms and pulse 2 at +2.5 ms). In addition, using rectified EMG as done here to determine onsets may slightly obscure the time resolution compared to other methods (Meunier et al. 1990; Condliffe et al. 2016; Ozyurt et al. 2019), and the presence or absence of excitation could further obscure differences since excitatory responses were common for FN but not Q stimulation.

The long duration of inhibition observed for both FN and Q stimulation suggest a contribution from direct activation of axons and mechanical activation of GTOs. Most authors (Meunier et al. 1990; Meunier et al. 1994; Barbeau et al. 2000) have concluded that the long inhibitory duration in humans can be explained primarily by recurrent inhibition based on direct inhibitory recordings in the cat lasting up to about 50 ms with some exceptional cases of 100 ms reported (Eccles et al. 1954). However, more recent evidence in animals (Obeidat et al. 2014) and humans (Ozyurt et al. 2019) has shown that the duration of homonymous recurrent inhibition is much shorter when motoneurons are actively firing with increasing intensity compared to rest, suggesting long duration effects could be limited to rest or during very low contraction levels. Therefore, these findings raise the possibility that the inhibition observed in this study during SOL active contraction of approximately 20% maximal effort could be less influenced by recurrent inhibition than previously assumed. Another consideration we acknowledge but are unable to reconcile in this study are reports

demonstrating that recurrent inhibitory inputs influence motor unit firing synchrony rather than an overt inhibition (Hamm 1990; Obeidat et al. 2014; Edgley et al. 2021). An additional explanation for prolonged inhibition could be from Ib afferent feedback which, as noted above, is expected to persist (i.e. continuously fire) at least until the quadriceps twitch contraction peak which typically occurred after more than 100 ms. Data from the cat indicate mechanical activation of GTO receptors with stretch or twitch contractions can cause heteronymous inhibition recorded as reduced force for at least 100 ms (Wilmink and Nichols 2003; Lyle and Nichols 2019). This inhibition is apparent across varying levels of motoneuron activation and in fact inhibitory magnitude is generally larger onto motoneuron populations with higher levels of recruitment (Wilmink and Nichols 2003; Lyle and Nichols 2018).

Potential Functional utility of using muscle stimulation to examine heteronymous feedback

—Sensorimotor circuitry from muscle spindles, GTOs and Renshaw cells projects extensively between muscles and thus has the ability to influence limb coordination (Sainburg et al. 1995; Akay et al. 2014; Buhrmann and Di Paolo 2014). A current barrier to identifying the unique influence of specific heteronymous circuits is lack of specificity when stimulating a peripheral nerve. The present study provides evidence that muscle stimulation offers an alternative to nerve stimulation for evaluating heteronymous inhibition between human lower limb muscles. Prior studies in the cat and human provide additional support for this noninvasive approach to study heteronymous feedback (Knikou and Conway 2002; Aguiar and Baker 2018; Lyle and Nichols 2019). The muscle stimulation approach as used in this study may help clarify whether exaggerated heteronymous excitation in persons with stroke (Dyer et al. 2014) is due to enhanced effects from muscle spindle feedback and/or reduced heteronymous inhibition. Moreover, modulation of muscle spindle feedback during walking has been shown to differ between soleus H-reflex and stretch evoked responses during some phases (Andersen and Sinkjaer 1999; Thompson et al. 2019), likely due to increased sensitivity of nerve evoked responses to presynaptic inhibition (Morita et al. 1998). These findings may also apply to heteronymous inhibitory responses; therefore, mechanical activation of GTOs from muscle stimulation evoked twitch contractions is expected to increase functional insights over nerve stimulation alone.

We have found several advantages of using muscle stimulation to study heteronymous feedback. First, quadriceps muscle stimulation evoked twitch responses for a given intensity are extremely repeatable over time, whereas caution must be exercised for FN stimulation since small leg movements can make the surface electrodes shift relative to the nerve. In addition, whereas several muscle nerves are not easily accessible (e.g., sciatic) or activate several muscles (e.g., tibial nerve, femoral nerve), muscle stimulation can be applied to nearly any muscle group or individual muscles (e.g., vastus lateralis) in isolation. Thus, when compared to nerve stimulation, we propose the muscle stimulation approach used in the present study can be a feasible and more flexible option to evaluate the functional role of heteronymous inhibition during various locomotor tasks. A potential drawback of muscle stimulation is discomfort at the site of stimulation. A strategy to reduce the likelihood of discomfort is stimulating over motor points (Botter et al. 2011). In this study of healthy young adults, only one participant reported mild discomfort at the highest intensity;

however, there remains a possibility that discomfort could influence the feasibility of using muscle stimulation as described here in populations with more subcutaneous tissue (e.g. older adults) or stimulation of other muscle groups (e.g., triceps surae, Bergquist et al. 2011a).

