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Effects of posture and coactivation on corticomotor excitability of ankle muscles

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Abstract

Background—The use of transcranial magnetic stimulation (TMS) to evaluate corticomotor excitability of lower limb (LL) muscles can provide insights about neuroplasticity mechanisms underlying LL rehabilitation. However, to date, a majority of TMS studies have focused on upper limb muscles. Posture-related activation is an important under-investigated factor influencing corticomotor excitability of LL muscles.

Objective—The purpose of this study was to evaluate effects of posture and background activation on corticomotor excitability of ankle muscles.

Methods—Fourteen young neurologically-unimpaired participants (26.1 ± 4.1 years) completed the study. TMS-evoked motor evoked potentials (MEPs) were recorded from the tibialis anterior (TA) and soleus during 4 conditions – standing, standing coactivation, sitting, and sitting coactivation. TA and soleus MEP amplitudes were compared during: (1) standing versus sitting; (2) standing coactivation (standing while activating both TA and soleus) versus sitting coactivation; and (3) standing coactivation versus standing. For each comparison, background EMG for TA and soleus were matched. Trial-to-trial coefficient of variation of MEP amplitude and coil-positioning errors were additional dependent variables.

Results—No differences were observed in TA or soleus MEP amplitudes during standing versus sitting. Compared to sitting coactivation, larger MEPs were observed during standing coactivation for soleus but not TA. Compared to standing, the standing coactivation task demonstrated larger MEPs and reduced trial-to-trial MEP variability.

Conclusion—Our findings suggest that incorporation of measurements in standing in future TMS studies may provide novel insights into neural circuits controlling LL muscles. Standing and

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Conflicts of interest

The authors have no conflicts of interests related to the contents of the manuscript.

standing coactivation tasks may be beneficial for obtaining functionally-relevant neuroplasticity assessments of LL musculature.

Keywords

Activation; coactivation; lower limb; motor evoked potentials; neuroplasticity; posture

1. Introduction

The use of transcranial magnetic stimulation (TMS) to evaluate corticomotor excitability of lower limb (LL) muscles can provide insights about neuroplasticity mechanisms underlying LL rehabilitation and gait retraining (Bowden, Woodbury, & Duncan, 2013; Butler & Wolf, 2003; Chieffo, Comi, & Leocani, 2016; Merton & Morton, 1980; Rothwell, 1997). However, to date, the majority of TMS studies have focused on upper limb muscles (Borich, Neva, & Boyd, 2015; Butler, Kahn, Wolf, & Weiss, 2005; Darling, Wolf, & Butler, 2006). Due to differences in strength of descending cortical monosynaptic projections onto spinal segmental circuitry and spinal reflexes (Barthelemy, Grey, Nielsen, & Bouyer, 2011; Phillips, 1967), as well as types of functional tasks associated with upper limb versus LL muscles, training-induced neural plasticity mechanisms may differ markedly between upper limb and LL muscles. Moreover, because the motor homunculus of LL muscles is located deep within the inter-hemispheric fissure, with greater proximity between representations of neighboring muscles, TMS of LL muscles presents constraints with regards to accessibility and specificity of TMS-induced electric fields (Smith, Stinear, Alan Barber, & Stinear, 2017). Therefore, to characterize mechanisms underlying therapeutic interventions that target gait and posture, there is a need for more systematic TMS investigations of LL muscles.

The facilitatory effect of a low-level voluntary contraction on TMS-evoked MEPs is well established in both upper limb (Darling et al., 2006; Kischka, Fajfr, Fellenberg, & Hess, 1993; Rossini, Zarola, Stalberg, & Caramia, 1988) and LL muscles (Ackermann, Scholz, Koehler, & Dichgans, 1991; Benecke, Meyer, Gohmann, & Conrad, 1988; Lavoie, Cody, & Capaday, 1995; Valls-Sole, Alvarez, & Tolosa, 1994). Similarly, postural activation may modulate TMS-evoked MEPs. While the motor cortex plays an important role during volitional contractions (Ashe, 1997; Georgopoulos, Ashe, Smyrnis, & Taira, 1992), subcortical motor structures play a greater role during postural control (Prentice & Drew, 2001; Soto, Valls-Sole, Shanahan, & Rothwell, 2006). Posture-related activation is an important yet under-investigated factor influencing TMS-evoked MEPs of LL muscles. Additionally, posture-related effects on corticomotor excitability may differ based on the functional role of the muscle. The soleus, an anti-gravity ankle extensor, shows posture-related EMG activation during standing, and is a modulator of postural sway (Nardone & Schieppati, 1988). In contrast, the tibialis anterior (TA), an ankle flexor, does not demonstrate activation during quiet standing, but is important for controlling foot position during walking (Barthelemy et al., 2011). Furthermore, there may be differences in neural control circuitry between the TA and soleus, with soleus receiving fewer or weaker direct corticospinal projections than TA (Brouwer & Ashby, 1990; Trinastic et al., 2010).

