Corticospinal excitability remains unchanged in the presence of residual force enhancement and does not contribute to increased torque production

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Abstract

Following active muscle stretch, muscle force is enhanced, which is known as residual force enhancement (rFE). As earlier studies found apparent corticospinal excitability modulations in the presence of rFE, this study aimed to test whether corticospinal excitability modulations contribute to rFE. Fourteen participants performed submaximal plantar flexion stretch-hold and fixed-end contractions at 30% of their maximal voluntary soleus muscle activity in a dynamometer. During the steady state of the contractions, participants either received subthreshold or suprathreshold transcranial magnetic stimulation (TMS) of their motor cortex, while triceps surae muscle responses to stimulation were obtained via electromyography (EMG), and net ankle joint torque was recorded. B-mode ultrasound imaging was used to confirm muscle fascicle stretch during stretch-hold contractions in a subset of participants. Following stretch of the plantar flexors, an average rFE of 7% and 11% was observed for contractions with subthreshold and suprathreshold TMS, respectively. 41-46 ms following subthreshold TMS, triceps surae muscle activity was suppressed by 19-25%, but suppression was not significantly different between stretch-hold and fixed-end contractions. Similarly, the reduction in plantar flexion torque following subthreshold TMS was not significantly different between contraction conditions. Motor evoked potentials, silent periods and superimposed twitches following suprathreshold TMS were also not significantly different between contraction conditions. As TMS of the motor cortex did not result in any differences between stretch-hold and fixed-end contractions, we conclude that rFE is not linked to changes in corticospinal excitability, making rFE a muscle mechanical property rather than a combined neuro-muscular property.

Keywords: eccentric contraction, active stretch, neural control, torque enhancement, inhibition, transcranial magnetic stimulation

Introduction

It is well known that stretch of an active muscle results in increased force production during the isometric steady state following stretch compared with the steady-state force produced at the same muscle length and activation level during a fixed-end contraction. This is referred to as residual force enhancement (rFE), which was initially investigated *in situ* (*Abbott & Aubert*, 1952). Decades later, rFE was investigated in isolated muscle fibres and two opposing mechanisms were suggested: (1) that rFE following active muscle stretch results from non-uniformities in sarcomere lengths (*Julian & Morgan*, 1979), and (2) that rFE is due to the recruitment of a parallel non-contractile element during active stretch (*Edman, Elzinga & Noble*, 1978). Since then, numerous studies have investigated the development of rFE and suggested additional potential underlying mechanisms. These suggestions include stretch-induced increases in the number of cross-bridge attachments and/or the attachment of the second myosin head, and an increase in the average cross-bridge force and strain (*Brunello* et al., 2007; *Rassier*, 2012; *Herzog*, 2014). Lately, the recruitment of a parallel non-contractile element during active stretch has been related to titin, which might increase its force contribution in the presence of calcium and by interacting with actin (*Nishikawa*, 2016; *Herzog*, 2018; *Freundt & Linke*, 2019).

Complementary to *in vitro* research, several *in vivo* studies have investigated rFE (as approximated by net joint torque) during electrically-stimulated and voluntary contractions (*Seiberl, Power & Hahn*, 2015; *Chapman* et al., 2018). rFE during voluntary contractions has been observed for small muscles of the thumb (Lee and Herzog, 2002), for large muscles of the lower limb (*Pinniger & Cresswell*, 2007; *Seiberl* et al., 2010), and during multi-joint multi-muscle contractions of the lower limbs (*Hahn* et al., 2010). The potential relevance of rFE during human movement was demonstrated in studies that investigated rFE during

submaximal voluntary contractions and at joint angle configurations that mimicked those of human locomotion (*Hahn* et al., 2010; *Seiberl* et al., 2013; *Paternoster* et al., 2016).

Besides rFE, several *in vivo* studies have documented an activation reduction (AR) during the steady state following active muscle stretch. In contrast to rFE, which is observed when muscle activity is matched between stretch-hold (STR) and fixed-end reference (REF) contractions, AR occurs when force or torque is matched between contraction conditions. AR refers to a reduced muscle activity level needed to maintain a given force/torque following active muscle stretch compared with fixed-end contractions at the same final muscle length. Several studies have reported AR and concluded that neuromuscular efficiency following active muscle stretch is increased, which might be explained by enhanced passive forces due to increased titin forces (*Oskouei & Herzog*, 2005; *Altenburg* et al., 2008; *Seiberl* et al., 2012; *Joumaa & Herzog*, 2013; *Jones, Power & Herzog*, 2016; *Mazara* et al., 2018; *Paquin & Power*, 2018). However, *Paquin & Power* (2018) also found a rightward shift in the EMG-torque relationship following active stretch compared with fixed-end reference conditions, which indicates that the neuromuscular activation strategy might be altered in the presence of rFE.

Changes in EMG following active stretch have motivated a number of studies to investigate the neural control and/or neural modulations that occur during the isometric steady state following active muscle stretch (*Altenburg* et al., 2008; *Hahn* et al., 2012; *Paquin & Power*, 2018; *Sypkes* et al., 2018; *Contento, Dalton & Power*, 2019). For example, *Altenburg* et al. (2008) examined single motor unit behaviour of the vastus lateralis muscle during AR following active muscle stretch. These authors found similar discharge rates of VL motor units between STR and REF conditions, which led them to conclude that a derecruitment of motor units might have occurred during AR (Altenburg et al. 2008).

Two further studies used motor evoked potentials (MEPs) and cervicomedullary motor evoked potentials (CMEPs) to investigate excitability modulations of cortical and spinal sites in the presence of rFE (*Hahn* et al., 2012; *Sypkes* et al., 2018). In the first study, *Hahn* et al. (2012) found larger MEPs and larger V-waves, but unchanged CMEPs following stretch of the plantar flexors during maximal voluntary contractions. Based on the enlarged MEPs and unchanged CMEPs, it was interpreted that cortical excitability increased, but spinal excitability was unchanged in the rFE state. The enlarged V-waves were considered to represent greater cortical motoneuronal output and/or an increase in spinal stretch reflex excitability following active muscle stretch (*Hahn* et al. (2012). In the second study, *Sypkes* et al. (2018) found smaller CMEPs and an unchanged MEP/CMEP ratio in the presence of rFE during submaximal dorsiflexion contractions, which they interpreted as reduced spinal excitability with no change in cortical excitability. Therefore, the only two studies on corticospinal excitability suggest opposing cortical and spinal modulations in the presence of rFE. While this could be attributed to the different muscles and contraction levels tested, the findings from both studies should be considered with caution for reasons outlined below.

Based on supplementary material provided by *Sypkes* et al. (2018), their data was reanalysed to assess differences in MEP amplitudes when normalised to the maximal M-wave (M_{max}) obtained from the same contraction condition. This analysis, which was not performed by the authors, revealed unchanged normalised MEPs in the presence of rFE when compared with the fixed-end reference contractions (Fig. 1). As MEPs represent both spinal and cortical excitability, the combined results of their reduced CMEPs and unchanged MEPs should be interpreted as reduced spinal excitability (as *Sypkes* et al. 2018 did) and increased cortical excitability, which does not align with the authors interpretation of unchanged cortical excitability. Taken together, both studies on neural excitability (*Hahn* et al. 2012 and *Sypkes* et al. 2018) indicate increased cortical excitability in the presence of rFE.