Methodological considerations: An important procedural consideration is ensuring that heteronymous reflex effects are neural in origin. Similar to previous studies, the ankle-foot was secured to a device that was fixed to the floor which made the knee and ankle mechanically coupled. Thus, the dependent variable of ongoing SOL EMG could be influenced by knee extension. In the present study, a pad was firmly placed against the tibia to prevent the knee from extending due to the FN and Q muscle stimulation evoked twitch responses. Even though the excitatory and inhibitory heteronymous interactions (e.g., latency, durations) observed in the present study were consistent with prior reports suggesting the findings were neural, we had 3 participants repeat the experiment with an ankle immobilizer which effectively decoupled the ankle and knee. We found remarkably similar excitatory and inhibitory onset and magnitudes between the ankle fixation methods providing strong support for a neural origin for the heteronymous effects in this study.

Nerve and muscle stimulation are both noninvasive, feasible approaches to evoke heteronymous feedback between muscle groups. An open question remains regarding the extent to which the stimulation methods used and the effects recorded reflect actual behavior or a version of network capacity. A fundamental reality is that activation of muscle spindle, Ib and motor axons via electrical stimulation is resulting in non-physiological, synchronous inputs to the spinal cord. Therefore, the strength of any nerve stimulation evoked effect is expected to differ, perhaps markedly, from movement related afferent or motor firing as has been previously noted (e.g., Morita et al. 1998; Andersen and Sinkjaer 1999). The stimulation evoked twitch contractions used in this study are expected to cause a more naturalistic physiological firing of GTOs with due consideration that muscle stimulation evoked twitch contractions differ in some ways to natural contractions (e.g., recruitment order) (Bergquist et al. 2011b).

Further study is needed to clarify several factors not addressed in this study. Examining the influence of longer muscle stimulation pulse widths on heteronymous responses would be helpful in the future to determine to what extent the short pulse width Q stimulation and/or location (muscle vs nerve) reduced the chance of excitatory responses observed in the present study. The goal here was to examine intensities above motor threshold though evaluating below motor threshold would additionally provide insight about activation of sensory axons in isolation. The present study was completed in healthy young adults. Future work is needed to determine if the results generalize to older adults and patient populations. Lastly, this study identified similar inhibition magnitudes evoked by Q and FN stimulation, but this study was not designed to identify the origin of inhibition. Identifying the afferent pathways responsible, and whether the relative contribution differs between muscle and nerve stimulation, would be an important contribution necessary to clarify the functional role in health and disease.

Conclusions

This study examined the differential effects of evoking heteronymous feedback by stimulating a peripheral nerve and transcutaneous muscle stimulation. We found that the frequency and magnitude of heteronymous excitation was much reduced for muscle stimulation when compared to nerve stimulation. Muscle and nerve stimulation resulted in similar magnitudes of heteronymous inhibition that scaled with increasing stimulation evoked torque magnitudes. This study provides novel evidence that muscle stimulation can be used to selectively examine inhibitory heteronymous feedback between muscles in the human lower limb compared to nerve stimulation.

Acknowledgements

The authors wish to thank Dr. T. Richard Nichols for stimulating discussions and helpful suggestions on the manuscript. The authors also thank Ignacio Novoa for creating Figure 5, and DPT students who helped during the data collection sessions.

Funding

Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number K01HD100588 and 1R01HD095975-01A1, as well as National Institute of Neurological Disorders and Stroke awards 5U01NS086607-05, U01NS166655, and 1U01NS102353-01. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Data availability

The data from the current study are available from the corresponding author on reasonable request.

