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Knee extension motor imagery paired with single-pulse TMS facilitates corticospinal excitability in the tibialis anterior muscle, but not in the rectus femoris muscle

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Keywords

Motor imagery, Transcranial magnetic stimulation, Corticospinal excitability, Rectus femoris muscle, Tibialis anterior muscle, Motor evoked potential

Abbreviations

ANOVA, analysis of variance; BEMG, background electromyograph; BF, biceps femoris; EMG, electromyograph; LTP, long-term potentiation; MEP, motor evoked potential; MG, medial gastrocnemius; MI, motor imagery; MI+TMS, motor imagery and transcranial magnetic stimulation; MI-only, MI alone; MVC, maximum voluntary contraction; PAS, paired associative stimulation; Post 0, 0 minutes after MI+TMS; Post 15, 15 minutes after MI+TMS; Post 30, 30 minutes after MI+TMS; Pre, before MI+TMS; RF, rectus femoris; RMS, root mean square; rMT, resting motor threshold; SD, standard deviation; SOL, soleus; TA, tibialis anterior; TMS, transcranial magnetic stimulation; tsDCS, transcutaneous spinal direct current stimulation; VL, vastus lateralis

Abstract

The rectus femoris (RF) muscle is recruited during a wide range of movements and restoring its function is relevant to both rehabilitation and sports performance. However, previous studies have reported difficulty in inducing facilitatory changes in corticospinal excitability of the RF muscle. We investigated whether and how a method combining motor imagery and transcranial magnetic stimulation (MI+TMS), which has been effective for low plasticity muscles in the upper limbs, modulates the corticospinal excitability in the RF muscle and other lower limb muscles. Twenty healthy participants underwent an MI+TMS protocol consisting of 120 repetitions of knee extension MI paired with single-pulse TMS. Motor evoked potentials (MEPs) were recorded from the RF, biceps femoris (BF), tibialis anterior (TA), and soleus (SOL) muscles at baseline (Pre), immediately after (Post 0), and at 15 (Post 15) and 30 minutes (Post 30) post-MI+TMS. MI+TMS did not significantly modulate MEPs in the RF muscle, an agonist for knee extension, and RF muscle activity during maximum voluntary contraction was significantly reduced. By contrast, MEPs in the TA muscle were significantly increased at Post 0 and Post 15 compared to Pre, whereas no significant changes were observed in the BF and SOL muscles. Additional measurements with 15 participants in the MI-only condition showed no significant changes in MEP amplitude in any muscle. These findings indicate that MI+TMS facilitates corticospinal excitability in a muscle specific manner and suggest that the corticospinal pathway to the RF muscle may be less susceptible to facilitatory plastic changes.

1. Introduction

The rectus femoris (RF) muscle is involved in knee extension and hip flexion and therefore plays a critical role in daily activities such as walking, standing, and jumping, as well as in athletic performance. Because impairments in the neuromuscular function of the RF muscle are closely associated with movement disorders and the risk of falls (Carter et al. 2002), developing effective methods to improve its neuromuscular function is clinically important.

One potential target for such interventions is the corticospinal tract, which conveys motor commands from the primary motor cortex to the muscles via the spinal cord and whose excitability can be plastically modulated through repeated training and rehabilitation (Gómez-Feria et al. 2023). A range of approaches has been used to modulate corticospinal excitability, including transcranial magnetic stimulation (TMS), which applies external stimulation to motor-related cortical areas (Hand et al. 2023), and peripheral afferent stimulation to muscles or nerves (Knash et al. 2003; Mang et al. 2011). These interventions have frequently been shown to alter the amplitude of motor-evoked potentials (MEPs), which are commonly used as an index of corticospinal excitability. In addition, paired associative stimulation (PAS), which combines cortical TMS with peripheral afferent input at specific intervals, has also been used to modulate corticospinal excitability (Stefan et al. 2000).

Despite these advances, no neuromodulation techniques have been reported that facilitate corticospinal excitability in the RF muscle. For example, in the lower limbs, PAS increased corticospinal excitability in the biceps femoris (BF), tibialis anterior (TA), soleus (SOL), and medial gastrocnemius (MG) muscles, whereas no significant changes were observed in the RF and vastus lateralis (VL) muscles (Kaneko et al. 2021). These findings suggest that inducing plasticity in the corticospinal pathway to the RF muscle may be more difficult than in other lower limb muscles. In line with these findings, studies using transcutaneous spinal direct current

stimulation (tsDCS) have also reported no significant modulation of MEPs in the quadriceps femoris, including the RF muscle, compared with sham stimulation (Stępień et al. 2024). Although tsDCS acts through spinal reflex and afferent circuits and has been shown to facilitate corticospinal excitability in upper limb muscle (Lim and Shin 2011), it has not produced comparable effects in the RF muscle. Taken together, these findings suggest that the optimal approach for inducing plasticity may differ across muscles and that alternative strategies are needed to facilitate corticospinal excitability in the RF muscle.