Moreover, the two studies share some limitations that need to be considered. These limitations include that the observed changes in CMEPs (*Sykpes* et al. 2018) and MEPs (*Hahn* et al. 2012) while significant, were rather small. Further, only a few responses (i.e. MEPs) to transcranial magnetic stimulation (TMS) were averaged (n = 6 in *Hahn* et al. 2012 and n = 4 in *Sypkes* et al. 2018), although it is recommended to average a minimum number of ten MEPs, but as many as 20 MEPs for excellent intra- and inter-session reliability (*Biabani* et al., 2018). Finally, the sample sizes of the two studies were rather small (n = 10 and n = 11, respectively), and so far, neither of these results have been replicated. Therefore, a secondary re-analysis was performed where the data from the two studies were pooled (n = 29; n = 11 for tibialis anterior from *Sypkes* et al. 2018 and n = 9 for each, soleus and medial gastrocnemius, from *Hahn* et al. 2012). This re-analysis revealed no significant change in CMEPs ($p = 0.344$, -0.019 ± 0.107 mean difference) and no significant change in MEPs ($p = 0.339$, -0.018 ± 0.1 mean difference) in the presence of rFE compared with the fixed-end reference contractions (Fig. 1). Accordingly, we think that corticospinal excitability is unaltered in the presence of rFE.

Therefore, the aim of this study was twofold. First, we wanted to partly replicate the above-mentioned studies and investigate whether we could observe corticospinal excitability changes in the presence of rFE. Second, this study was designed to investigate whether cortical inhibition affects force production in the presence of rFE differently to fixed-end reference contractions. This was examined by activating and inhibiting the motor cortex via transcranial magnetic stimulation (TMS) in the presence (STR) and absence (REF) of rFE.

Based on a critical evaluation and re-analysis of previous research (*Hahn* et al., 2012; *Sypkes* et al., 2018), we predicted that the activation of cortical interneurons and pyramidal neurons by suprathreshold TMS (*Rothwell*, 1997; *Rossini* et al., 2015) would result in similar MEPs and superimposed twitches in the presence of rFE compared with the fixed-end reference

contractions with matched background muscle activity. Further, we predicted that inhibiting motor cortical neurons by subthreshold TMS (*Davey* et al., 1994; *Petersen* et al., 2001; *Zuur* et al., 2010) would lead to a similar suppression in muscle activity (EMG) and plantar flexion torque in the presence of rFE compared with fixed-end reference contractions with matched background muscle activity. If both predictions hold, the results would indicate that corticospinal excitability is not altered and does not contribute to the increased torque production in the presence of rFE.

Materials and methods

Participants

Fourteen recreationally-active participants (six women, 26.7 ± 5.3 yrs., 1.77 ± 0.11 m, and 74.0 ± 16.8 kg) voluntarily participated in this study after providing free written informed consent. Participants were free of any neuromuscular disorders and injuries to their right lower limb. The experimental protocol was approved by the local Ethics Committee of the Faculty of Sport Science at Ruhr University Bochum, Germany.

Experimental setup

During the experiment, participants laid prone on a dynamometer bench with their upper body supported by their forearms and their right foot tightly strapped onto the footplate attachment of a dynamometer (IsoMed 2000, D&R Ferstl GmbH, GER). The axes of rotation of the ankle joint and the dynamometer were aligned prior to testing and the foot was firmly secured to the footplate using three straps (forefoot, ankle, heel) to minimize heel lift during the plantar flexion contractions. Additionally, the participants' hips were secured to the dynamometer bench with a belt. A computer monitor positioned in front of the participants was used to provide visual feedback of their soleus muscle activity (moving 0.25-s root-mean-square amplitude calculation) during the plantar flexion contractions. Net ankle joint torques and

ankle joint angles of the right leg were sampled at 1 kHz using a 16 bit Power 3 1401 and Spike2 data collection system (v.8.01, Cambridge Electronics Design, Cambridge, UK)

Surface electromyography

Muscle activity and responses to TMS from soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles were recorded via bipolar surface electromyography (EMG). EMG of the antagonistic tibialis anterior (TA) was not obtained as it has been shown that TA EMG during fixed-end plantar flexion is more likely from crosstalk than coactivation (*Raiteri, Cresswell & Lichtwark*, 2015). After skin preparation, self-adhesive surface electrodes (Ag/ AgCl, Kendall, ECG Electrodes, 8 mm recording diameter) were positioned with an inter-electrode distance of 20 mm over the triceps surae muscle bellies following the recommendations of SENIAM (Hermens et al., 1999). A reference electrode was placed over the right fibular head. Cables were fixed to the skin with tape to prevent motion artefacts. EMG signals were amplified 1000 times by a multichannel analogue amplifier (AnEMG12, Bioelectronica, Turin, IT) and band-pass filtered between 10 Hz and 4.4 kHz, prior to being sampled at 5 kHz. EMG and torque/angle data were synchronised and sampled using the same analogue-to-digital converter and software described above.

Transcranial Magnetic Stimulation (TMS)

TMS (MagPro Compact, MagVenture, Farum, Denmark) was used to either inhibit or activate the motor cortical area of the right plantar flexor muscles in the left hemisphere, slightly left of the vertex, via a double-cone coil (D-B80 Butterfly Coil, MagVenture, Farum, Denmark). The coil had monophasic current running through its centre in an anterior-posterior direction. The vertex was marked on the scalp and defined as halfway between the left and right processus zygomaticus ossis temporalis and halfway between the os nasale and the external occipital protuberance. The vertex location helped to find the optimal location for TMS of the motor cortical area innervating the right plantar flexor muscles (TMS hotspot), which is

generally defined as the position in which a single stimulation evokes the largest peak-to-peak MEP amplitude in the target muscle (Siebner and Ziemann, 2007). In order to find the TMS hotspot, several stimuli were delivered while the TMS coil was slightly left, in front or behind the vertex, while participants performed fixed-end plantar flexion contractions at 30% of their maximal voluntary activity (MVA) as measured during maximal voluntary fixed-end contractions (see experimental protocol). Once the TMS hotspot was located, the wings of the coil were marked on the scalp with a semi-permanent marker. Subthreshold and suprathreshold intensities were then determined by either decreasing or increasing the stimulator output. To achieve a suppression of soleus muscle activity, stimulator output was reduced in small increments until the active motor threshold (AMT) was reached. This threshold was defined as the point when stimulation during 30% MVA resulted in visible MEP responses in only five of ten consecutive trials (*Petersen* et al., 2001). Once the AMT was determined, stimulation intensity was slightly reduced again to 34 ± 3 % of maximal stimulator output so that MEPs were no longer elicited. For activation of the motor cortex, stimulator output was increased to 58 ± 9 % of maximal stimulator output until MEPs were clearly visible in comparison to the immediately preceding background EMG in at least five consecutive trials.