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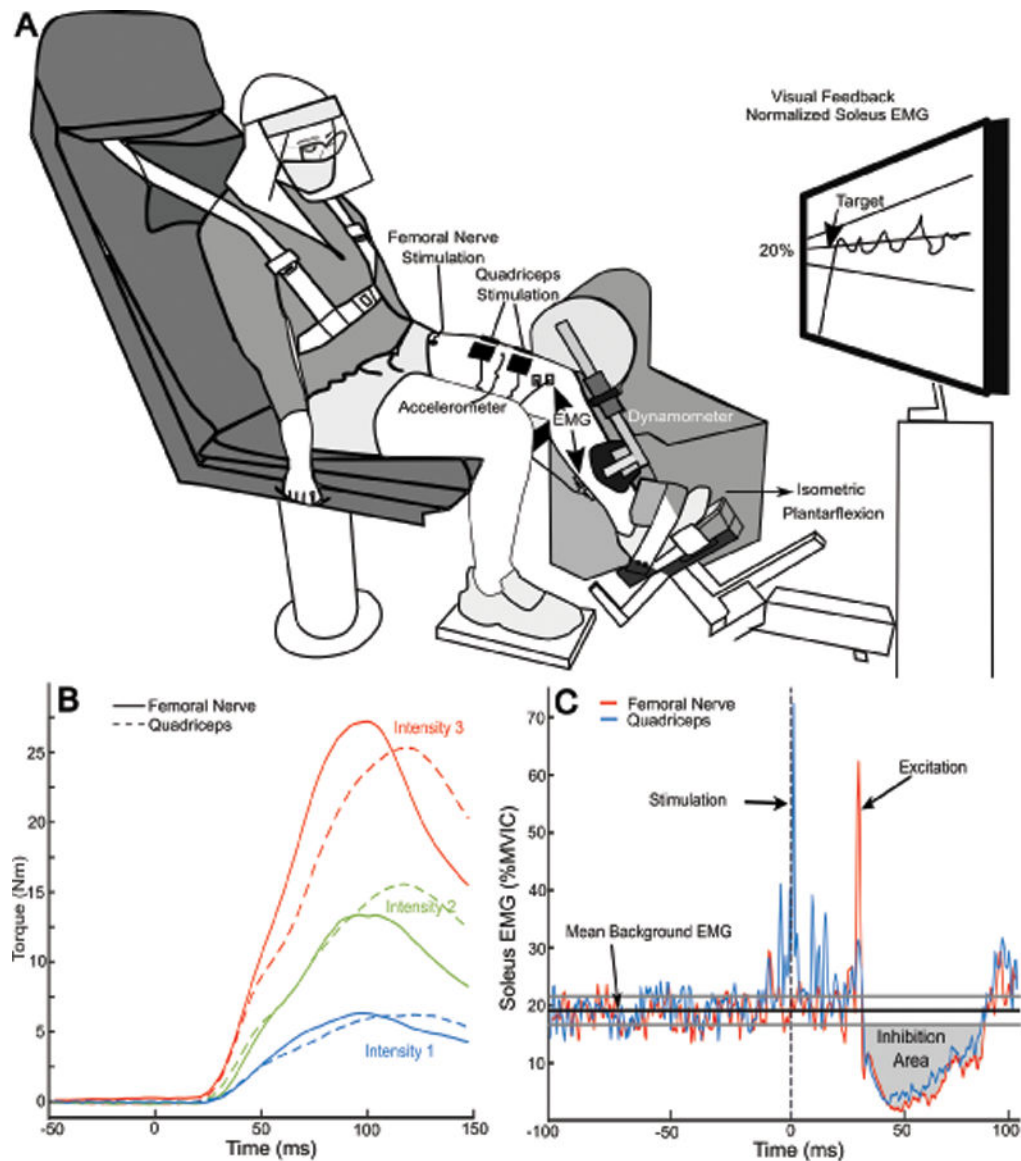


Figure 1.

Experimental set-up, stimulation-torque response, and data analysis

A) Schematic of the experimental set-up and EMG feedback. **B)** Torque-time profiles from a participant across 3 stimulation intensities for femoral nerve (solid lines) and quadriceps stimulation (dashed lines). Stimulation was applied at time 0. The torque recordings were completed with the knee at 90° degrees. **C)** Example traces representing the average-rectified SOL EMG from 35 trials from a single participant illustrating the heteronymous effects from femoral nerve and quadriceps stimulation onto ongoing SOL EMG. The solid black horizontal line represents the mean SOL EMG during a 400 ms period prior to stimulation (feedback target was 20% MVIC). The solid grey horizontal lines represent 1 SD above and below the mean background SOL EMG. Excitatory onset was determined as the SOL EMG trace exceeding the mean + 1 SD for 2 ms and excitatory end when returning below the mean + 1 SD line for 2 ms. Only excitatory traces with onset 23