The approach proposed by Foysal and Baker (2020) may be effective for this purpose. They applied a combined TMS and motor imagery (MI) approach to the wrist extensors, which had failed to exhibit plastic changes with PAS (Foysal and Baker 2019), and succeeded in increasing corticospinal excitability. MI is a mental simulation or rehearsal of a movement without any motor output and reflects internal activation of motor-related cortical regions. Indeed, MI elicits neural activity similar to that during actual movement (Decety et al. 1994; Hanakawa et al. 2003). Therefore, combining MI with TMS may provide a useful strategy for inducing plastic changes in the RF muscle. MI+TMS may be particularly promising for the RF muscle because our previous study (Ishikawa et al. 2024) revealed that the modulation of corticospinal excitability in lower limb muscles during MI depends on the target muscle and the type of MI. In particular, MEPs in the RF muscle increased significantly during knee extension MI, indicating that the corticospinal excitability in the RF muscle is facilitated during MI.

Therefore, this study aimed to investigate whether MI+TMS can facilitate corticospinal excitability in the RF muscle, in which the induction of plasticity appears to be difficult. If MI+TMS fails to significantly modulate the corticospinal excitability in the RF muscle, this would support the hypothesis that the corticospinal pathway to the RF muscle may have limited plasticity. Conversely, if MI+TMS is effective, it may represent a novel neurorehabilitation strategy for

targeting the RF muscle.

2. Materials and Methods

2.1 Participants

A total of thirty healthy individuals with no history of neurological or musculoskeletal disorders participated in this study. Twenty participants (5 females, age: 24.8 ± 2.0 years, height: 171.0 ± 6.6 cm, weight: 66.4 ± 10.8 kg; mean $\pm$ standard deviation [SD]) participated in the MI+TMS condition, and 15 participants (3 females, age: 22.3 ± 3.2 years, height: 170.2 ± 2.6 cm, weight: 60.4 ± 9.8 kg; mean $\pm$ standard deviation [SD]) participated in the MI alone condition. Five participants participated in both conditions. All participants received verbal and written explanations and gave written consent before participating in the experiment. All experimental procedures were approved by the local ethics committee of the University of Tokyo (approval numbers: 831-2). This study was performed following the Declaration of Helsinki (2013).

2.2 Data Acquisitions

2.2.1 Electromyographic (EMG) Activity

Electromyographic (EMG) activity was recorded from the right RF, BF, TA, and SOL muscles. All participants verbally confirmed that their right foot was dominant. After cleaning the skin with alcohol to reduce impedance, two bipolar Ag/AgCl surface electrodes (Vitorode F-150S, Nihon Kohden, Japan) were placed following the SENIAM recommendations (Hermens et al. 2000). A ground electrode was placed around the right knee. All EMG signals were filtered with a band-pass filter (5-1000 Hz) and amplified ($\times 1000$) using a multi-channel amplifier (MEG-6108, Nihon Kohden, Japan). The signals from 100 ms before the TMS to 300 ms after were digitized at a sampling frequency of 4000 Hz using an analog-to-digital (A/D) converter (PowerLab/16SP, ADInstruments, Australia) and stored on the computer for offline analysis.

2.2.2 Transcranial Magnetic Stimulation (TMS)

TMS was delivered over the left primary motor cortex using a mono-phasic magnetic stimulator (Magstim200, Magstim Co., UK) with a double cone coil (outside diameter of 110 mm; Magstim Co., UK). The optimal stimulus location was defined as the hotspot where the largest MEPs were elicited from the RF. The coil position and orientation were monitored throughout the experiment using a neuronavigation system (Brainsight, Rogue Research, Canada) to ensure the exact coil placement. The coil's position was kept at the same position for all trials, and all trials were conducted within an error of 1 mm. The resting motor threshold (rMT) was defined as the minimum TMS intensity for which MEPs had peak-to-peak amplitudes $> 50 \mu\text{V}$ and were evoked in at least five of ten successive trials (Rossini et al. 2015). In MI+TMS and MEPs evaluation sessions, all the experimental stimulus intensity was set at 120% of the rMT level, which generally corresponds to the upward phase of the TMS recruitment curve (Temesi et al. 2014). This upward phase is thought to be sensitive to reflect facilitatory or inhibitory effects of corticospinal excitability. MEPs were measured from four lower limb muscles.

2.3 Experimental Procedures

Participants were seated comfortably on a chair (Fig. 1A). Twelve MEPs were recorded at each of the following time points: before the MI+TMS (Pre), and at 0 min (Post 0), 15 min (Post 15), and 30 min (Post 30) post-MI+TMS (Fig. 1B). Before starting the experiment, a recruitment curve was recorded to confirm the validity of the stimulus intensity. MEPs were obtained five times at each intensity ranging from 90% rMT to 140% rMT in 10% increments. In the recruitment curve, the MEPs elicited by 120% rMT were confirmed to be located in the ascending phase. In the MI+TMS condition, participants were instructed to avoid any muscle contraction and to alternate between the resting and MI states every 4 seconds. In the resting state, they were asked to relax without internal imagery. In the MI state, they performed MI of maximal

effort knee extension, initiated by auditory and visual cues (“Motor Imagery”). TMS was applied during the MI state, and this concurrent application of TMS and MI was repeated 120 times. The rest and MI states alternated every 4 seconds, resulting in an 8-second cycle that was repeated 120 times. The duration of the MI+TMS was approximately 16 minutes. The maximum voluntary contraction (MVC) of knee extension was also measured at each time point. The MVC of knee extension was measured by manually stabilizing the knee joint to prevent movement and recording muscle activity during a 3-second maximal isometric contraction. Additionally, after all evaluations were finished, MVC of knee flexion, foot dorsiflexion, and plantar flexion were measured.