Ultrasound imaging

Muscle fascicle behaviour of the MG from three participants was examined during familiarisation sessions using B-mode ultrasound imaging (LS128 CEXT-1Z, Telemed, Vilnius, Lithuania) to ensure muscle fascicle stretch occurred during ankle rotation in the stretch-hold condition. Ultrasound images were recorded using a flat, linear, 128□element transducer (LV7.5/60/128Z□2, 6 MHz, 60 x 50 mm (width x depth)) operating at ~60 fps in EchoWave II software (Telemed, Vilnius, Lithuania). The transducer was placed on the skin over the MG mid-muscle belly in the longitudinal plane and rotated to obtain a clear image

with continuous aponeuroses and muscle fascicles. The position of the probe was marked on the skin to ensure consistent placement during the stretch-hold contractions.

Contraction conditions

The experiment involved participants performing submaximal plantar flexion contractions at 30% MVA. The background activity level was controlled throughout the contractions by having participants visually match their SOL EMG amplitude (moving 0.25-s root-mean-square (RMS) amplitude calculation) to a horizontal cursor on a computer monitor. The conditions involved fixed-end reference contractions (REF) at 20° dorsiflexion (DF) and active stretch-hold contractions (STR) from 0°-20° DF (0° refers to the sole of the foot being perpendicular to the shank).

All contractions started with a 2-s linear ramp from 0-30% MVA. Following the ramp, the isometric steady state during REF was maintained for 13-s. During STR, the ramp was followed by a 1-s isometric steady state at the initial ankle joint angle (0° DF), before the plantar flexor muscles were actively stretched to an ankle joint angle of 20° DF at an angular velocity of $30°s^{-1}$. Following active stretch, participants maintained the subsequent isometric steady state for ~11-s, resulting in an overall contraction duration of 15-s (Fig. 2).

Experimental protocol

Participants attended two sessions on two different days. In the first session, participants were familiarised with the test protocol and trained to perform maximal voluntary fixed-end contractions (MVC) and the submaximal contractions described above. Additionally, participants were familiarised with TMS.

The second session consisted of the test protocol (Fig. 3). After a short warm-up (eight submaximal plantar flexion contractions with increasing torque), at least two MVCs were performed to determine 100% MVA. Participants were instructed to push their forefoot into the footplate as hard as possible and to maintain the contractions so that a torque plateau was

clearly visible. MVC torque was calculated as the difference between peak torque during the contraction and the mean baseline over 500 ms prior to the beginning of contraction. To ensure that the MVCs were repeatable, peak-to-peak torques were required to be within a 5% range. 100% MVA was determined as the smoothed (moving 0.25-s RMS amplitude calculation) SOL EMG amplitude at peak MVC torque from the MVC with the highest peak-to-peak torque. Three minutes of rest was provided between MVCs to minimise fatigue.

Following the MVCs, the TMS hotspot and the subthreshold and suprathreshold TMS intensities were determined during sustained fixed-end contractions at 30% MVA. Once hotspot and stimulation intensities were determined, 100 subthreshold stimulations were delivered for each contraction condition to study motor cortex inhibition. For this purpose, contractions from each condition (i.e. STR or REF) were separated into ten sets with ten TMS (1 Hz) during the isometric steady state phase of the contractions at 20° DF starting 5-s following contraction onset (Fig. 2). Contractions were randomised and a minimum of three minutes rest was provided between each set. Activation of the motor cortex was investigated by providing 20 suprathreshold stimulations per contraction condition. Suprathreshold stimulations were delivered at the same time points as the subthreshold stimulations, but participants performed only two contractions per condition. The contractions with suprathreshold TMS were always performed after all sets of the subthreshold stimulations had been completed. During all contractions, trials were excluded and repeated if participants could not maintain their soleus EMG activity within 25-35% MVA.

Data analysis

Residual Force Enhancement (rFE)

rFE for the subthreshold and suprathreshold conditions was determined by calculating the difference between the isometric steady-state torque during the STR and REF conditions. Net plantar flexion torque was averaged across contractions for each contraction condition and

mean rFE was calculated during the isometric steady state at 20° DF, from 500-990 ms after each TMS stimulus. While most rFE studies have analysed torque or force with a 500 ms time window, we excluded the final 10 ms to ensure that the stimulations delivered at 1 Hz would not affect our analysis. Stimulations delivered before the isometric steady state of SOL muscle activity were excluded. Participants were excluded from further analysis when their torque following stretch was smaller compared with their torque during the fixed-end reference contractions (i.e. when there was no rFE).

TMS inhibition

Suppression of muscle activity (SOL, MG and LG) following subthreshold TMS was determined via averaged rectified raw EMG signals from each muscle and contraction condition. Waveform averages were calculated over a time window of 100 ms after stimulation and again, stimulations delivered before the isometric steady state of SOL muscle activity were excluded. The latency and duration of EMG amplitude suppression were determined using the evaluation methods by *Petersen* et al. (2001) and *Zuur* et al. (2010). The onset of EMG amplitude suppression was marked when the EMG amplitude first dropped under the background EMG amplitude (mean value calculated 15–25 ms following stimulation) for at least 4 ms. The offset of EMG amplitude suppression was calculated when the EMG amplitude rose above the background EMG amplitude for at least 1 ms (*Zuur* et al., 2010) (Fig. 4). The mean EMG amplitude between the onset and offset of suppression was calculated and compared with the background EMG amplitude right before suppression.

Torque production following TMS-induced EMG amplitude suppression was analysed via moving correlations (50-ms time intervals) between the torque of the STR and REF conditions. A reduction in torque due to TMS-induced EMG amplitude suppression was identified when torque data of REF and STR contractions was correlated ($r \geq 0.7$, i.e. a very large effect). This data analysis was based on the random fluctuations in torque steadiness that

should result in non-correlated torque signals. However, when torque was affected by the TMS-induced EMG amplitude suppression in a similar manner, large positive correlations were expected. Once the time window of torque reduction was identified, the torque offset between the REF and STR conditions was removed by subtracting the mean torque difference between REF and STR over the first 10 ms after the onset of torque reduction. Finally, the mean torque during the period of TMS-induced torque reduction (i.e. during the times where $r \geq 0.7$) was calculated for REF and STR and compared between contraction conditions.

TMS activation

MEPs of SOL, MG and LG following suprathreshold TMS were calculated as peak-to-peak amplitudes from the raw EMG signals and averaged across contractions for each muscle and contraction condition (Lewis et al., 2014) (Fig. 5). Additionally, the silent period (SP) duration was analysed for both contraction conditions as the time from stimulation to the end of the SP. The end of the SP was defined as the time when the EMG signal crossed the threefold standard deviation (SD) of the SP raw EMG following the MEP for the first time (Fig. 5). SD during the SP was determined as the smallest SD of the raw EMG over a moving 30-ms window. The sizes of superimposed twitch torques following suprathreshold TMS were calculated separately as the difference in torque between the peak of the twitch and the torque at the time of stimulation and averaged for each contraction condition.