ms after stimulation were considered as arising from Ia feedback (Hultborn et al. 1987; Meunier et al. 1990) (e.g., muscle stimulation caused stimulation artifacts in some cases as shown in the example). The area (shown in grey for inhibition area only on the figure was calculated using trapz in matlab and referenced to the mean background EMG) and duration of excitation were calculated using the onset and end time points. The same analyses were completed for inhibition using mean – 1SD.

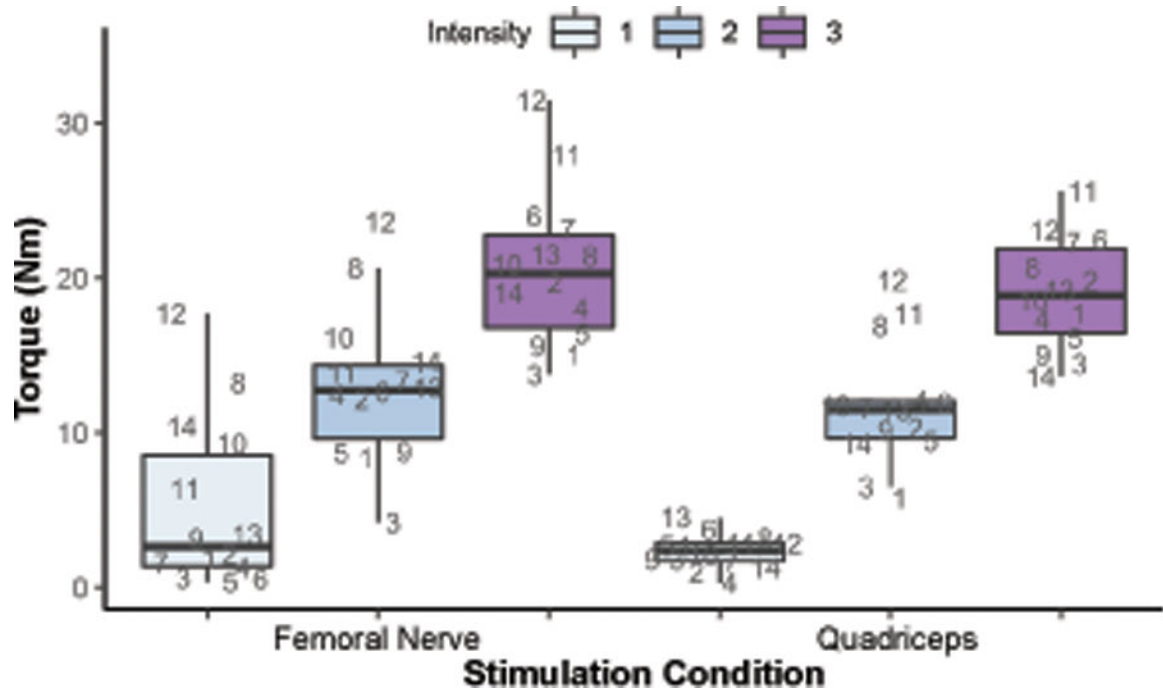


Figure 2. Femoral nerve and quadriceps stimulation evoked torque responses across intensities. The box plot indicates the distribution of torque magnitudes for each intensity. Each participant is shown as a number.

