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MAIN TEXT

Inhibition of tibialis anterior spinal reflex circuits using frequency-specific neuromuscular electrical stimulation

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Abstract

Background: Neuromuscular electrical stimulation (NMES) can generate muscle contractions and elicit excitability of neural circuits. However, the optimal stimulation frequency for effective neuromodulation remains unclear.

Methods: Eleven able-bodied individuals participated in our study to examine the effects of: (1) low-frequency NMES at 25 Hz, (2) high-frequency NMES at 100 Hz; and (3) mixed-frequency NMES at 25 and 100 Hz switched every second. NMES was delivered to the right tibialis anterior (TA) muscle for 1 min in each condition. The order of interventions was pseudorandomized between participants with a washout of at least 15 min between conditions. Spinal reflexes were elicited using single-pulse transcutaneous spinal cord stimulation applied over the lumbar enlargement to evoke responses in multiple lower-limb muscles bilaterally and maximum motor responses (M_{max}) were elicited in the TA muscle by stimulating the common peroneal nerve to assess fatigue at the baseline and immediately, 5, 10, and 15 min after each intervention.

Results: Our results showed that spinal reflexes were significantly inhibited immediately after the mixed-frequency NMES, and for at least 15 min in follow-up. Low-frequency NMES inhibited spinal reflexes 5 min after the intervention, and also persisted for at least 10 min. These effects were present only in the stimulated TA muscle, while other contralateral and ipsilateral muscles were unaffected. M_{max} responses were not affected by any intervention.

Conclusions: Our results indicate that even a short-duration (1 min) NMES intervention using low- and mixed-frequency NMES could inhibit spinal reflex excitability of the TA muscle without inducing fatigue.

KEYWORDS
neuromuscular electrical stimulation, spinal reflex, stimulation frequency, tibialis anterior, transcutaneous spinal cord stimulation

Suzufumi Arai and Atsushi Sasaki contributed equally to this work and are designated as co-first authors.

1 | INTRODUCTION

Neuromuscular electrical stimulation (NMES) can be used not only to generate muscle contractions without voluntary commands but also to modulate cortical and spinal neural excitability by activating sensory and motor pathways.[1] Interventions using NMES could help to restore functional movements and improve neuromotor function after neurological impairments such as spinal cord injury and stroke.[2] Moreover, NMES has been widely used for spasticity management.[3] While NMES has been applied successfully in clinical settings, the underlying mechanisms are still not well understood.

Neurophysiological effects induced by NMES depend on the stimulation parameters such as pulse amplitude, width, duty cycle, and the frequency.[4] Mang et al. reported that corticospinal excitability modulation in the tibialis anterior (TA) muscle was larger after the NMES intervention at 100 Hz compared to when stimulation was applied at 10, 50, and 200 Hz.[5] While NMES at 100 Hz could efficiently increase corticospinal excitability, it was also reported that high-frequency (>80 Hz) NMES resulted in stronger muscle contractions and more muscle fatigue,[6,7] whereas low-frequency (≤50 Hz) stimulation led to lesser muscle fatigue.[8] Similarly, it was reported that the rate of force decline during NMES at 20 Hz was lower compared to that at 80 Hz,[9] suggesting that low-frequency NMES induces less muscle fatigue compared to high-frequency stimulation. Based on these previous studies on the frequency-dependent effects of NMES on neural excitability and muscle fatigue, it could be considered that a combination of high- and low-frequency NMES would enhance the modulation of neural excitability with less fatigue. Moreover, because it is known that low-frequency and high-frequency NMES, respectively, recruit different motor unit populations,[1,10,11] their combined intervention may induce a more diverse afferent inputs. However, there have been no studies to date to examine the effects of mixed-frequency NMES.