Due to task-specificity of training-induced neuroplasticity (Kleim & Jones, 2008), for LL muscles, TMS measurements obtained during functionally-relevant postures such as standing may provide more sensitive indices of corticomotor excitability compared to measurements in sitting. However, most LL TMS studies to date have acquired measurements during sitting (Forrester, Wheaton, & Luft, 2008; Perez, Lungholt, Nyborg, & Nielsen, 2004; Sivaramakrishnan, Tahara-Eckl, & Madhavan, 2016; Wheaton, Villagra, Hanley, Macko, & Forrester, 2009). The few studies that obtained TMS-evoked MEPs during standing showed markedly inconsistent findings (Ackermann et al., 1991; Lavoie et al., 1995; Obata, Sekiguchi, Nakazawa, & Ohtsuki, 2009; Soto et al., 2006). Furthermore, although coil-targeting accuracy is improved by neuronavigation-guided TMS (Julkunen et al., 2009; Schmidt et al., 2015), previous investigations that delivered TMS during standing did not use neuronavigation to monitor coil-positioning accuracy (Ackermann et al., 1991; Lavoie et al., 1995; Obata et al., 2009; Soto et al., 2006). The current study aims to address gaps in previous literature related to the effects of posture and background activation on TMS-evoked responses of LL muscles. Here, we used neuronavigation to confirm consistent coil-positioning between postures, rigorously matched background EMG activation for between-posture comparisons of TMS-evoked MEP response amplitude, and evaluated TMS-evoked MEPs for both TA and soleus muscles.

Additionally, for the first time, we evaluated TA and soleus corticomotor excitability during a novel ‘standing coactivation’ task, which, in contrast to quiet standing, requires volitional activation of TA and postural activation of soleus. Agonist-antagonist coactivation serves to stabilize a joint during external perturbations or motor learning (J. Nielsen & Kagamihara, 1992; J. B. Nielsen, 1998; Perez, Lundbye-Jensen, & Nielsen, 2007). Coactivation is associated with modulation of spinal (presynaptic inhibition, H-reflex amplitude, Renshaw inhibition) and supraspinal pathways (J. Nielsen & Kagamihara, 1992; J. B. Nielsen, 1998; Perez et al., 2007), which can influence the output of the lower motor neuron pool in response to the descending TMS-induced volley. The standing coactivation task evaluated in our present study could provide methodology for gaining new insights about LL motor control circuitry. TMS measures obtained during coactivation may also have clinical relevance due to greater agonist-antagonist coactivation observed in individuals with neurological lesions such as stroke (Lamontagne, Malouin, Richards, & Dumas, 2002).

Here, we systematically evaluated the effects of posture and voluntary activation on corticomotor excitability of ankle muscles through 3 comparisons, in which background EMG activation for TA and soleus were rigorously matched between comparisons. Specifically, we compared TMS-evoked MEP amplitudes for TA and soleus during: (1) standing (normal or quiet standing) versus sitting; (2) standing coactivation (standing with voluntary activation of the TA and postural activation of the soleus) versus sitting coactivation; and (3) standing coactivation versus standing.

2. Materials and methods

This study was approved by the Emory University Institutional Review Board (IRB). All participants provided informed written consent in accordance with the Declaration of Helsinki.

2.1. Participants

Nineteen young, neurologically intact subjects with no history of orthopedic or neurological conditions were enrolled in this study. Exclusion criteria included neurologic disorders, contraindications to TMS, and episode of syncope in the past 12 months (Rossi, Hallett, Rossini, Pascual-Leone, & Safety of, 2009). Five participants did not complete the study protocol or were excluded due to contraindications to TMS, including a syncope incident (Kesar, McDonald, Eicholtz, & Borich, 2016), medications, or previous concussion. The remaining 14 participants (11 females, age: 26.1 ± 4.1 years) completed the study protocol. Data were obtained during a single session.

2.2. Electromyography procedures

After standard skin preparation procedures to minimize skin impedance, bipolar, circular, self-adhesive surface electromyography (EMG) sensors (11-mm diameter, 11-mm inter-electrode distance, Biopac systems Inc., USA) were attached according to the SENIAM guidelines (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000) to skin overlying the belly of the right TA and soleus muscles, with a common ground sensor attached over the right lateral malleolus. Appropriate EMG sensor placement was confirmed by checking EMG signals while the participant contracted each muscle. EMG data were sampled at 1000 Hz (bandpass 10–500 Hz, amplification 2000) (AcqKnowledge software, Biopac Systems, Inc.).

2.3. EMG activity during maximum voluntary contractions (MVCs)

EMG data were collected while the participant performed 3 MVCs of 5-second duration. TA and soleus MVCs were measured at the beginning of the session. For the soleus MVC, the participants were instructed to stand on the right foot and maximally plantarflex their ankle. For the MVC, in a seated position, the participant maximally dorsiflexed against resistance. A 3-second window during peak contraction was used to calculate the average root mean square (RMS) EMG amplitude, and the maximal value of 3 contractions was used as the MVC. Note that this modified MVC methodology provided an estimate of background activation during maximal activation, and not necessarily an accurate representation of the maximal force generating ability. The MVC EMG was used to set a background activation level (10% MVC), selected to mimic the magnitude of activation observed during standing (Ackermann et al., 1991), that the participants maintained during TMS.