As the present study used a combined MI and TMS, an investigation was also conducted to determine whether a condition with MI alone (MI-only) modulates the lower-limb corticospinal excitability. Their lower limb MEPs were recorded before and after 120 repetitions of knee extension MI. The experiment was performed with the same procedures as in the MI + TMS condition: subjects alternated between the MI state, in which they performed maximum effort knee extension MI for 4 seconds, and the resting state, in which they did not imagine anything for 4 seconds. To investigate the effect of MI alone, TMS was not performed during the MI state. MEPs were evaluated before the MI-only (Pre), and at 0 min (Post 0), 15 min (Post 15), and 30 min (Post 30) after MI-only.

2.4 Data Analysis

All signals were analyzed using a custom-written script in MATLAB (MATLAB 2022a, The MathWorks Inc., Natick, MA, USA). MEPs peak-to-peak amplitudes in the RF, BF, TA, and SOL muscles were calculated for each trial. Muscles for which the average MEPs amplitude at Pre was less than 50 μ V were excluded from the analysis. As a result, the number of analyses conducted were RF: $n = 20$, BF: $n = 8$, TA: $n = 19$, and SOL: $n = 15$ for the MI+TMS condition,

and RF: n = 15, BF: n = 7, TA: n = 15, and SOL: n = 13 for the MI-only condition. The root mean square (RMS) of EMG signals measured during MVC was calculated for each 50-ms window, and the maximum RMS value was used as the MVC in each muscle. The EMG RMS value of the 50 ms before TMS was defined as the background EMG (BEMG) activity for each muscle. Trials with raw BEMG (RMS) > 10 μ V were removed (De Luca 2003). At each time point, the remaining MEPs amplitudes and BEMG were averaged. The BEMG was normalized according to the EMG activity during MVC.

2.5 Statistical Analysis

All statistical analyses were conducted using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan). The statistical analysis included MEPs as a marker of corticospinal excitability. Since BEMG affects MEPs, a statistical analysis of BEMG activity was performed to ensure that BEMG did not differ between time points.

The Shapiro-Wilk test showed that the normalized MEPs amplitudes and normalized BEMG according to MVC (%MVC) were not normally distributed. For the normalized MEPs amplitudes in the RF, BF, TA, and SOL muscles, the Friedman test, a non-parametric equivalent for repeated-measures analysis of variance (ANOVA), was used to compare MEPs at Pre, Post 0, Post 15, and Post 30. If the Friedman test revealed a significant effect, multiple comparisons using the Steel test, a non-parametric equivalent for the Dunnett test, were conducted to compare MEPs at Pre versus Post 0, Post 15, and Post 30. The same statistical method was used for the EMG of the RF during knee extension MVC. The significance level for all tests was set to $p < 0.05$. The same statistical methods were applied to the MEPs amplitudes, BEMG, and RF EMG during knee extension MVC in the MI-only condition.

3. Results

Table 1 summarizes the non-normalized and normalized RMS values for BEMG activity in the RF, BF, TA, and SOL muscles before TMS. MVC values for the RF muscle at Pre, Post 0, Post 15, and Post 30 were 0.39 ± 0.10 mV, 0.38 ± 0.14 mV, 0.36 ± 0.12 mV, and 0.37 ± 0.14 mV, respectively in the MI+TMS condition. MVC values for the BF, TA, and SOL muscles were 0.34 ± 0.21 mV, 0.58 ± 0.24 mV, and 0.27 ± 0.17 mV, respectively. For the normalized BEMG activity (%MVC) of all the recorded muscles, the Friedman test showed no significant difference between the time points [RF: $\chi^2(3) = 4.35$, $p = 0.223$; BF: $\chi^2(3) = 3.78$, $p = 0.286$; TA: $\chi^2(3) = 1.26$, $p = 0.739$; SOL: $\chi^2(3) = 5.58$, $p = 0.134$]. For the MI-only condition, the Friedman test also showed no significant difference in the normalized BEMG activity of all muscles [RF: $\chi^2(3) = 4.26$, $p = 0.235$; BF: $\chi^2(3) = 2.97$, $p = 0.396$; TA: $\chi^2(3) = 2.30$, $p = 0.513$; SOL: $\chi^2(3) = 2.22$, $p = 0.528$].

Table 2 summarizes the average MEPs amplitudes in the RF, BF, TA, and SOL muscles at each time point. Fig. 2 indicates the group mean of the normalized MEPs amplitudes for each muscle. For the normalized MEPs amplitudes of the RF, BF, and SOL muscles, the Friedman test showed no significant difference between the time points [RF: $\chi^2(3) = 1.50$, $p = 0.682$; BF: $\chi^2(3) = 3.75$, $p = 0.290$; SOL: $\chi^2(3) = 3.56$, $p = 0.313$]. For the normalized TA MEPs amplitudes, the Friedman test showed a significant effect between the time points [$\chi^2(3) = 8.49$, $p = 0.037$]. Steel tests showed that the normalized MEPs amplitudes at Post 0 and Post 15 were significantly increased compared with those at Pre [Post 0, $t = 2.7$, $p = 0.02$; Post 15, $t = 2.7$, $p = 0.02$]. There was no significant difference in MEPs amplitudes between at Pre and at Post 30 [$t = 2.1$, $p = 0.09$].