Previous research investigating the effects of NMES on spinal excitability have focused primarily on the ankle plantar flexor soleus (Sol) muscle. For instance, it was reported that 20 Hz or 100 Hz NMES intervention to the Sol muscle could inhibit H-reflex excitability.[12] Moreover, it was reported that spinal excitability not only of the stimulated Sol muscle but also in nonstimulated thigh muscles was inhibited immediately after NMES interventions.[13] These studies suggest that NMES applied to the Sol could inhibit spinal excitability in the stimulated and other nonstimulated muscles. However, the effects of TA NMES on spinal excitability remain unclear despite the importance of ankle dorsiflexion activated by the TA muscle groups in daily activities such as walking. This lack of clarity may be due to difficulties in measuring spinal excitability (H-reflex) in the TA muscle.[14]

Transcutaneous spinal cord stimulation (tSCS) is a technique that can be utilized to reliably evoke spinal reflexes in multiple lower-limb muscles that share common features as the H-reflex.[15] tSCS could measure spinal reflexes not only from the TA, which is difficult to measure using the H-reflex, but also from multiple other muscles bilaterally and simultaneously.[13,15,16] Therefore, the objective of the present study was to investigate the effect of TA NMES with different frequencies on the excitability of spinal reflex circuits in multiple lower-limb muscles using the tSCS technique. We hypothesized that NMES interventions would inhibit spinal excitability as previously reported in the Sol muscle.[13,17] We also hypothesized that combining high- and low-frequency would more effectively modulate spinal excitability due to less fatigue and a more diverse afferent input induced by NMES.[1,10,11]

2 | METHODS

2.1 | Participants

Eleven able-bodied individuals (age: 21–25 years) participated in the study after providing informed consent (Table 1). None of the participants had any history of neurological disorders and impairments. Right leg dominance was approved using questionnaires: (i) kick a ball, (ii) stamp out a simulated fire, (iii) pick up a marble, and (iv) trace shapes using their foot.[18] The experimental procedures were approved by the university ethics committee at Osaka University (approval: L020).

2.2 | Study protocol

During the study, participants remained in the supine position. Three different conditions, each lasting 1 min, were tested: (1) low-frequency NMES at 25 Hz; (2) high-frequency NMES at 100 Hz; and (3) mixed-frequency NMES with the 25 and 100 Hz frequencies switched every second. For each condition, assessments examining spinal reflex excitability in multiple lower-limb muscles bilaterally and maximum motor response (M_{max}) of right TA were performed at the baseline (Pre) and immediately (Post0), 5 min (Post5), 10 min (Post10), and 15 min (Post15) after each intervention. The order of experimental conditions was pseudorandomized between participants with a washout of at least 15 min between conditions (Figure 1B).

TABLE 1 Participant age, weight, height, neuromuscular electrical stimulation (NMES) intensity for low-frequency, high-frequency and mixed-frequency stimulation, as well as transcutaneous spinal cord stimulation (tSCS) intensity and vertebral level of the cathode for spinal reflex assessments.

				NMES intensity (mA)				tSCS setup	
				Low frequency	High frequency	Mixed-frequency			
Participant	Age (years)	Weight (kg)	Height (cm)	at 25 Hz	at 100 Hz	at 25 Hz	at 100 Hz	Intensity (mA)	Level
1	24	65	171	27	26	29	24	35	L1–L2
2	25	65	176	27	26	27	26	40	T12–L1
3	24	59	165	20	20	20	20	60	L2–L3
4	24	59	167	20	20	20	20	50	L2–L3
5	21	61	167	24	27	27	26	60	L2–L3
6	24	61	170	29	30	30	29	70	L2–L3
7	24	80	172	27	24	29	27	80	L2–L3
8	24	75	176	21	24	23	23	60	L2–L3
9	21	52	168	24	23	23	21	60	L1–L2
10	23	61	171	27	26	27	24	60	L1–L2
11	24	69	176	35	33	33	32	60	L2–L3
Mean	23.5	64.3	170.8	25.5	25.4	26.2	24.7	57.7	
SD	1.2	7.5	3.7	4.2	3.7	4.0	3.6	11.9	

2.3 | Data acquisition

Electromyography (EMG) was recorded from: (a) TA; (b) Sol; (c) vastus medialis (VM); (d) biceps femoris (BF) muscle groups bilaterally (right: R-TA, R-Sol, R-VM, R-BF; and left: L-TA, L-Sol, L-VM, L-BF) by placing a pair of surface EMG electrodes (Ag/AgCl; Vitrode F-150s, Nihon Kohden, Japan) on the belly of each muscle with an approximate 2 cm separation. The ground electrode (Disposable Earth Electrodes 45400-SK; GE Healthcare, Japan) was placed around the right knee (Figure 1A). Prior to electrode placement, the skin was cleaned using alcohol to reduce skin impedance. All signals were band-pass filtered (15–1000 Hz) and amplified (×1000) using a biosignal amplifier (MEG-6108, Nihon Kohden, Japan). Data were digitized at a sampling frequency of 4000 Hz using an analog-to-digital converter (Powerlab/16SP, AD Instruments, Australia) and saved on the computer for post-processing.