2.4. TMS procedures

Single TMS pulses were delivered using a custom figure-of-eight, angled, batwing coil (70-mm diameter, Magstim, UK) connected to a monophasic stimulator (Magstim 2002). Stereotaxic neuronavigation (Brainsight, Rogue Research, Canada) was used to track coil position and orientation, with the participant's head co-registered to a standard MNI-152 template brain throughout the experiment. During TMS, the coil was held tangential to the scalp with the handle parallel to the interhemispheric fissure, to induce a postero-anterior current within the cortex. The hotspot for the right TA within the contralateral (left) primary motor cortex (M1) was determined as the optimal coil position that elicited maximal MEP responses from the right TA (Rossini et al, 2015). For each participant, coil position and orientation at the hotspot was recorded and used as the target throughout the session. Next,

using a computerized adaptive algorithm (Borckardt, Nahas, Koola, & George, 2006), the resting motor threshold (rMT) was determined with an MEP amplitude criterion of 80 μ V (Rossini et al., 2015). The criterion MEP amplitude for rMT was increased from 50 μ V, which is commonly used (Rossini et al., 2015), to 80 μ V to ensure that discernible MEPs would be obtained at rest from both TA and soleus muscles. Next, TMS intensity was set to a suprathreshold level (120% rMT). At this suprathreshold intensity, we confirmed that measurable MEPs were elicited from both TA and soleus. For 3 participants, TMS intensity was increased to 130% of rMT to enable elicitation of measurable MEPs from the soleus. Because the current study involved repeated-measures within-subject comparisons, to prevent the effect of stimulation intensity from influencing study results, the same TMS intensity was used for each participant across all testing conditions.

2.5. EMG biofeedback for maintaining consistent background EMG pre-activation

Raw EMGs from TA and soleus were channeled via a data-acquisition board into a custom biofeedback program (USB-6343, LabVIEW, National Instruments) to visually display the RMS value of the ongoing EMG activation for the participant in real-time. According to the testing condition (e.g. sitting with soleus activated), participants were provided biofeedback to maintain the muscle's activation within the target window (10% MVC, tolerance: $\pm$ 5%). When the testing condition required that both muscles be activated (e.g. sitting or standing with coactivation), biofeedback visual display was provided for both muscles simultaneously. The participants were provided verbal instructions before each testing condition. For the coactivation trials, the visual feedback paradigm was explained to the participants by informing them that the two lines on the visual display screen represented their ongoing activation for ankle muscles that lift the ankle upward (TA) and push the toes downward (soleus) respectively. Participants' goal was to activate the muscles to a low-level to reach the target (shown by a black line and shaded area on the screen). During seated coactivation, if additional instruction was needed, they were told to stiffen their ankle in order to activate both muscles. During standing coactivation, participants were told that the plantarflexor would be activated automatically in standing, and they simply had to maintain consistent postural orientation to keep the soleus EMG consistent. To activate TA during standing, they were instructed (similar to sitting) to pull their toes upward. EMG biofeedback ensured that participants maintained desired background EMG pre-activation levels for each condition, and that background EMG was consistent across comparable testing conditions (e.g. between sitting and standing).

2.6. Data collection during different postural testing conditions

A custom-written software program (Acknowledge, Biopac Inc.) was used to deliver 10 TMS pulses at 0.2 Hz. Data acquisition was delineated so that 50-ms of data were collected before and 450-ms were collected after the TMS pulse, with a total acquisition time of 500-ms for each TMS pulse (Fig. 2). TMS-evoked MEPs were collected during 4 conditions (Fig. 1):

1. sitting with TA at rest and soleus activated (*Sitting*)
2. sitting with both TA and soleus activated (*Sitting coactivation*)

3. quiet or normal standing with TA at rest and soleus activated (*Standing*)
4. standing with both TA and soleus activated (*Standing coactivation*).

Sitting conditions were completed first in randomized order, followed by the standing conditions in randomized order. Data from these 4 conditions were used to make 3 comparisons: (1) standing versus sitting, (2) standing coactivation versus sitting coactivation, and (3) standing versus standing coactivation. For the first 2 comparisons (effects of posture), our methodology was designed to match background EMG for TA and soleus between the compared conditions. For the 3rd comparison (effect of coactivation during the standing posture), soleus background EMG was matched but TA background activation would differ between the 2 conditions. Controlling for background activation of both TA and soleus was important due to the known effects of background activation on TMS-evoked MEPs.

2.6.1. Seated posture—Participants sat in a chair with the back supported, neck unsupported, knee and hip at 90° flexion, and ankle at 0° dorsiflexion. The alignment of the participant's feet was marked with tape on a floor-mat to ensure consistent foot placement during the session. An ankle weight was positioned over the foot for stabilization.

2.6.2. Standing posture—Participants stood with arms relaxed by their side and feet on the floor-mat in the same alignment as during sitting. Hip, knee, and ankle joints were in a neutral alignment. Participants were instructed to distribute their weight symmetrically on both legs. Similar to sitting, an ankle weight was positioned over the dorsum of foot for stabilization.

2.7. Data analysis

2.7.1. Visual inspection and calculation of background EMG activation—Pre-stimulation EMG background RMS amplitude for both muscles was calculated for a 50-ms window prior to the delivery of TMS (Fig. 2). Background EMGs were reviewed for each comparison, and erroneous trials where the background did not match the condition being tested were removed. Rigorous checking of individual and group background EMG RMS data was performed to ensure that comparisons between the standing and seated posture were not confounded by differences in background EMG activity. Several criteria were used to evaluate background EMG data. In addition to evaluating individual subject data. First, individual subject data were evaluated to confirm that the background EMG RMS for each muscle matched the condition appropriately. Second, paired *t*-tests were conducted on the background EMG RMS data to confirm that the background EMG matched the condition being tested. For instance, paired *t*-test should show a significantly greater background EMG activation for the TA muscle during the sitting versus the sitting coactivation condition. The statistical results for the background EMG data are included in the results section. Third, change scores of background EMG RMS amplitude were assessed and points above 2.5 standard deviation of the mean change score were excluded as outliers. Removal from analysis implies that if background EMG was deemed inappropriate, MEP data were removed from analysis for that particular comparison.