Statistical analysis

Paired t-tests or Wilcoxon signed-rank tests, based on the normality of paired differences as assessed by a Shapiro-Wilk test, were used to test for significant differences (alpha level of 0.05) in triceps surae muscle activity, plantar flexion torque, EMG amplitude suppression, torque reduction, MEP amplitude, SP duration and superimposed twitch torque amplitude between REF and STR conditions. Statistical analysis was performed using Prism 9 software (GraphPad, USA). Values are presented as mean ± SD in the text.

Results

Five and four participants were excluded from further analysis as they did not show rFE following the subthreshold (n=9) and suprathreshold (n=10) stimulations, respectively.

Contraction conditions

During both contraction conditions, participants largely managed to maintain a constant level of muscle activity (~30% MVA). EMG of SOL, MG and LG did not significantly differ between STR and REF conditions during the isometric steady state of contractions with suprathreshold TMS (SOL: P = 0.535, MG: P = 0.492, LG: P = 0.351). However, for subthreshold TMS, while EMG of SOL or LG was not significantly different between conditions (SOL: P = 0.476, LG: P = 0.245), EMG of MG was significantly higher in the REF compared with STR condition (P = 0.012; see Supplementary Fig. 1). Ultrasound imaging confirmed that the muscle-tendon unit stretch during the 20° dynamometer rotation of the stretch-hold contraction resulted in fascicle stretch of the MG muscle at 30% MVA (n=3).

Contractions with subthreshold TMS

The isometric steady-state net plantar flexion torque in the STR condition exceeded the time-matched steady-state torque in the REF condition (STR: 105.9 ± 34.5 Nm; REF: 99.0 ± 33.4 Nm, P = 0.002), which resulted in mean rFE of 7.3 ± 4.2% (Fig. 6A).

Subthreshold stimulation of the motor cortex led to significant (P < 0.001) EMG amplitude suppression for SOL, MG and LG without any significant difference between STR and REF conditions (SOL: P = 0.970; MG: P = 0.753; LG: P = 0.802). The mean EMG amplitude suppression ranged between 19-25% of the background EMG amplitude, it occurred 41-46 ms after stimulation, and it lasted 10-15 ms in total (Table 1). After accounting for the torque

offset between the REF and STR conditions, the net plantar flexor torque following EMG amplitude suppression did not significantly differ (P = 0.228) between STR and REF conditions.

Contractions with suprathreshold TMS

The isometric steady-state net plantar flexion torque in the STR condition exceeded the time-matched steady-state torque in the REF condition (STR: 89.5 ± 26.0 Nm; REF: 82.2 ± 27.2 Nm, P = 0.001), which resulted in mean rFE of 10.8 ± 10.0 % (Fig. 6A). For all muscles analysed (SOL, MG, LG), MEP and SP following suprathreshold TMS did not significantly differ between STR and REF conditions (MEP: SOL: P = 0.304, MG: P = 0.652, LG: P = 0.453, Fig. 6B; SP: SOL: P = 0.325, MG: P = 0.286, LG: P = 0.359, Table 2). TMS-evoked superimposed twitches following suprathreshold stimulations were 9.1 ± 3.6 Nm (STR) and 8.6 ± 3.5 Nm (REF), and did not significantly differ between STR and REF conditions (P = 0.131, Fig. 6C).

Discussion

The aims of this study were to determine whether corticospinal excitability is modulated in the presence of rFE and to assess whether an inhibition of motor cortical neurons affects muscle activity and the increased torque production in the presence of rFE compared with fixed-end reference contractions without rFE. To achieve this, we compared reductions in EMG activity and torque production following inhibition of motor cortical neurons, and MEPs, SPs and superimposed twitch torques following activation of motor cortical neurons between submaximal stretch-hold and fixed-end contractions.

Participants largely managed to keep muscle activity constant (30% MVA) throughout the fixed-end reference contractions and during the isometric steady state of the stretch-hold contractions. As the observed differences in MG background EMG were rather small (2%

MVA), they are considered negligible regarding the interpretation of the results. Mean rFE magnitudes of 7% (subthreshold TMS) and 11% (suprathreshold TMS) were observed during the steady state in STR compared with REF, which is in line with former studies (*Oskouei & Herzog*, 2005; *Pinniger & Cresswell*, 2007; *Seiberl* et al., 2013; *Paternoster* et al., 2016). Inhibition of motor cortical neurons by subthreshold TMS caused reductions in EMG activity and net plantar flexion torque, however the reductions did not differ significantly between contraction conditions. Similarly, MEP amplitudes, SP durations and superimposed twitch torque amplitudes evoked by suprathreshold TMS were not significantly different between STR and REF conditions.

Following subthreshold TMS, we found a suppression of triceps surae muscle activity by 19-24% relative to the background activity. This is similar to the ~15% EMG amplitude suppression in SOL induced by subthreshold TMS during walking and jumping (*Petersen* et al., 2001; *Zuur* et al., 2010), but smaller compared with the 50% suppression in hand and arm muscles during voluntary fixed-end contractions (*Davey* et al., 1994). Also, the latency and the duration of the observed EMG amplitude suppression was similar to values reported previously (*Petersen* et al., 2001; *Zuur* et al., 2010). Importantly, the TMS-induced EMG amplitude suppression showed no significant difference between STR and REF conditions, which also resulted in similar magnitudes of torque reduction for both contraction conditions. This supports our prediction that inhibiting motor cortical neurons by subthreshold TMS would not affect the STR and REF conditions differently.

Our data also supports our prediction that suprathreshold TMS does not elicit larger MEP amplitudes and SP durations, or larger superimposed twitch torque amplitudes in the presence of rFE compared with fixed-end reference contractions (twitch amplitude of ~9 Nm for both contraction conditions). The unchanged MEP amplitudes and SP durations indicate that corticospinal excitability was unaltered in the presence of rFE, which, based on a re-analysis

of the previously published data is in line with the earlier studies on corticospinal excitability in the presence of rFE (*Hahn* et al. 2012; *Sypkes* et al. 2018).

Interestingly, we found that activation of the motor cortex with a given suprathreshold stimulation intensity elicited comparable twitch torques for both contraction conditions, despite steady-state torques following active stretch being enhanced because of rFE. This is opposite to what would be expected based on the work of Merton (*Merton*, 1954), who showed that superimposed twitch size decreases as the pre-stimulus torque of the voluntary contraction increases. Accordingly, the equally-sized superimposed twitches following TMS in the presence of rFE support the idea that rFE is not based on a higher number of cross bridges, but on passive structural elements within the muscle that are engaged during stretch (*Edman, Elzinga & Noble*, 1978; *Nishikawa*, 2016; *Herzog*, 2018; *Freundt & Linke*, 2019). The contribution of such passive structural elements to the increased force and torque production following stretch could also explain the observed reduction in EMG activity following stretch observed in force/torque-matched contractions (*Oskouei & Herzog*, 2005; *Seiberl* et al., 2012).