2.4 | Neuromuscular electrical stimulation

A portable electrical stimulation system Motionstim 8 (Medel, Hamburg, Germany) was used to deliver NMES continuously for 1 min to the R-TA muscle by applying rectangular, biphasic, symmetric charge-balanced stimulation pulses with a 300 μs pulse width via surface electrodes (anode: 5 × 5 cm and cathode: 5 × 5 cm). Specifically, a single pulse consisted of a 300 ms positive current followed by a 300 ms negative current. The motor threshold was identified by gradually increasing the stimulation intensity with 1 mA increments by checking for palpable contraction. The motor threshold was determined for each participant and condition prior to each condition as motor thresholds may change during the experiment. For the mixed-frequency condition, two motor thresholds were identified separately for the 25 Hz and 100 Hz stimulation and used during the intervention. The current intensity for each intervention was set at 150% of the motor threshold.[13] Retrospective analysis showed no significant effect of NMES frequency on the stimulating intensities ($F(3, 40) = 0.237$, $p = 0.870$; Table 1).

2.5 | Transcutaneous spinal cord stimulation (tSCS)

tSCS was used to assess the spinal reflex excitability in multiple lower-limb muscles bilaterally using a constant current electrical stimulator (DS7R, Digitimer Ltd., UK) to apply a single monophasic 1 ms pulse width square pulse.[13,16] The anode electrode (7.5 × 10 cm) was positioned on the trunk above the umbilicus, and the

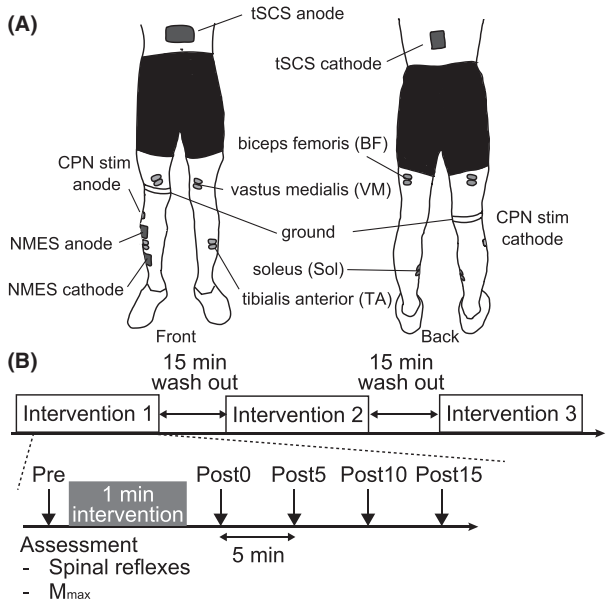

FIGURE 1 (A) Electromyography (EMG) electrodes were placed bilaterally on the TA, soleus (Sol), vastus medialis (VM), biceps femoris (BF) muscles. Electrodes for transcutaneous spinal cord stimulation (tSCS) were placed on the lumbar spine to induce spinal reflexes. Electrodes for NMES were placed on the right TA muscle. To induce the maximum motor response (M_{max}) of the TA muscle, the electrodes were placed on the right common peroneal nerve (CPN). (B) Experimental protocol included spinal reflexes and M_{max} assessments. Experimental data were obtained: at the baseline (Pre) and immediately (Post0), 5 min (Post5), 10 min (Post10), 15 min (Post15) after the intervention. During the intervention, NMES was delivered continuously for 1 min using different frequencies: (1) low-frequency at 25 Hz, (2) high-frequency at 100 Hz; and (3) mixed-frequency NMES with the 25 and 100 Hz frequencies switched every second. The order of experimental conditions was pseudorandomized between participants and separated by 15 min washout between conditions.