2.7.2. Calculation of peak to peak MEP amplitudes—For each MEP, the difference between the maxima and minima within the MEP window was calculated as the peak to peak (P-P) amplitude. The average of 10 MEP amplitudes was used for each condition. For each condition, trial-to-trial variability in MEP amplitude was indexed by calculating the coefficient of variation (CV) (standard deviation of the MEP amplitudes divided by the mean MEP amplitude x100).

2.7.3. Coil-position accuracy—The position error (distance between coil-center and hotspot location) and angular error (difference in coil tilt/rotation versus hotspot coil orientation) of the TMS coil position were extracted from Brainsight for all trials. Coil-positioning error thresholds were set based on our previous experience and studies; for the current study, TMS samples that demonstrated a coil-position error >2-mm and angular error >3° were removed from subsequent analyses.

2.8. Statistical analysis

The primary dependent variables were MEP amplitudes for TA and soleus. Because variations in background EMG can influence TMS-evoked MEPs, background EMG RMS data were checked for individual trials and participants, as well as evaluated as a secondary dependent variable. Additional secondary variables included TMS coil position and angular errors with respect to the hotspot, and trial-to-trial CV of MEP amplitude.

A 2-way ANOVA was conducted to evaluate the effect of posture (standing, sitting) and activation (soleus active, coactivation) on MEP amplitudes for each muscle (TA and soleus). To make the 3 specific comparisons consistent with the study objectives, for each muscle (TA and soleus), 3 planned paired *t*-tests were performed: standing versus sitting, standing coactivation versus sitting coactivation, and standing versus standing coactivation. Additionally, for each paired comparison, within-subject, between-condition change scores were calculated as the difference in MEP amplitude for the two compared conditions (e.g. MEP amplitude in standing minus MEP amplitude in sitting) (Fig. 3c and 3d). MEP change scores were plotted for each participant to evaluate inter-individual variability, and determine whether the 95% confidence interval (CI) of the change scores included zero (Gardner & Altman, 1986). The CI and individual subject change scores were reported consistent with recommendations that reporting CIs along with statistical *p*-values enables a more thorough interpretation of the main effect, and is helpful for incorporating the study results into future meta-analyses (Gardner & Altman, 1986; Hirji & Fagerland, 2011). Similarly, change scores for background EMG RMS were computed and plotted to exclude outliers, i.e. participants with change scores exceeding 2.5 times standard deviation of the mean.

For the 3 comparisons conducted in the study, a 1-way repeated-measures ANOVA with *post-hoc* paired comparisons was performed to determine whether the magnitude of change in MEP amplitude (i.e. MEP change score) differed across the 3 comparison types. SPSS version 21 was used for statistical analysis. Alpha-level was set at 0.05.

3. Results

Fourteen participants (11 females, age: 26.1 ± 4.1 years) completed the study protocol. The 2-way ANOVA with posture (standing, sitting) and activation as independent variables, and MEP amplitude for the TA as dependent variable, showed a main effect for posture ($p = 0.03$), activation ($p < 0.001$), and no interaction effect ($p = 0.51$). Similarly, the 2-way ANOVA for soleus MEP amplitudes showed a trend for main effect for posture ($p = 0.08$), significant effect of activation ($p = 0.03$), and no interaction effects ($p = 0.86$). Additionally, the 3 comparisons listed in the study objectives were evaluated using planned statistical comparisons, and are provided below.

3.1. Comparison between sitting and standing

Two participants were excluded from the analysis for this comparison because their background EMG root mean square (RMS) amplitude was not matched between standing and sitting, or the change scores (standing minus sitting) for EMG background RMS exceeded 2.5 standard deviations of the mean. Data from 12 participants were included in this analysis.

3.1.1. MEP amplitudes for TA and soleus—The paired 2-tailed *t*-test showed that TA MEP amplitudes were not different during sitting versus standing ($N = 12$, $p = 0.30$) (Fig. 3a). Individual participant change scores showed that although the majority of participants exhibited an increase in MEP amplitude during standing compared to sitting (positive change score), there was high inter-individual variability, and the 95% confidence interval (CI) of the mean change scores included zero (Fig. 3c). There was no difference ($p = 0.123$) in trial-to-trial coefficient of variation (CV) of MEP amplitudes between sitting ($40.0 \pm 25.9\%$) and standing ($51.2 \pm 11.7\%$).

Soleus MEP amplitude was not different during sitting versus standing ($p = 0.15$) (Fig. 3b); individual subject change scores showed high inter-individual variability and the 95% CI of change scores included zero (Fig. 3d). There was no difference ($p = 0.26$) in trial-to-trial CVs of soleus MEP amplitudes between sitting ($30.2 \pm 8.5\%$) and standing ($34.8 \pm 9.8\%$).