Limitations

First, we only performed TMS of the motor cortex to assess corticospinal excitability, which does not allow us to distinguish between cortical and spinal aspects of corticospinal excitability. Accordingly, the unchanged MEP amplitudes and SP durations that we found in the presence of rFE compared with the reference contractions do not exclude potential spinal modulations in the presence of rFE, and the increased torques following stretch might induce tension-related inhibitory afferent feedback from Golgi tendon organs (*Contento, Dalton & Power* (2019). However, as the superimposed twitches following TMS were not reduced in the presence of rFE, we do not think that spinal excitability was reduced in the STR

conditions. Second, we did not obtain M-waves to normalise MEPs. Theoretically, we therefore might have missed potential changes in MEP amplitudes due to possible changes in M-wave amplitudes. However, we think that this is unlikely as earlier studies on this topic (*Hahn* et al. 2012; *Sypkes* et al. 2018) have not found differences in M-wave amplitudes in the presence of rFE. Further, the similar superimposed twitches in the STR and REF conditions again provide support that the overall excitability of the neuromuscular system was unchanged. Finally, we did not obtain TMS input-output curves, which would reveal the relative sizes of the measured MEPs. However, when setting up the individual stimulation intensities, we ensured that MEPs could still increase with increasing stimulation intensity so that changes due to the contraction conditions would be detectable.

Conclusion

In conclusion, we found that subthreshold and suprathreshold TMS of motor cortical neurons affected muscle activity and torque production, but the mechanical and neural responses to TMS did not differ between stretch-hold and fixed-end reference contractions. This is in line with our predictions and suggests that corticospinal excitability remains unaltered in the presence of rFE. This further suggests that the enhanced torque production following active muscle stretch is not due to changes in corticospinal excitability, but that rFE is a mechanical property of muscle, likely because of the stretch-induced engagement of passive structural elements.

Disclosures

The authors report no conflicts of interest.

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Tables

Table 1. Mean ± SD values of EMG amplitude suppression following subthreshold transcranial magnetic stimulation from soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles during stretch-hold (STR) and fixed-end reference (REF) contractions.

	SOL	**MG**	**LG**
EMG suppression REF [%]	24.0 ± 6.7	24.7 ± 7.2	19.4 ± 2.3
EMG suppression STR [%]	24.2 ± 5.4	23.7 ± 6.9	20.9 ± 6.4
Latency REF [ms]	44 ± 4	41 ± 5	44 ± 5
Latency STR [ms]	45 ± 3	43 ± 6	46 ± 5
Duration REF [ms]	14 ± 4	15 ± 4	12 ± 5
Duration STR [ms]	13 ± 6	12 ± 4	10 ± 4

Table 2. Mean ± SD values of motor evoked potentials (MEP) and silent periods (SP) after suprathreshold transcranial magnetic stimulation from soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles during stretch-hold (STR) and fixed-end reference (REF) contractions.

	SOL	**MG**	**LG**
MEP REF [V]	1.30 ± 0.78	2.54 ± 2.67	1.19 ± 0.46
MEP STR [V]	1.37 ± 0.72	2.70 ± 2.97	1.14 ± 0.50
SP REF [ms]	115 ± 31	112 ± 22	118 ± 29
SP STR [ms]	116 ± 31	115 ± 23	123 ± 34

Figure legends

Figure 1. Reanalysed data from Sypkes et al. (2018) and Hahn et al. (2012). The dark grey squares and error bars represent the means and 95% confident intervals of the normalised CMEP/Max and MEP/Mmax differences between the stretch-hold and fixed-end reference contraction conditions calculated from the Sypkes et al. (2018) supplementary data. The blue circles and error bars represent the normalised mean differences and 95% confident intervals calculated from the pooled CMEP/Max and MEP/Mmax data of the studies by Sypkes et al. (2018) and Hahn et al. (2012). Note that with the increased sample size obtained from pooling the data together, the CMEP/Mmax and the MEP/Mmax differences between the stretch-hold and fixed-end reference contraction conditions are no longer significant.

Figure 2. Example data from the stretch-hold (STR, blue) and fixed-end reference (REF, grey) contraction conditions. A: The traces show soleus (SOL) EMG (moving 0.25-s root-mean-square (RMS) amplitude calculations). Transcranial magnetic stimulation (TMS, vertical black lines) was delivered at 1 Hz from 5 s after contraction onset (marked as time zero). In case the first stimulation was delivered before SOL EMG reached the target level, the stimulation was excluded from analysis. B: Traces show the corresponding crank arm angles.

Figure 3. A: Schematic diagram of the experimental protocol. The protocol started and ended with maximal voluntary contractions (MVC, dark grey). Transcranial magnetic stimulation (TMS) hotspot and sub-/suprathreshold intensities (light grey) were then determined before data collection. Twenty contractions with subthreshold TMS (dark blue) and 4 contractions with suprathreshold TMS (light blue) were separated into three sets, with 5 min rest between each set. B: Schematic description of contractions within sets (blue bars) and delivery of TMS

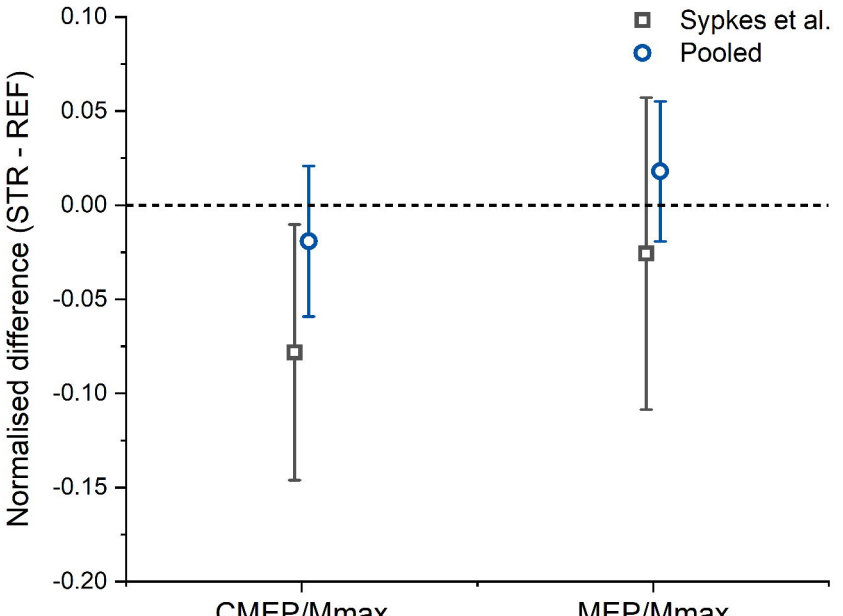
(same procedure for sub- and suprathreshold TMS). Vertical black lines indicate the timing of TMS during the submaximal contractions. Each set during subthreshold TMS consisted of 10 contractions of 15 s duration, followed by a 3 min rest.