Table 3 summarizes the normalized RMS values of EMG activities in the RF muscle during knee extension MVC. Fig. 3 indicates the group mean of the normalized RMS values for the RF muscle. The Friedman test showed a significant effect between the time points [$\chi^2(3) = 15.6$, $p = 0.001$]. The RMS of EMG in the RF muscle during knee extension MVC was significantly smaller immediately after and 15 minutes after the MI+TMS than before the

MI+TMS [Post 0, $t = 2.9$, $p = 0.01$; Post 15, $t = 4.6$, $p = 0.00001$]. There was no significant difference in MVC between at Pre and at Post 30 [$t = 1.7$, $p = 0.20$].

Fig. 4 shows the MEP results for the MI-only condition. The Friedman test showed no significant differences in the normalized MEPs amplitudes between the time points in any muscle [RF: $\chi^2(3) = 4.85$, $p = 0.183$; BF: $\chi^2(3) = 1.63$, $p = 0.653$; TA: $\chi^2(3) = 7.25$, $p = 0.064$; SOL: $\chi^2(3) = 4.50$, $p = 0.212$]. Fig. 5 shows the results of the RF muscle activity during the knee extension MVC task in the MI-only condition, and the Friedman test showed no significant difference between the time points [$\chi^2(3) = 5.68$, $p = 0.128$] (Fig. 5).

4. Discussion

After 120 repetitions of knee extension MI combined with single-pulse TMS, the MEP amplitude of the RF muscle showed no significant change at any post-intervention time point (Fig. 2), despite optimizing the MI task and TMS parameters for the RF muscle (Fig. 1). In contrast, MEPs amplitudes of the TA muscle significantly increased at Post 0 and Post 15, while those of the BF and SOL muscles were not significantly modulated. Background EMG activity during the pre-stimulus period remained consistently low and did not differ significantly between time points in any muscle (Table 1), indicating that the observed MEP changes were unlikely to be attributable to differences in pre-stimulus muscle activation. Additionally, RF muscle activity during knee-extension MVC was significantly reduced at Post 0 and Post 15.

The absence of significant modulation of corticospinal excitability in the RF muscle in the present study is consistent with the findings of Kaneko et al. (2021).

In that study, PAS, which was optimized for the RF muscle and combined transcutaneous spinal cord stimulation with TMS, also failed to modulate RF corticospinal excitability, despite successfully facilitating excitability in the BF, TA, SOL, and MG muscles.

Taken together, these findings suggest that facilitatory effects are more difficult to induce in the corticospinal pathway to the RF muscle than in other lower limb muscles. Because both PAS and MI+TMS were designed to induce facilitatory, long-term potentiation (LTP)-like plastic changes in corticospinal excitability, this pattern raises the possibility that the corticospinal pathway to the RF muscle is less susceptible to LTP-like plasticity.

One possible anatomical explanation for this interpretation relates to the proximal location and postural function of the RF. In general, the corticospinal tract preferentially innervates the distal limb muscles involved in fine voluntary control (Lawrence and Kuypers 1968; Porter and Lemon 1995). In contrast, proximal muscles engaged in postural control and antigravity function are thought to rely more heavily on reticulospinal input, which originates in the brainstem reticular formation (Lawrence and Kuypers 1968). Because MI and TMS primarily target the cortical and corticospinal circuits, these methods may have limited access to the neural pathways that contribute importantly to the motor control of the RF muscle. This may partly explain the limited LTP-like plastic changes observed in the corticospinal pathway to the RF muscle compared with muscles with stronger corticospinal innervation.

Another possible explanation for the non-significant group-level result is substantial inter-individual variability in responsiveness, rather than a uniform absence of plasticity. Fig. 2 shows a wide range of changes in the RF MEPs at Post 0. Some participants exhibited marked increases relative to baseline (200–330%), while others showed no change or a decrease. This heterogeneous pattern, which contrasts with the more uniform TA MEP changes, is consistent with previous reports showing considerable inter-individual variability in responsiveness to neuromodulatory interventions (Müller-Dahlhaus et al. 2008). This pattern may also reflect inter-individual differences in the relative contribution of descending pathways innervating the RF muscle. In particular, participants with comparatively stronger corticospinal projections may have

273 been more responsive to MI+TMS, which internally and externally stimulates the contralateral
274 corticospinal pathway to the RF muscle, than those relying more heavily on reticulospinal or
275 ipsilateral corticospinal pathways. This interpretation is consistent with known inter-individual
276 variability in corticospinal tract morphology (D'Amico et al. 2020) and with anatomical
277 evidence suggesting that the RF muscle is less strongly innervated by the corticospinal tract than
278 distal muscles (Eisner-Janowicz et al. 2023), although it remains speculative in the absence of
279 direct neuroimaging evidence. Taken together with the previous PAS findings, the present results
280 suggest that facilitatory plastic changes in the RF corticospinal pathway may be less consistently
281 induced by these protocols than in some other lower limb muscles, with inter-individual
282 variability as an additional contributing factor. Future studies with larger samples and individual-
283 level predictors, such as descending tract morphology assessed by diffusion tensor imaging or
284 baseline MEP characteristics, will be necessary to clarify these possibilities. Importantly, the
285 present findings primarily concern LTP-like plastic changes, and it remains unclear whether the
286 same interpretation applies to long-term depression-like plasticity in the corticospinal pathway to
287 the RF muscle.