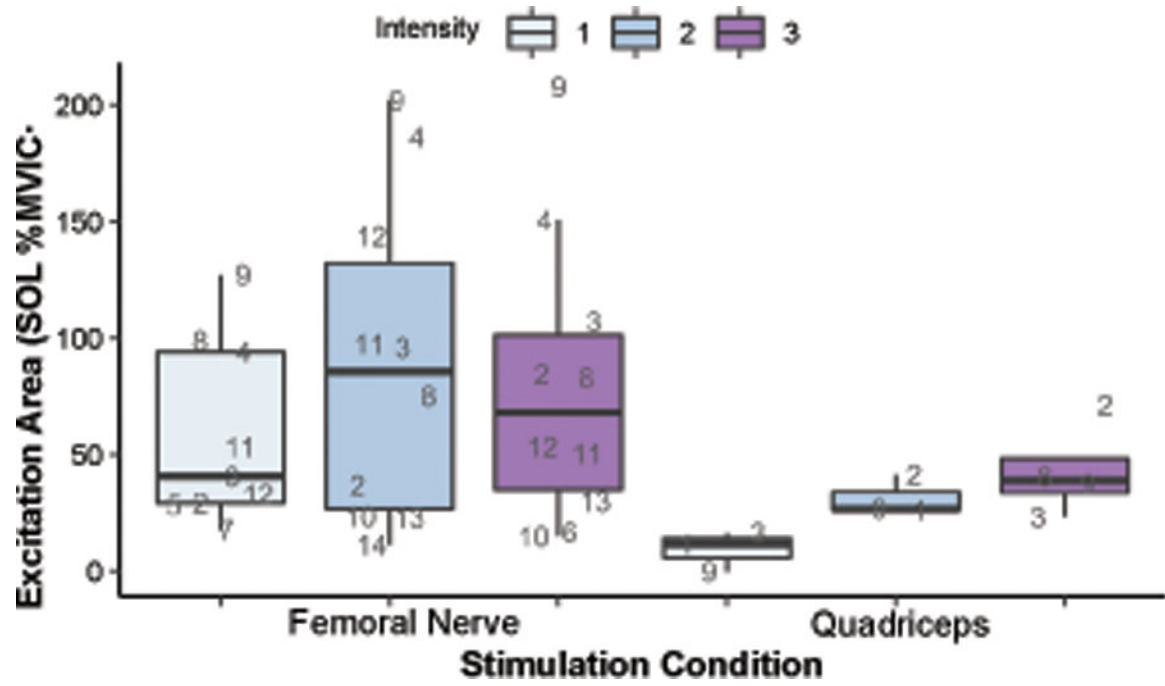


Figure 3. Heteronymous excitation from femoral nerve and quadriceps stimulation onto SOL across the three stimulation intensities. The box plot indicates the sample data distribution for each intensity, and the numbers represent each participant.

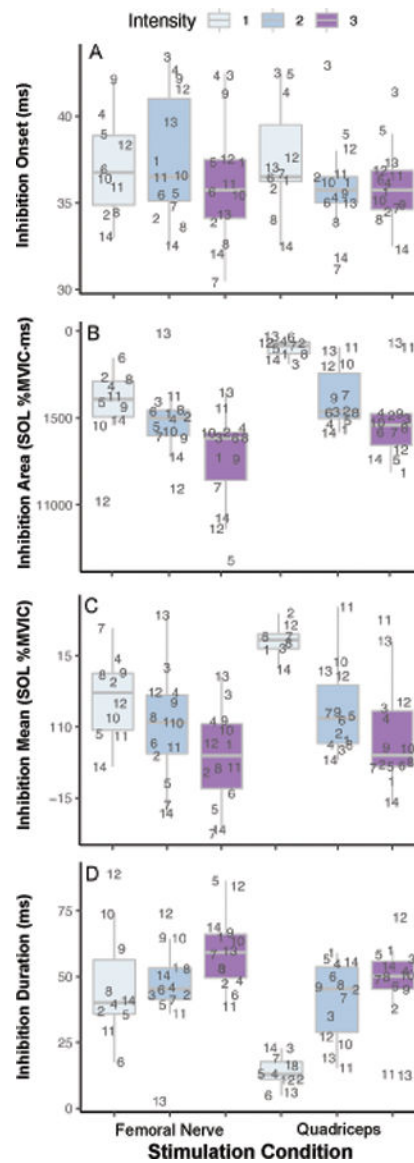


Figure 4.

Heteronymous inhibition onset, magnitude, and duration from femoral nerve and quadriceps stimulation onto SOL across the three stimulation intensities. **A)** Inhibition onset was determined as the time when SOL EMG went 1 SD below the mean background SOL EMG. The magnitude of heteronymous inhibition expressed as **B)** EMG area (%MVIC·ms) and **C)** as the mean background subtracted SOL EMG during a window from 35–73 ms after stimulation. **D)** The duration of heteronymous inhibition was determined as period that EMG went 1 SD below the mean background SOL EMG for 2 ms and returned above the 1 SD mean background EMG line for 2 ms. The box plots indicate the sample data distribution for each intensity and condition, and the numbers represent each participant.