cathode electrode (5 × 5 cm) was initially placed on the lumbar enlargement between the L1–L2 spinous process (Figure 1A).[19,20] The cathode was then repositioned, and the responses were tested with the cathode electrode over the L2–L3 and T12–L1 levels using the same stimulating intensities to determine the optimal stimulation location.[21] The site that induced the largest responses in all muscles was selected as the stimulation location (Table 1). To determine the tSCS intensity for the experiment, partial recruitment curves of the spinal reflex responses across all recorded lower-limb muscles were obtained by gradually increasing the tSCS intensity starting from 0 up to a maximum of 100 mA, in 10 mA increments. Based on these recruitment curves, the stimulus intensity was adjusted during the experimental setup to simultaneously elicit responses in all recorded muscles such as to be on the ascending part of the recruitment curve (i.e., below the plateau and above the threshold) where the responses could increase or decrease when the stimulus intensity is modified.[22,23] Using this setting, a paired-pulse stimulation protocol with two stimuli delivered with a 50 ms inter-stimulus interval was tested to confirm the reflex nature of the evoked responses by eliciting 10 paired stimuli for each participant. Analysis of the average first and second evoked response amplitudes demonstrated a significant suppression of the second response for all muscles across participants (Wilcoxon signed-rank test, all $p < 0.001$) (Figure 2). The suppression of the second responses demonstrated post-activation depression, confirming that spinal reflexes were evoked by the activation of the dorsal root afferent fibers.[15,16,19–21]

During the experiment, 10 single-pulse stimuli were recorded at each time point with an average stimulating intensity of 57.7 ± 11.9 mA (Table 1), which remained constant throughout the assessments.

2.6 | Maximum motor response (M_{max})

Maximum motor response (M_{max}) was used to assess the muscle fatigue in the R-TA muscle.[24] M_{max} was elicited by applying a single monophasic1 ms pulse width square pulse to the common peroneal nerve via surface electrodes placed on the proximal part of the head of the fibula (Figure 1A) through a constant current electrical stimulator (DS7R, Digitimer Ltd., UK). The intensity to induce the M-waves with maximum amplitude (i.e., M_{max}) was determined by gradually increasing the intensity until the M-wave reached a plateau in its maximum amplitude, where further increments in stimulus intensity no longer increased the peak-to-peak amplitude of the M-wave.[25] For eliciting M_{max} responses, the stimulus intensity was set at 20% above this intensity.[25] During the experiment, three M_{max} responses were recorded at each time point.

2.7 | Data analysis

The peak-to-peak amplitude of the spinal reflexes and M_{max} were computed offline in MATLAB (R2022a, Mathworks Inc., USA). Ten spinal reflexes and three for M_{max} repeated trials were averaged for each time point and each experimental condition. Spinal reflex and M_{max} amplitudes were then normalized as a percentage of the baseline (% of Pre) amplitude for each experimental condition.