3.1.2. Background EMG and TMS coil-positioning errors—There was no difference in background EMG between sitting versus standing for TA ($p = 0.84$) (Fig. 3e) or soleus ($p = 0.22$) (Fig. 3f). Significantly smaller ($p = 0.004$) coil-target position errors were observed during sitting (0.26 ± 0.17 mm) versus standing (0.53 ± 0.36 mm). Similarly, there was a smaller ($p = 0.01$) coil-target angular error in sitting (0.80 ± 0.30 degrees) versus standing (1.07 ± 0.45 degrees).

3.2. Comparison between sitting coactivation and standing coactivation

One participant was excluded due to the soleus background EMG activation change score being greater than 2.5 standard deviations of the group mean. Data for 13 participants were included in this comparison.

3.2.1. MEP amplitudes for TA and soleus—TA MEP amplitudes were not different ($p = 0.1$) during sitting coactivation (2.11 ± 1.40 mV) versus standing coactivation (2.61 ± 1.29

mV) ($N = 13$, Fig. 4a). Change scores for TA MEP amplitudes showed high inter-individual variability and the 95% CI of the mean change scores included zero (Fig. 4c). There was no difference ($p = 0.23$) in trial-to-trial CV of MEP amplitudes between sitting coactivation ($19.6 \pm 8.1\%$) and standing coactivation ($23.1 \pm 10.1\%$).

Significantly larger ($p = 0.02$) soleus MEP amplitudes were observed during standing coactivation (0.59 ± 0.26 mV) compared to sitting coactivation (0.51 ± 0.22 mV) (Fig. 4b). Although there was high inter-individual variability in the change scores for MEP amplitude, the 95% CI of the average change score did not include zero (Fig. 4d). No difference was detected ($p = 0.46$) in trial-to-trial CV between sitting coactivation ($21.6 \pm 8.8\%$) and standing coactivation ($20.1 \pm 6.95\%$).

3.2.2. Background EMG and TMS coil-positioning errors—Although participants were provided visual feedback to maintain targeted background EMG, significantly larger background EMG activation for the TA muscle was observed during standing coactivation versus sitting coactivation ($p = 0.033$) (Fig. 4e). There was no difference in soleus background EMG ($p = 0.877$) between conditions (Fig. 4f).

There was a significantly smaller ($p = 0.01$) coil-target position error during sitting coactivation (0.25 ± 0.14 mm) versus standing coactivation (0.49 ± 0.33 mm). Similarly, there was a smaller ($p = 0.01$) coil-target angular error during sitting coactivation (0.88 ± 0.28 degrees) versus standing coactivation (1.23 ± 0.49 degrees).

3.3. Comparison between standing and standing coactivation

Two participants were excluded from analysis for this comparison due to soleus background EMG not being matched between the two conditions.

3.3.1. MEP amplitudes for TA and soleus—Significantly larger TA MEP amplitudes ($p < 0.001$) were observed during standing coactivation (2.60 ± 1.35 mV) compared to standing (1.04 ± 0.68 mV) ($N = 12$, Fig. 5a). Individual change scores were positive for all participants (1.56 ± 0.98 mV), indicating a larger MEP amplitude for the standing coactivation condition across all participants, and the 95% CI of the change score did not include zero (Fig. 5c). Additionally, trial-to-trial CVs for TA MEP amplitude were significantly greater during standing ($51.3 \pm 11.8\%$) compared to standing coactivation ($22.8 \pm 10.4\%$) ($p < 0.0001$).

Greater soleus MEP amplitudes ($p = 0.03$) were detected during standing coactivation (0.59 ± 0.27 mV) compared to standing (0.44 ± 0.30 mV) (Fig. 5b). The 95% CI of change scores for soleus MEP amplitudes did not include zero (Fig. 5d). There was a significantly larger trial-to-trial CV of soleus MEP amplitudes ($p = 0.002$) during standing ($38.0 \pm 13.2\%$) versus standing coactivation ($20.5 \pm 7.2\%$).

3.3.2. Background EMG and TMS coil-positioning errors—Consistent with task requirements for the testing conditions, a significant increase ($p < 0.001$) in TA background EMG RMS was observed during standing coactivation (0.038 ± 0.023 mV) versus standing (0.005 ± 0.004 mV) (Fig. 5e). Soleus background EMG RMS did not show a difference ($p =$

0.12) between standing coactivation (0.009 ± 0.002 mV) and standing (0.011 ± 0.003 mV) (Fig. 5f).

Coil-position error was not significantly different ($p = 0.50$) between quiet standing (0.53 ± 0.36 mm) and standing coactivation (0.49 ± 0.33 mm). Similarly, there was no difference ($p = 0.13$) in coil-position angular error during standing (1.07 ± 0.45 degrees) versus standing coactivation (1.23 ± 0.49 degrees).

3.4. Evaluation of MEP change scores for the 3 study comparisons

To evaluate whether there were differences in the magnitude of within-subject modulation or change in MEP amplitude induced by changes in posture and background activation, we conducted a one-way repeated measures ANOVA with the 3 comparison types as the independent variable ((1) sitting versus standing, 2) sitting coactivation versus standing coactivation, 3) standing versus standing coactivation) and the within-subject between-condition MEP change score as dependent variable. The ANOVA for TA MEP change scores revealed a significant effect of comparison type ($p = 0.010$) (Fig. 6). Planned *post-hoc* pairwise comparisons showed larger change scores for the standing coactivation versus standing change score compared to the standing coactivation versus sitting coactivation change score ($p = 0.013$) (Fig. 6). Significantly larger change scores were also detected for standing coactivation versus standing compared to standing versus sitting change scores ($p = 0.008$) (Fig. 6). The 1-way repeated measures ANOVA for the soleus MEP change scores showed no significant effect of comparison type ($p = 0.687$).