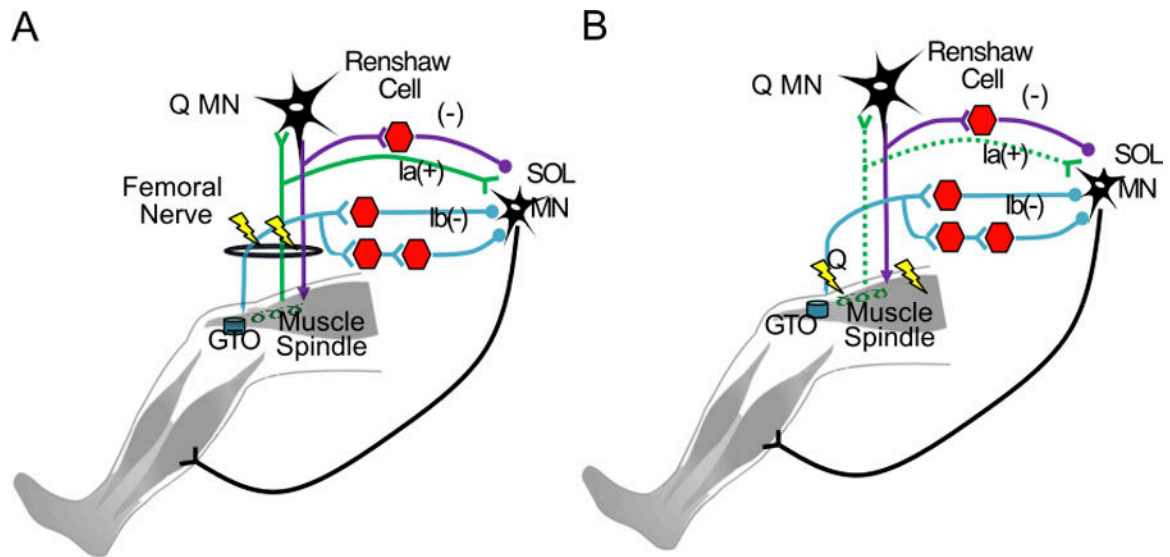


Figure 5.

Hypothesized heteronymous spinal circuit pathways involved during femoral nerve and quadriceps stimulation onto ongoing soleus EMG. A. During femoral nerve stimulation, activation of Ia axons (green lines) provide the most parsimonious explanation for heteronymous excitation. Activation of Ib axons (blue lines) and motor axons (purple line) acting via the Renshaw cell as recurrent inhibition are the most likely inputs responsible for heteronymous inhibition. The motor evoked twitch contraction is presumed to have additionally provided inhibitory feedback by mechanically activating the Golgi tendon organs.

B. Quadriceps stimulation with surface electrodes resulted in decreased frequency and magnitude of excitatory responses suggesting reduced activation of Ia axons (green dotted line) compared to femoral nerve stimulation and thus presumably also reduced direct activation of Ib axons. The most parsimonious explanation for heteronymous inhibition was Ib afferent firing (blue lines) due to the twitch contraction and recurrent inhibition due to motor axon antidromic inputs acting via Renshaw cells (purple line). Q: quadriceps; SOL: soleus; GTO: Golgi tendon organ; MN: motoneuron

Table 1.

Temporal characteristics of muscle contractions evoked across condition and stimulation intensities. The time of twitch onset is an estimate of when GTO receptors are mechanically activated and time of peak torque an estimate of the minimum duration that GTO receptors are expected to be firing.

	Intensity 1	Intensity 2	Intensity 3
Femoral N Stim: peak torque time (ms)	114.2 ± 19.5	103.9 ± 14.9	98.2 ± 11.5
Quadriceps stim: peak torque time (ms)	113.6 ± 8.9	116.2 ± 12.5	112.8 ± 12.0
Femoral N stim: VL twitch onset (ms)	17.6 ± 8.2	13.2 ± 7.1	9.5 ± 2.2
Femoral N stim: VM twitch onset (ms)	18.3 ± 11.2	12.6 ± 3.4	11.4 ± 2.4
Quadriceps stim: VM twitch onset (ms)	9.6 ± 3.2	5.6 ± 2.2	4.8 ± 2.4
Quadriceps stim: VL twitch onset (ms)	10.7 ± 5.9	6.3 ± 3.9	5.9 ± 4.0

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