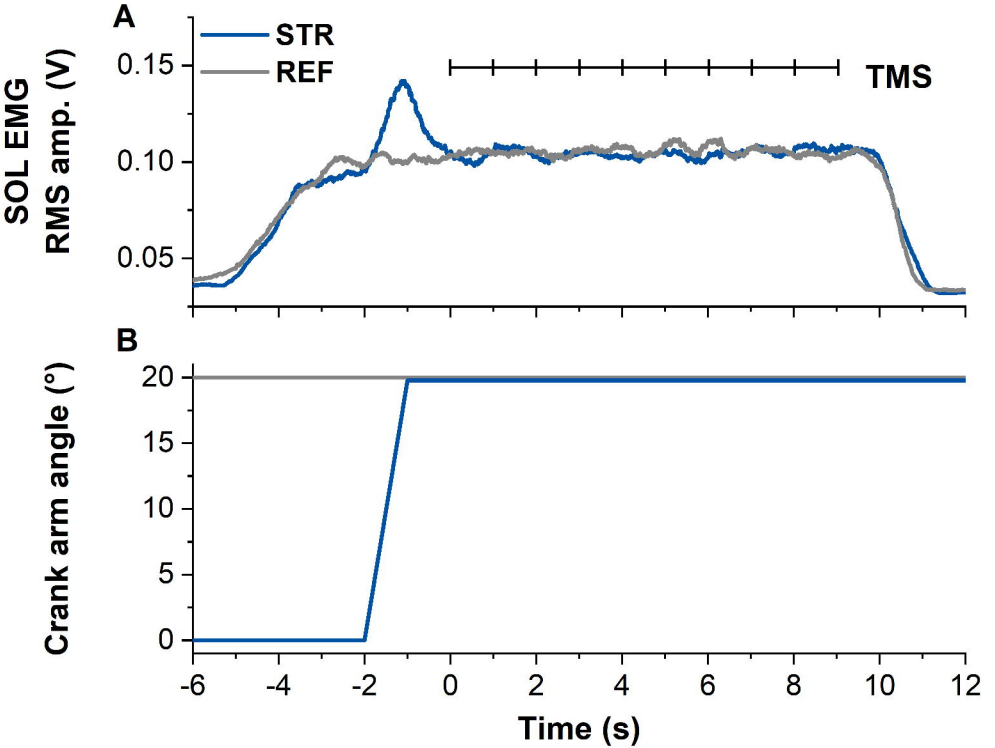
Figure 4. Example data of rectified and averaged soleus (SOL) EMG from contractions with subthreshold transcranial magnetic stimulation (TMS). The horizontal dotted line represents the averaged background EMG amplitude calculated from 15-25 ms following stimulation. Vertical dashed lines indicate onset and offset of EMG amplitude suppression following TMS. Time zero indicates the time of stimulation.

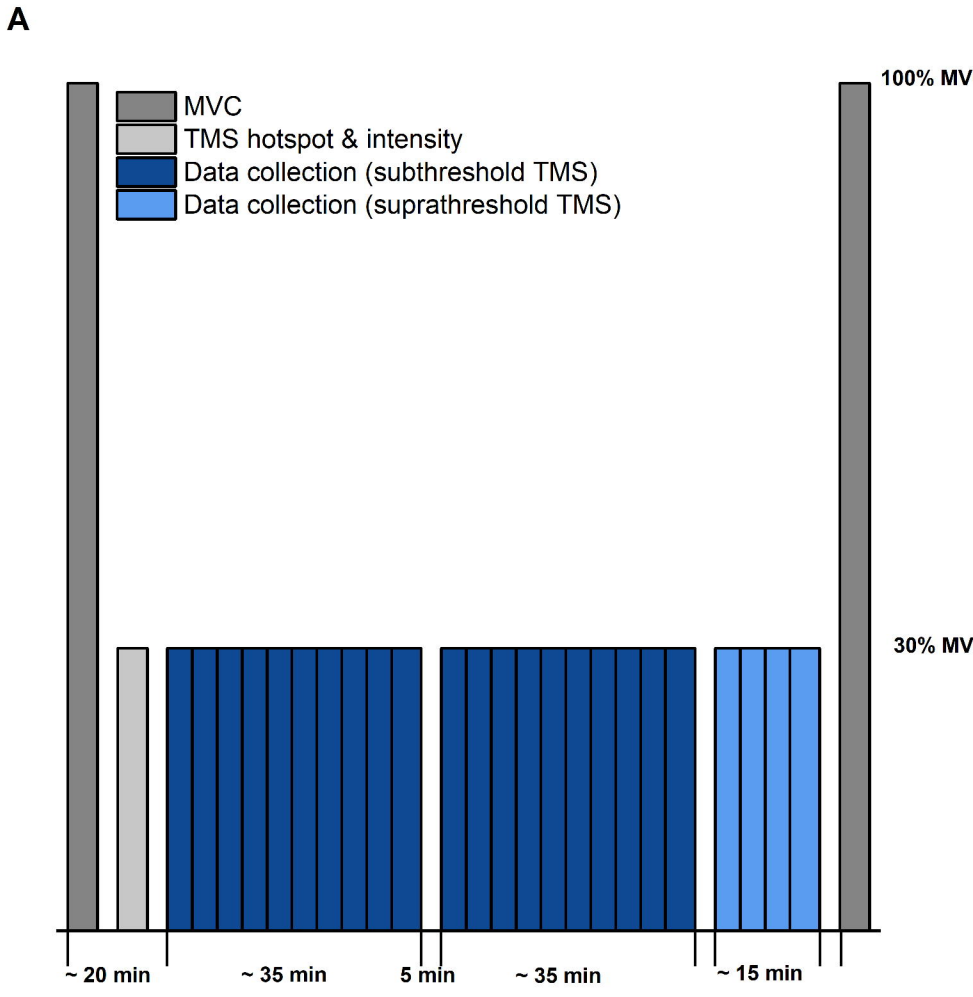
Figure 5. Representative example of motor evoked potentials (MEPs) and silent periods (SPs) from raw soleus (SOL) EMG signals elicited by suprathreshold transcranial magnetic stimulation (TMS). The light grey traces show the single MEPs and the dark blue trace shows the average of all SOL MEPs from one participant. The horizontal dashed black line indicates the duration of the silent period (SP) and the vertical black line indicates the peak-to-peak amplitude of the largest single MEP. Time zero indicates the time of stimulation.