4. Discussion

Our systematic evaluation of the effects of posture on corticomotor excitability of ankle muscles revealed no enhancement of corticomotor excitability of ankle muscles in the standing compared to seated posture. The standing coactivation task evaluated in our study provided innovative findings and potential methodological advantages for future LL TMS studies. The only significant posture-related effect observed in our study was an enhancement of soleus TMS-evoked MEPs during standing coactivation compared to sitting coactivation (Fig. 4). Additionally, evaluation of the effect of muscle activation in the standing posture showed that compared to standing, the standing coactivation condition resulted in larger MEPs and reduced trial-to-trial CV of MEP amplitudes (Fig. 5). Results for coil-positioning error reveal that delivering TMS in the standing posture may result in a small but significant reduction in coil-positioning accuracy compared to sitting, emphasizing the need for incorporating neuronavigation tools during LL TMS, especially in the standing posture. Comparison of MEP change scores revealed that transitioning from a quiet standing to the standing coactivation task elicited a greater modulation of TA MEP amplitude compared to MEP modulation induced by transitioning from the seated to standing posture (Fig. 6).

4.1. No difference in TA and soleus MEPs during standing versus sitting

For both TA and soleus, we found no difference in TMS-evoked MEP amplitudes obtained in the standing versus sitting postures, when background EMG activation for both muscles

was matched between postures (Fig. 3). Our findings are consistent with 2 previous studies showing no enhancement of ankle muscle corticomotor excitability in standing versus sitting (Lavoie et al., 1995; Soto et al., 2006). However, our results differ from Ackerman et al. and Obata et al., who reported enhancement of TMS-evoked MEPs in standing compared to sitting (Ackermann et al., 1991; Obata et al., 2009). However, because Ackerman et al. did not match EMG background activation between postures, the enhancement of soleus MEPs in standing in their study was likely related to greater soleus EMG during standing (Ackermann et al., 1991). Obata et al., however, matched soleus background EMG between postures, and showed an increase in recruitment curve plateau and slope for TA and soleus during standing (Obata et al., 2009). The differences in that study and the present report could be explained by methodological differences. Unlike our study, in which we recorded MEPs at 120% above rMT, Obata et al. delivered a range of TMS intensities (Obata et al., 2009). Second, Obata et al. did not utilize neuronavigation (Obata et al., 2009). The fact that neuronavigation was not used in any of the previous studies is especially noteworthy because our results showed that despite providing the experimenter holding the TMS coil with real-time feedback about coil position, there was a small but significant decrease in coil-positioning accuracy during standing. Thus, the testing posture can influence coil-targeting accuracy which could, in part, contribute to previous inconsistent study findings (Lavoie et al., 1995). Importantly, the inconsistency in the literature, and the contradictory findings of our study compared to Obata et al. (Obata et al., 2009) underscore the need for more rigorous investigation on the effects of testing conditions (e.g. posture, upper limb position, activation state of targeted and non-targeted muscles) on TMS-evoked responses of LL muscles.

4.2. Compared to sitting coactivation, larger MEPs were observed during standing coactivation for soleus but not TA

A unique contribution of our study was that in addition to measuring TMS-evoked MEPs during quiet or normal standing, we also recorded TMS-evoked MEPs during a standing coactivation task. During standing coactivation, participants were provided visual feedback and instructed to maintain low-level voluntary EMG activation of the TA, while also maintaining posture-related activation of the soleus within a specified target. For the TA, no difference was observed in TMS-evoked MEP amplitudes between sitting coactivation and standing coactivation (Fig. 4). Notably, despite providing visual feedback about background EMG, there was a significantly larger TA background EMG during standing coactivation versus sitting coactivation. However, the fact that we failed to show enhancement of TA TMS-evoked MEPs in standing despite the standing posture showing greater TA background EMG than sitting, further supports our conclusion about lack of enhancement of TA corticomotor excitability during standing.

We observed larger soleus MEPs during standing coactivation compared to sitting coactivation, and soleus background EMG was similar for both postural conditions (Fig. 4). The increase in soleus corticomotor excitability observed during standing coactivation versus sitting coactivation merits more study, and may be hypothesized to be related to several factors. During standing coactivation, the juxtaposition of subcortically-driven postural activation and cortically-driven motor control for maintaining the ongoing

background EMG within the prescribed target window may result in larger TMS-evoked MEPs. To maintain soleus EMG within the target EMG activation window, the standing coactivation task may require more postural compensatory adjustments of soleus activation, which are cortically-mediated (Taube et al., 2006). During standing coactivation, to activate the right TA, participants may demonstrate a compensatory weight-shift to the contralateral leg and increase in left soleus activation, which could lead to facilitation of right soleus MEPs (Smith et al., 2017). Notably, the enhancement of corticomotor excitability during standing coactivation versus sitting coactivation was seen in soleus but not TA, providing support for differential neural control mechanisms for extensor and flexor muscles.