288 Although the present results indicate that MI+TMS does not reliably induce LTP-like
289 changes in the contralateral corticospinal pathway to the RF muscle at the group level, this does
290 not preclude the possibility that RF function may be improved through alternative neural
291 mechanisms. Reports from Paralympic athletes suggest that functional reorganization involving
292 the RF muscle can occur (Nakazawa 2022). For example, during movement with a prosthesis,
293 athletes with an amputation showed activation of the motor cortex ipsilateral to the amputated
294 limb, despite the fact that voluntary movement is typically controlled predominantly by the
295 contralateral corticospinal pathway. Such reorganization is likely shaped by both disability-related
296 factors and long-term repetitive training. These observations raise the possibility that, in the RF

muscle, plastic changes may occur more readily in descending pathways other than the contralateral corticospinal tract, including the ipsilateral corticospinal and reticulospinal pathways. If so, these pathways may represent alternative targets for future interventions aimed at improving RF function.

The significant decrease in the RF muscle activity during knee-extension MVC after MI+TMS may be consistent with central fatigue induced by repetitive MI and TMS (Fig. 3). This interpretation is consistent with a previous upper-limb study showing that repeated motor imagery reduced muscle activity during the MVC (Nakashima et al. 2021). For some participants, this central fatigue may have attenuated the facilitatory effects of MI+TMS on corticospinal excitability. In the MI-only condition, no obvious change in muscle activity during knee-extension MVC was observed.

In contrast to the RF muscle, corticospinal excitability in the TA muscle was significantly increased at Post 0 and Post 15 following MI+TMS (Fig. 2). Our previous study demonstrated that corticospinal excitability in the TA muscle was facilitated during knee-extension MI, although this effect was no longer significant after correction for multiple comparisons (Ishikawa et al. 2024). This suggests that the corticospinal pathway innervating the TA muscle was already in a facilitated state during the MI task used in the present study. The combination of this facilitated state and repeated TMS pulses may have induced LTP-like plastic changes in the TA corticospinal pathway. One possible explanation for the increased corticospinal excitability is the proximity of the TA and RF representations within the primary motor cortex. Accordingly, knee-extension MI may have influenced not only the RF representation but also neighboring cortical regions associated with the TA muscle, consistent with spillover effects reported in the upper-limb (Foysal and Baker 2020). In addition, because TMS delivered at the RF hotspot also elicited measurable TA MEPs in the present study, repeated MI+TMS may have

stimulated not only the RF-related cortical representation but also adjacent TA-related regions. The relatively strong corticospinal involvement of the TA muscle among lower limb muscles likely also contributed to the consistent plastic response, in line with evidence that corticospinal involvement differs across lower limb muscles and is generally stronger in more distal muscles (Eisner-Janowicz et al. 2023). Taken together, these findings support the idea that MI+TMS can induce plastic changes in corticospinal excitability more readily in muscles receiving greater corticospinal input.

By contrast, the absence of significant modulation in the BF and SOL muscles suggests that such internally generated corticospinal facilitation is not sufficient for all lower limb muscles. MI+TMS did not significantly modulate the corticospinal excitability in the BF and SOL muscles (Fig. 2). This contrasts with our previous findings (Kaneko et al. 2021), in which PAS significantly increased corticospinal excitability in both muscles. Because both protocols included single-pulse TMS, the different outcomes are more likely attributable to the accompanying input than to the cortical stimulation itself. In our previous studies, peripheral nerve stimulation was confirmed to provide sufficient input to the SOL and BF pathways based on reflex responses (Kaneko et al. 2021), whereas knee-extension MI did not produce sufficient facilitatory effects on corticospinal excitability in these muscles (Ishikawa et al. 2024). Together, these findings suggest that, at least under the present conditions, cortical facilitation during knee-extension MI was insufficient to induce plastic changes in the BF and SOL corticospinal pathways.

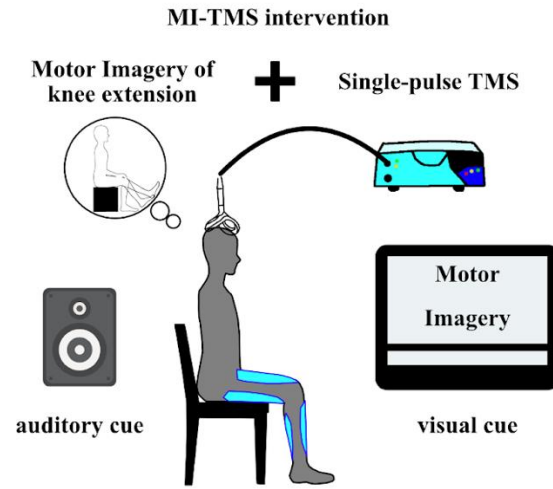
Additional measurements conducted in the MI-only condition revealed no significant change in MEP amplitude in any muscle (Fig. 4), nor in RF muscle activity during the knee extension MVC task (Fig. 5). These findings are consistent with previous research indicating that repeated MI without combined TMS may result in a decrease rather than an increase in corticospinal excitability in upper-limb muscles (Nakashima et al. 2021), and with an earlier study

reporting no significant change in MVC of the elbow flexor muscles following 80 intermittent maximum imagined contractions (Rozand et al. 2014). Together, these findings suggest that the facilitation of TA corticospinal excitability observed following MI+TMS cannot be readily explained by repeated MI alone, although the effects of the two conditions were not directly compared.