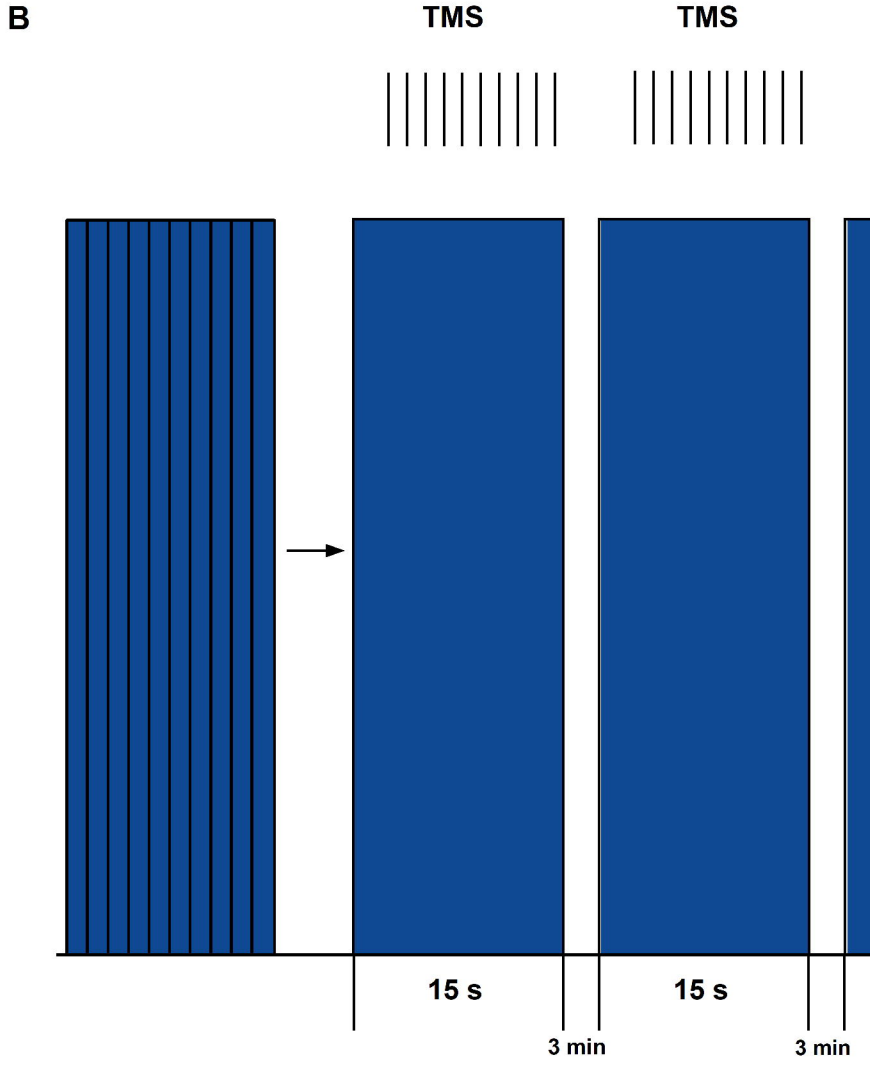
Figure 6. A: Residual force enhancement during stretch-hold (STR) contractions normalized to the time-matched torque during fixed-end reference (REF) contractions. The open circles and error bars represent the means and 95% confident intervals for the contractions with subthreshold transcranial magnetic stimulation (TMS) (left) and suprathreshold TMS (right), respectively. The grey dots represent the individual data points. B: Normalised MEP amplitude differences between STR and REF. The open circles and error bars represent the means and the 95% confident intervals and the grey dots represent the individual data points for soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles,

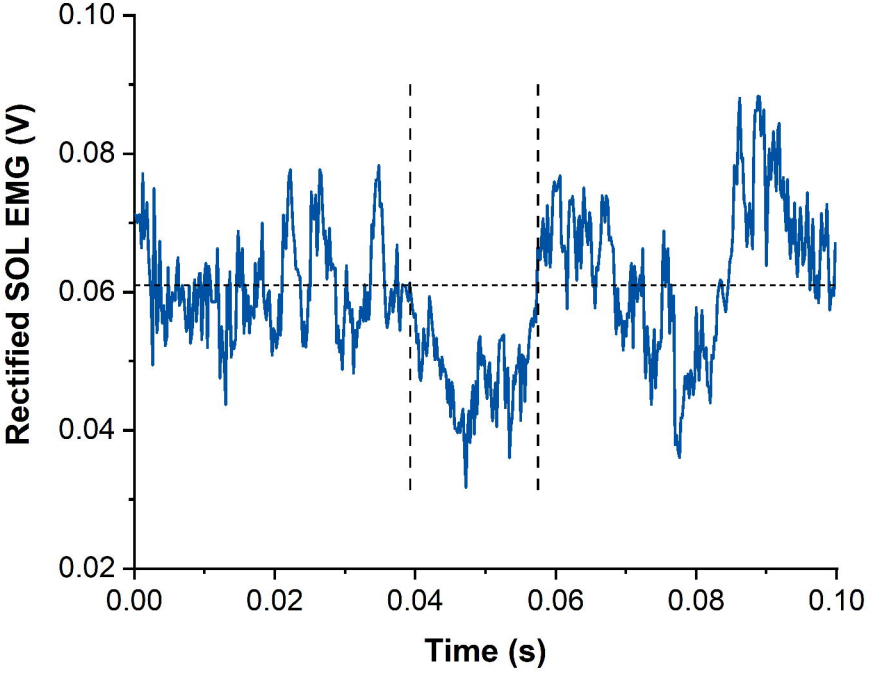
respectively. C: Superimposed twitch torques after suprathreshold TMS. The open circles and error bars represent the means and the 95% confident intervals and the grey dots represent the individual data points for REF (left) and STR (right).

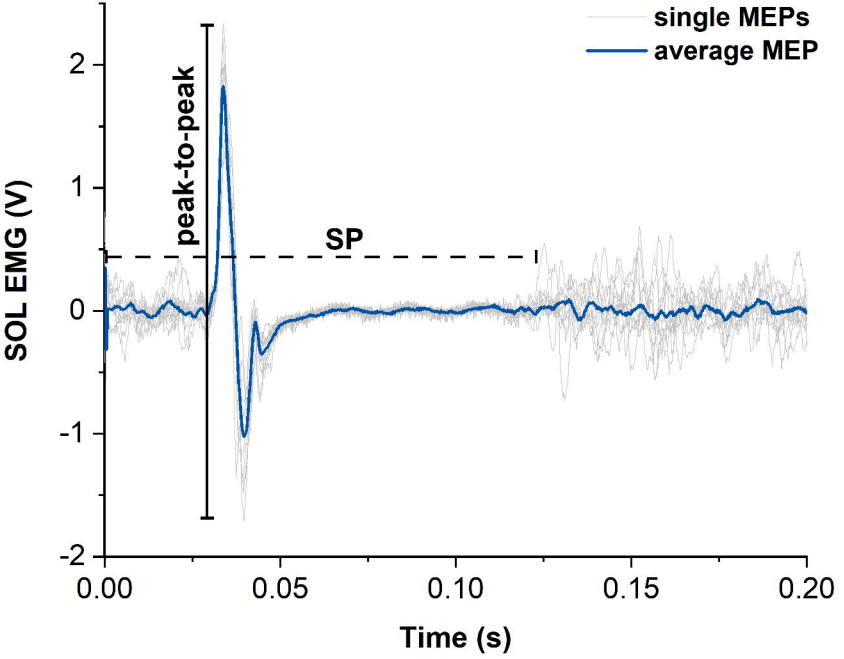

Sypkes et al.
Pooled
Normalised difference (STR - REF)
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
CMEP/Mmax
MEP/Mmax