4.3. Compared to standing, standing coactivation condition showed larger MEPs and reduced trial-to-trial MEP variability

We compared TMS-evoked MEPs during two standing conditions requiring different types of muscle activation – standing and standing coactivation. For both TA and soleus, transitioning from standing (only soleus active) to standing coactivation (both TA and soleus active) resulted in larger TMS-evoked MEPs and reduced trial-to-trial CVs (Fig. 5). The enhancement of TA MEPs during standing coactivation is consistent with the well-reported increased corticospinal excitability when the target muscle is pre-activated (Kischka et al., 1993). Larger soleus MEPs, in contrast, occurred despite similar soleus background EMG for both conditions, and may be caused by greater activation of the antagonist (TA) during standing coactivation (Geertsen, Zuur, & Nielsen, 2010). Also, in contrast to normal standing, which requires a more ‘automatic’, subcortically-mediated postural activation of the soleus, standing coactivation requires more deliberate, cortically-mediated control of EMG activation, which may contribute to larger MEP amplitudes. The larger soleus MEPs during standing coactivation may also reflect the important role played by the soleus in stabilizing postural sway during dynamic postural tasks. Compared to standing, the standing coactivation condition demonstrated reduced trial-to-trial MEP variability. The reduction in soleus MEP variability during standing coactivation compared to standing is likely caused due to the coactivation task providing greater control of background muscle activation for the agonist and antagonist muscles surrounding the ankle. Our analysis of MEP change scores revealed that among the 4 testing conditions evaluated in the current study, transitioning from standing to standing coactivation may be the best methodological strategy to enhance MEP amplitudes (Fig. 6). For future LL TMS studies, especially in individuals with neurologic disorders who show elevated TMS motor thresholds, the standing coactivation task may provide the dual advantage of greater soleus excitability and reduced trial-to-trial variability.

4.4. Methodological implications of our findings for TMS evaluations of LL muscles

In addition to addressing the research gap related to the effect of posture on cortical excitability of LL muscles, our findings have implications for future TMS studies. Here, in contrast to previous investigations of corticomotor excitability of LL muscles, we systematically compared coil-position errors. Although significantly greater coil-position errors were observed during standing, the differences in coil position error between standing and sitting were of small (and potentially negligible) magnitudes (<0.5 degree and 0.5 mm error). Physical parameters of the coil (location, tilt, rotation) are major non-physiological

factors influencing MEP amplitude (Schmidt et al., 2015), and may require greater consideration during LL TMS. Our findings provide support for incorporating measurements in the standing posture during LL TMS studies. No significant differences in trial-to-trial CV in MEP amplitude were observed between standing and sitting, supporting the reliability of TMS data collected in standing. The standing coactivation task introduced may be advantageous as a testing condition due to the larger MEP amplitudes and smaller trial-to-trial MEP variability observed. The standing coactivation condition may help increase the probability of generating a TA or soleus MEP before maximal stimulator intensity is reached in individuals post-stroke who are unable to voluntarily activate ankle muscles in sitting. Additionally, assessment of corticospinal excitability in a coactivation state may have clinical relevance due to prevalence of greater agonist-antagonist coactivation in individuals with neurological lesions such as stroke (Lamontagne et al., 2002). Moreover, compared to seated or supine postures, TMS-derived measures of corticomotor excitability obtained in the standing posture may be more task-specific, relevant, and sensitive to neuroplasticity induced by gait training interventions. TMS assessments for LL muscles in functionally-relevant postures are lacking in the literature, and our current study data can guide the development of methodological strategies for increasing the probability of inducing MEPs from LL muscles, a major methodological challenge for LL TMS. For example, Smith et al. recently demonstrated that activating the contralateral leg muscles may help enhance the probability of eliciting TMS-evoked MEPs from LL muscles (Smith et al., 2017). We posit that future TMS studies that include MEP measurements from LL muscles during a range of tasks/postures (e.g. seated, contralateral activation (Smith et al., 2017), standing coactivation) may reveal new insights about LL corticomotor control mechanisms. Standing and standing coactivation are postural tasks with clinical relevance, and our examination of how coactivation and posture influence TMS induced MEP amplitudes provides valuable normative data for the field, and lays foundations for further study in individuals with neurological lesions.

4.5. Study limitations

Although we attempted to maintain consistent foot position and joint alignment during the experiment, TMS data collected in the standing posture may be influenced by variations in weight-bearing asymmetry, postural sway, and activation of hip, knee, and trunk muscles. We controlled for background activation of the targeted (right) ankle muscles, but without EMG data from the contralateral leg or center of pressure data regarding weight-bearing, controlling these factors during standing presents a challenge. Despite these sources of variation, however, we did not detect a significant increase in trial-to-trial CV of MEPs collected during standing versus sitting. We evaluated rMT in the seated condition, and identical suprathreshold stimulation intensities were delivered for each participant during sitting and standing, with the assumption that, consistent with previous findings (Obata et al., 2009), there would be no difference in rMT between sitting and standing. TMS was delivered to the same hotspot location for TA and soleus, instead of attempting to isolate separate hotspot locations for each muscle. Because rMT was not determined separately for soleus, we cannot determine where the TMS intensity used in our study was located with respect to TMS input-output curves of the soleus muscle (Lavoie et al., 1995; Obata et al., 2009). TMS-evoked MEP amplitudes could be influenced by modulation of excitability

within the cortex (volitional drive, intra-cortical inhibition/facilitation), descending pathways (reticulospinal pathway activation, pre-synaptic inhibition), as well as excitability of spinal interneurons and lower motoneurons. In an attempt to identify the specific site and mechanism underlying the effects of posture and coactivation, future studies may evaluate the effects of age (Papegaaij, Taube, Baudry, Otten, & Hortobagyi, 2014), postural challenge (Papegaaij et al., 2016), and pathology, as well as additional measures such as cortical silent period and MEP duration (Brum, Cabib, & Valls-Sole, 2015).