5. Conclusion

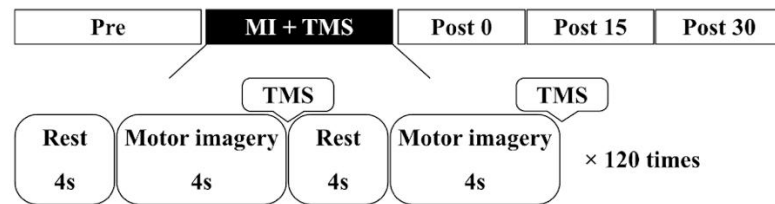
In this study, we investigated whether a combination of knee extension MI and TMS can modulate corticospinal excitability in lower limb muscles with particular focus on the RF muscle. MI+TMS did not significantly modulate corticospinal excitability in the RF muscle, indicating that the corticospinal pathway projecting to the RF muscle may be less susceptible to facilitatory plastic changes. These findings provide insight into muscle-specific differences in neuromodulatory responsiveness and suggest that alternative approaches may be required to facilitate corticospinal excitability in the RF muscle.

A



B

MI + TMS condition



MI - only condition

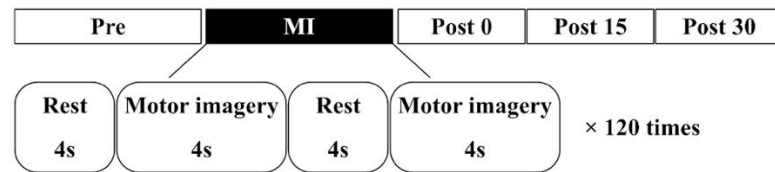


Fig. 1

(A) Experimental setup

Participants were asked to sit on a comfortable chair and perform motor imagery (MI) with auditory and visual cues. MI and rest were alternated every 4 seconds, and TMS was applied during MI. This sequence was repeated 120 times.

(B) Time course

Corticospinal excitability was assessed by TMS before the MI+TMS (Pre), immediately after (Post 0), and at 15 and 30 minutes post-MI+TMS (Post 15 and Post 30).

Table 1 Background EMG before TMS

Average non-normalized (μV) and normalized RMS values (% of MVC) for background EMG in the recorded muscles 50 ms before TMS in each condition.

		Non-normalized RMS value (μV)	Normalized RMS value (%MVC)
Background EMG (RF)	Pre	6.54 ± 3.25	1.87 ± 0.95
	Post 0	5.55 ± 3.33	1.46 ± 0.94
	Post 15	7.88 ± 3.48	2.07 ± 0.89
	Post 30	8.88 ± 4.01	2.40 ± 0.98
Background EMG (BF)	Pre	8.89 ± 5.01	2.26 ± 2.01
	Post 0	9.11 ± 7.81	2.78 ± 3.00
	Post 15	8.12 ± 7.77	2.39 ± 2.92
	Post 30	9.12 ± 9.01	2.68 ± 4.60
Background EMG (TA)	Pre	3.74 ± 2.12	1.61 ± 1.45
	Post 0	3.96 ± 2.62	1.60 ± 1.43
	Post 15	4.48 ± 2.75	1.70 ± 1.56
	Post 30	4.04 ± 2.53	1.56 ± 1.36
Background EMG (SOL)	Pre	5.61 ± 3.84	2.93 ± 3.89
	Post 0	7.70 ± 9.37	4.01 ± 6.92
	Post 15	6.06 ± 4.24	2.79 ± 1.97
	Post 30	6.53 ± 4.16	3.43 ± 3.95

Table 2 Corticospinal excitability of lower limb muscles in MI+TMS condition

Changes in average MEPs amplitudes expressed as a percentage of those measured before MI+TMS (mean $\pm$ standard deviation). Asterisks denote significant differences compared to those measured at Pre (Steel test).

	Post 0	Post 15	Post 30
RF	127.3 ± 65.2	120.4 ± 70.4	135.6 ± 92.0
BF	133.0 ± 75.6	127.9 ± 50.1	172.3 ± 109.8
TA	121.4 ± 60.0 *	122.5 ± 36.1 *	138.2 ± 70.5
SOL	111.1 ± 60.6	111.5 ± 29.9	126.3 ± 70.0