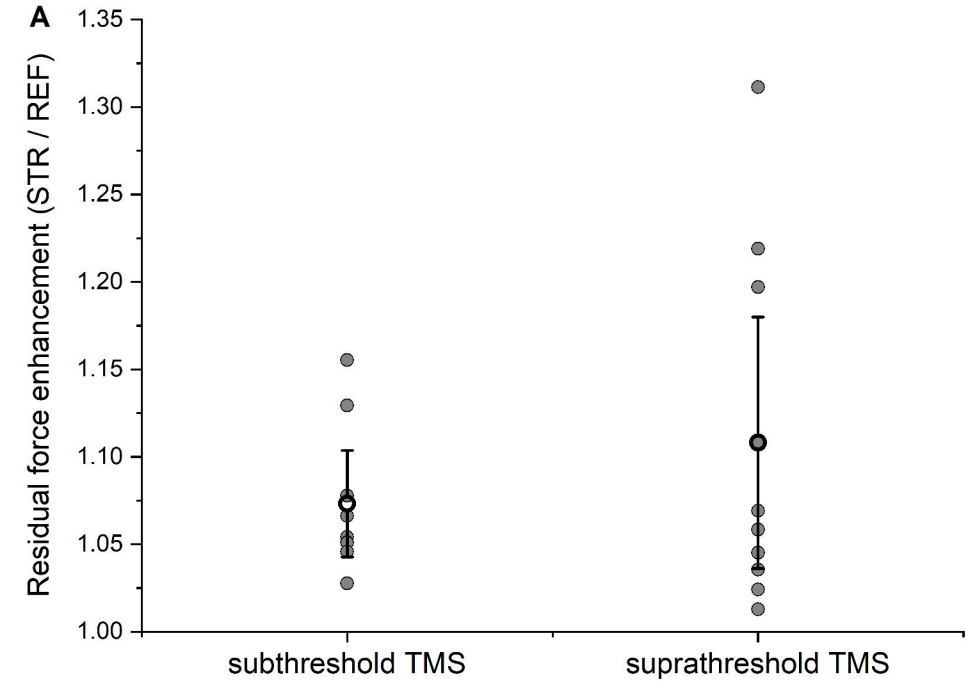

A
STR
REF
TMS
SOL EMG
RMS amp. (V)
0.15
0.10
0.05
B
Crank arm angle (°)
20
15
10
5
0
-6
-4
-2
0
2
4
6
8
10
12
Time (s)

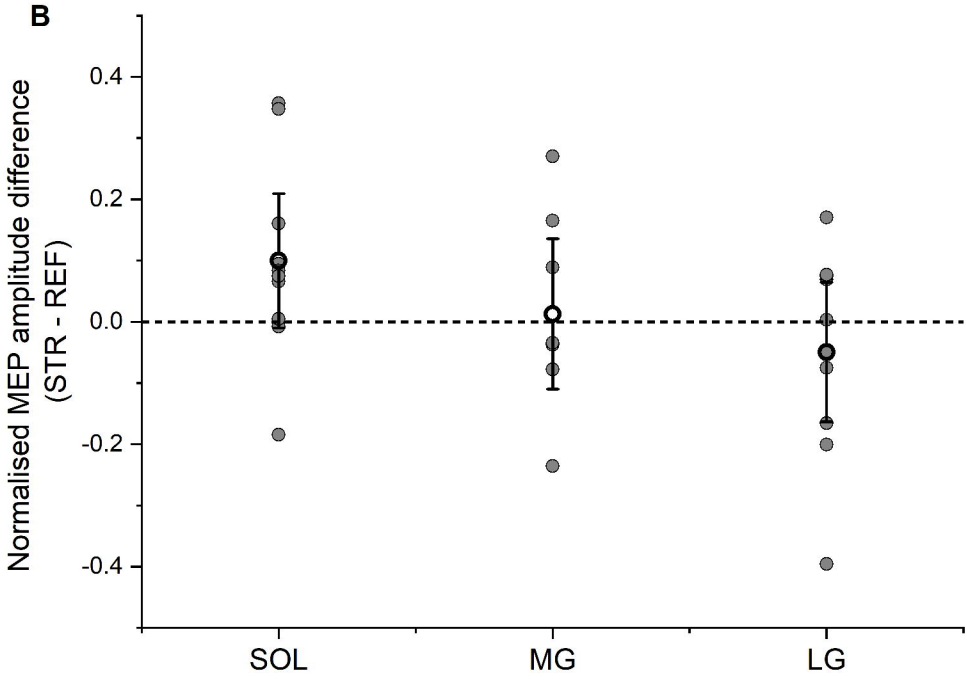

A
MVC
TMS hotspot & intensity
Data collection (subthreshold TMS)
Data collection (suprathreshold TMS)
100% MVC
30% MVC
~ 20 min
~ 35 min
5 min
~ 35 min
~ 15 min

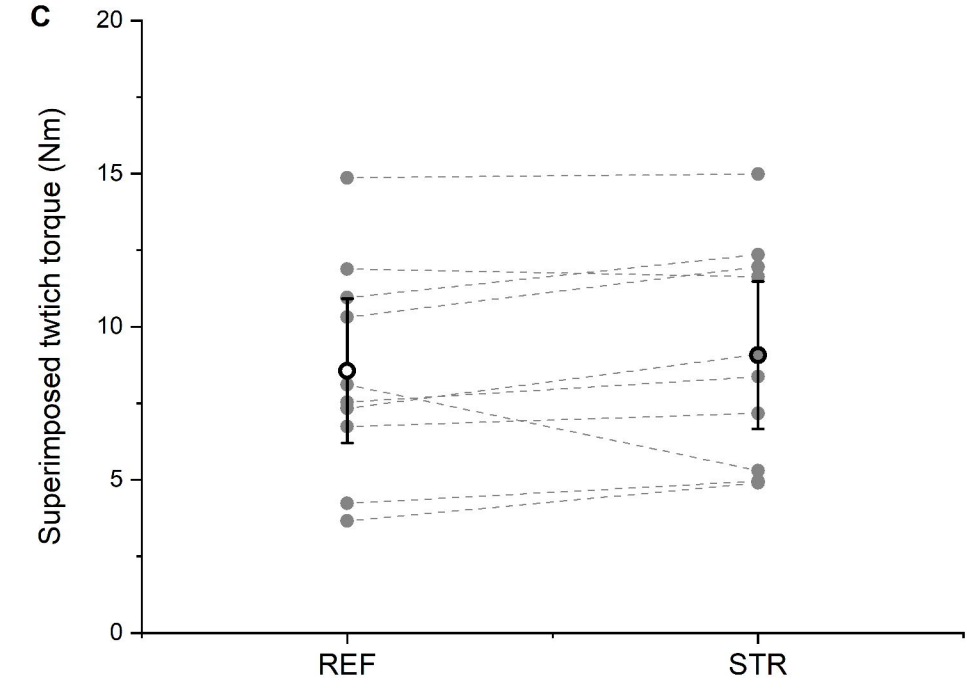

B
TMS
TMS
15 s
15 s
3 min
3 min


0.10
0.08
0.06
0.04
0.02
Rectified SOL EMG (V)
0.00
0.02
0.04
0.06
0.08
0.10
Time (s)


single MEPs
average MEP
peak-to-peak
SP
SOL EMG (V)
2
1
0
-1
-2
0.00
0.05
0.10
0.15
0.20
Time (s)


A
Residual force enhancement (STR / REF)
1.35
1.30
1.25
1.20
1.15
1.10
1.05
1.00
subthreshold TMS
suprathreshold TMS


B
Normalised MEP amplitude difference
(STR - REF)
0.4
0.2
0.0
-0.2
-0.4
SOL
MG
LG


C
Superimposed twtich torque (Nm)
20
15
10
5
0
REF
STR