5. Conclusions

When background EMG activation was matched between sitting and standing, no enhancement of corticomotor excitability of the TA or soleus muscles was observed during standing. Compared to sitting with TA-soleus coactivation, standing with coactivation of TA and soleus resulted in larger MEP amplitudes for the soleus, but not the TA. Compared to quiet standing, the standing coactivation condition provided the advantage of larger MEP amplitudes as well as smaller trial-to-trial variability in MEP amplitudes. Further investigation is needed to evaluate the influence of neurological lesions such as stroke and gait rehabilitation on posture-related effects on cortico-motor excitability of LL muscles.

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Fig. 1. Testing conditions. The schematic depicts the posture (sitting, standing) and muscle activation (background EMG activation for TA and soleus) for the 4 conditions tested in our study.

Fig. 2.

Raw data for the TA (left panels) and soleus (right panels) for each of the 4 testing conditions. Individual MEPs (gray) and the averaged MEP trace (black) from a single representative subject for the sitting (a), sitting coactivation (c), standing (e), and standing coactivation (g) for the TA are shown in the left column. Individual MEP traces (gray) with the averaged MEP (black) from a single representative subject for the sitting (b), sitting coactivation (d), standing (f), and standing coactivation (h) for the Soleus are shown in the right column. 12-ms latency between TMS delivery and the TMS artifact. Background EMG

activation was calculated from the 50-ms window before delivery of TMS (between –50 and 0 ms).

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Fig. 3.

Comparison between sitting and standing. (a–b) Box and whiskers plots representing the group data ($N = 12$) for the TA (a) and soleus (b) MEP amplitudes. Maximum and minimum amplitude values are shown by the whiskers, with the upper box representing data points between the median and 75th quartile and the lower box representing data points between the median and the 25th quartile. Average group data represented by filled circles. (c–d) Change scores for MEP amplitudes for the TA (c) and soleus (d) represent the difference in MEP amplitude between the standing and sitting for each individual subject (empty circles) and the average change score (filled circle $\pm$ 95% CI). For both muscles, the 95% CI included zero. (e–f) Average EMG background RMS for the TA (e) and SOL (f) during the 50-ms window prior to the TMS trigger, with no significant difference in background EMG between conditions for TA or soleus.

Fig. 4. Comparison between sitting coactivation and standing coactivation. (a–b) Box and whiskers plots representing the group data ($N = 13$) for MEP amplitudes are shown for TA (a) and soleus (b). Maximum and minimum MEP amplitude values are shown by the whiskers, with the upper box representing data points between the median and 75th quartile and the lower box representing data points between the median and the 25th quartile. Average group data represented by the filled circles. *Significantly larger soleus MEP amplitude during standing coactivation versus sitting coactivation ($p < 0.05$). (c–d) Change score plots for the TA (c) and soleus (d), representing the difference in MEP amplitude between the standing coactivation and sitting coactivation conditions, show values for individual subjects (empty circles) and the average change score (filled circle $\pm$ 95% CI). 95% CI of the change scores for TA included zero, but for soleus did not include zero. (e–f) Bar plots showing mean

background EMG RMS for TA (e) and SOL (f) during a 50 ms window prior to the TMS trigger. *Significant increase in the TA background during standing coactivation versus sitting coactivation ($p < 0.05$). No difference in soleus background between conditions.

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Fig. 5.

Comparison between standing and standing coactivation. (a–b) Box and whiskers plots show the group data ($N = 12$) for MEP amplitudes for the TA (a) and Soleus (b). Maximum and minimum MEP amplitude values are shown by the whiskers, with the upper box representing data points between the median and 75th quartile and the lower box representing data points between the median and the 25th quartile. Average is represented by the filled circles. *Both TA and soleus showed significant increases in MEP amplitude during standing coactivation versus standing ($p < 0.05$). (b–c) Scatter plots showing the change scores for TA (c) and soleus (d) MEP amplitudes (difference in MEP amplitude between the standing coactivation and standing conditions) for each participant (empty circles) and the average change score (filled circle $\pm$ 95% CI). For both TA and soleus, the

95% CI for change scores did not include zero. (e–f) Bar plots showing mean background EMG RMS for TA (e) and Soleus (f) during the 50-ms prior to the stimulation. Consistent with the requirements of the testing conditions, there was a significant increase in TA background EMG during standing coactivation versus standing. No difference in soleus background EMG between conditions.

Fig. 6.

MEP change scores for the 3 comparisons. Bar-plots showing the mean $\pm$ standard deviation ($N = 12$) for MEP change scores for TA (left) and soleus (right). The 3 bars indicate the change (or modulation) of MEP amplitude for each of the 3 comparisons conducted in the study: (1) standing minus sitting, 2) standing coactivation minus sitting coactivation, and 3) standing coactivation minus standing. *indicates a significant difference in MEP change score ($p < 0.05$).