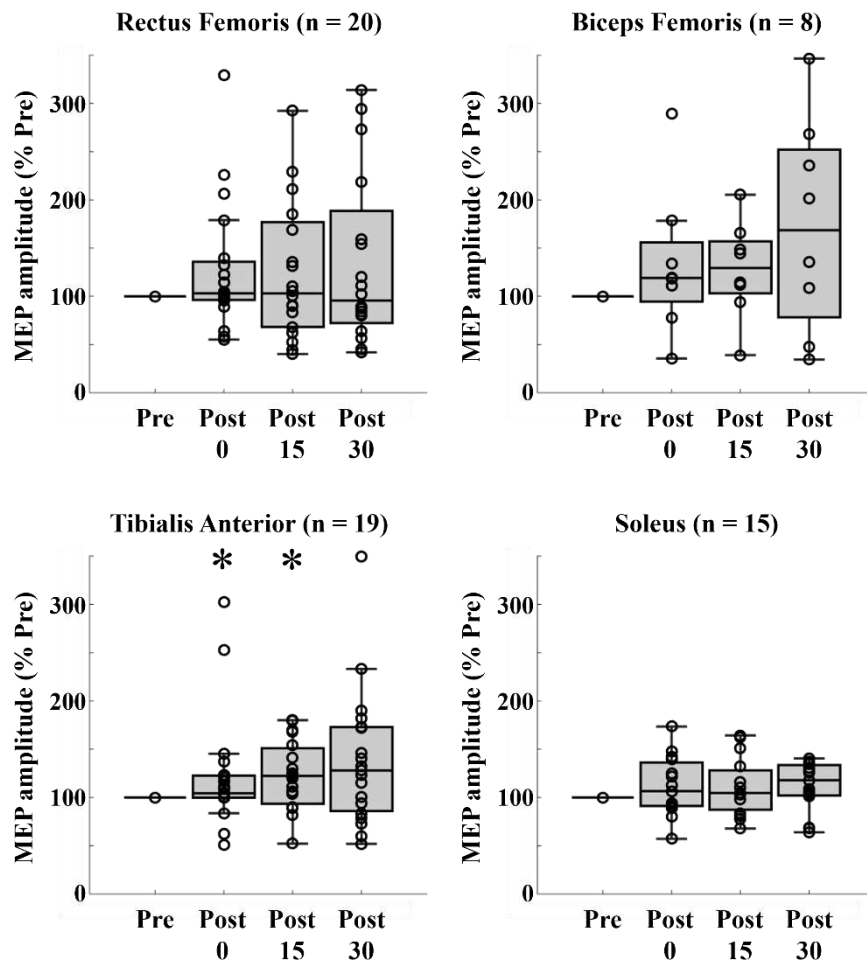


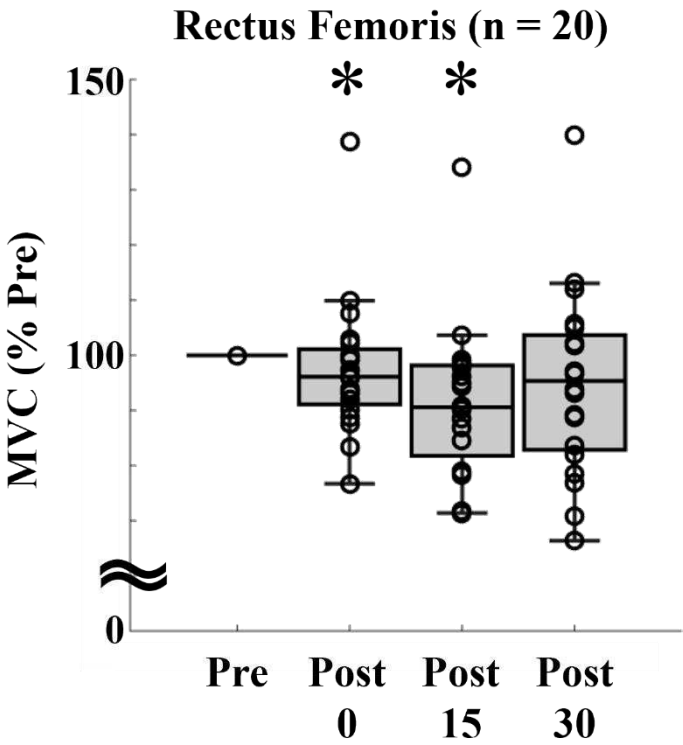
Fig. 2 Corticospinal excitability of lower limb muscles in MI+TMS condition

Each plot displays the average MEPs amplitudes of the RF, BF, TA, and SOL muscles at each time point in MI+TMS condition. MEPs amplitudes were normalized for the MEPs amplitude during the Pre. Asterisks (*) indicate significant differences in MEPs compared to MEPs at Pre (Steel test p-value < 0.05).

Table 3 Muscle activity of RF muscle in MI+TMS condition

Changes in average RMS of RF EMG during knee extension MVC as the percentage of those measured before MI+TMS (mean $\pm$ standard deviation). Asterisks denote significant differences compared to those measured at Pre (Steel test).

	Post 0	Post 15	Post 30
RF	96.1 $\pm$ 12.5 *	90.6 $\pm$ 13.7 *	95.3 $\pm$ 16.8



396

397 **Fig. 3 Muscle activity of RF muscle in MI+TMS condition**

398 Each plot displays the average RMS value of the RF muscle EMG during knee extension MVC
399 at each time point in MI+TMS condition. RMS values were normalized for the RMS value of the
400 RF muscle EMG during knee extension MVC at Pre. Asterisks (*) indicate significant differences
401 in MVC compared to MVC at Pre (Steel test p-value < 0.05).

402

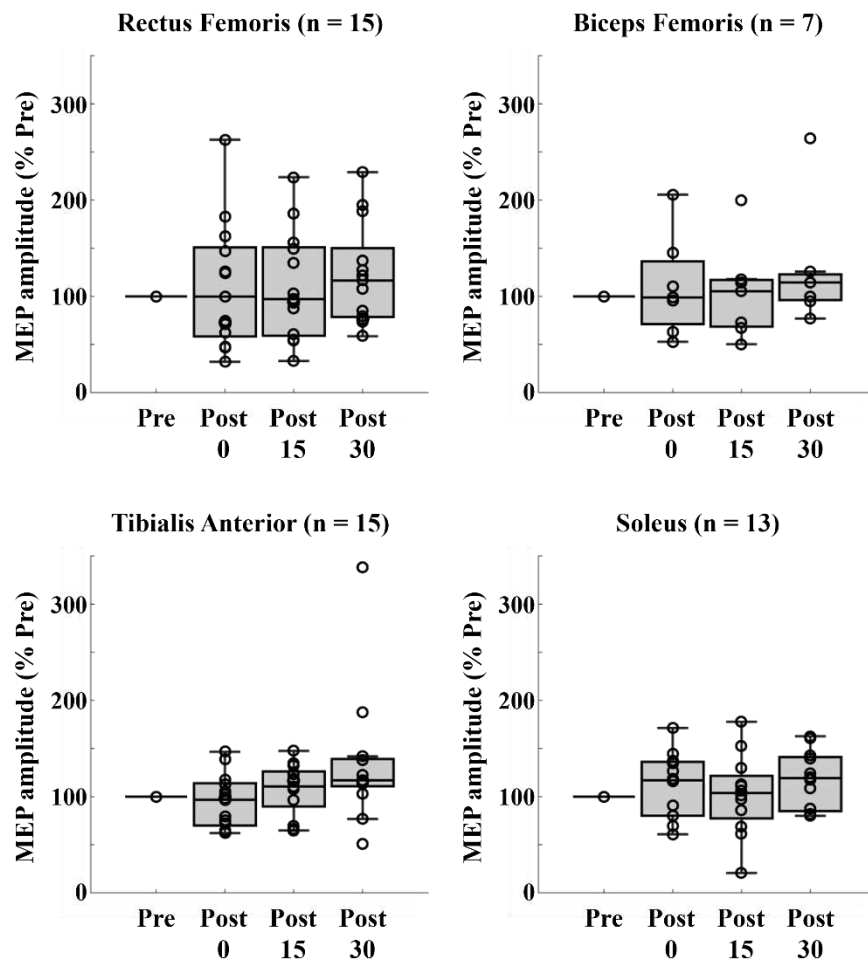
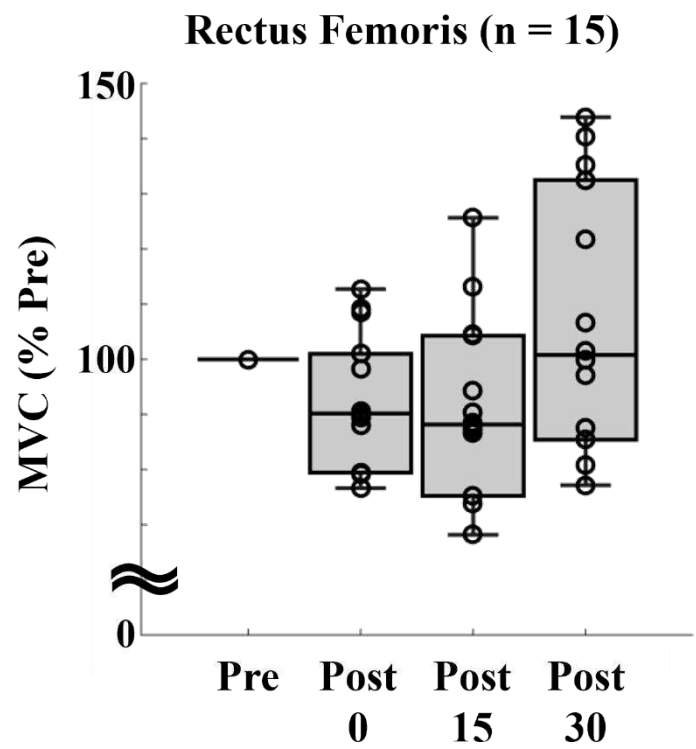


Fig. 4 Corticospinal excitability of lower limb muscles in MI-only condition

Each plot displays the average MEPs amplitudes of the RF, BF, TA, and SOL muscles at each time point in MI-only condition. MEPs amplitudes were normalized for the MEPs amplitude during the Pre.

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413 **Fig. 5 Muscle activity of RF muscle in MI-only condition**

414 Each plot displays the average RMS value of the RF muscle EMG during knee extension MVC
415 at each time point in MI-only condition. RMS values were normalized for the RMS value of the
416 RF muscle EMG during knee extension MVC at Pre.

417

418

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Statements and Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval

This study was performed in accordance with the principles of the Declaration of Helsinki and its later amendments. Approval was granted by the Ethical Review Committee for Experimental Research involving Human Subjects, Graduate School/College of Arts and Sciences, The University of Tokyo (Approval No. 831-2).

Consent to Participate

Written informed consent was obtained from all participants before their participation in the study.

443

444 **Data Availability**

445 The datasets generated and/or analyzed during the current study are available from the
446 corresponding authors on reasonable request.

447

448 **Author Contributions**

449 Keiichi Ishikawa: Conceptualization, Methodology, Formal analysis, Investigation, Data
450 curation, Writing – original draft, Visualization, and Funding acquisition. Naotsugu
451 Kaneko: Conceptualization, Methodology, Investigation, Writing – review and editing,
452 Supervision, and Funding acquisition. Kimitaka Nakazawa: Project administration,
453 Supervision, and Writing – review and editing. All authors read and approved the final
454 manuscript.

455

456 **Code Availability**

457 The custom MATLAB code used for data analysis is available from the corresponding
458 authors on reasonable request.

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