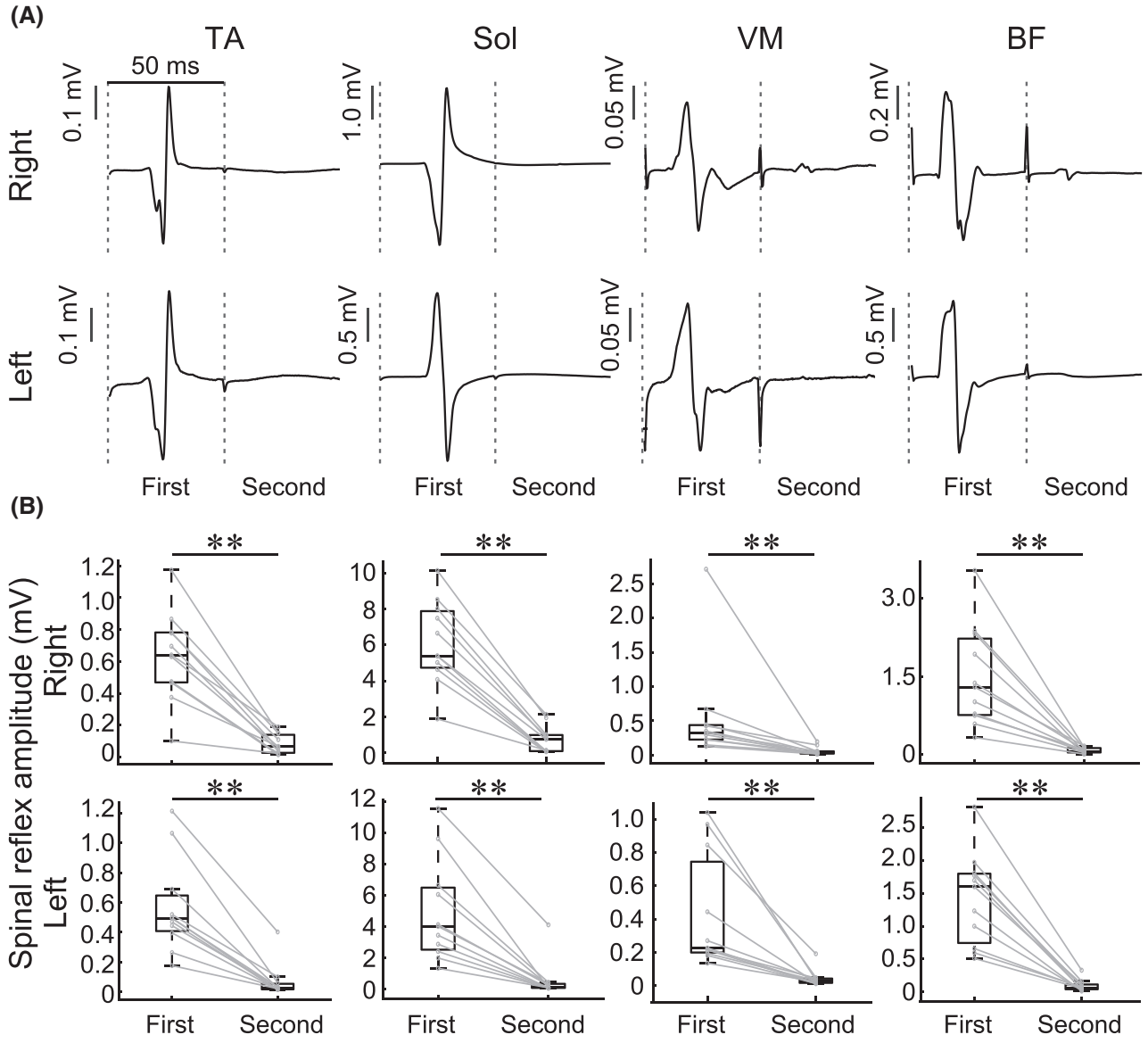

FIGURE 2 (A) Averaged spinal reflex responses during the paired-pulse stimulation protocol in a representative participant. (B) Group data of the first and second responses. Data are shown for the TA, soleus (Sol), vastus medialis (VM), biceps femoris (BF) muscles bilaterally. $^{**}p < 0.001$.

2.8 | Statistical analysis

The Friedman test, a nonparametric alternative to repeated measures analysis of variance (ANOVA), was utilized to compare the spinal reflex and M_{max} amplitude between different time points (Pre, Post0, Post5, Post10, and Post15) for each muscle and each experimental condition. Nonparametric tests were preferred due to the nonnormal distribution of all the measures as indicated by the Shapiro–Wilk test. When significant results were obtained on the Friedman test, multiple comparisons were conducted using the Wilcoxon signed-rank test with Bonferroni corrections to compare each time point (i.e., Pre (100%), Post0, Post5, Post10, and Post15). Additionally, the Wilcoxon signed-rank test was used to compare the first and second responses of the paired-pulse stimulation protocol. A significance level of $p < 0.050$ was set for the study. All statistical analyses were performed using Rstudio (2022.12.0, Rstudio Team).

3 | RESULTS

3.1 | Effects of NMES interventions

3.1.1 | Spinal reflexes

The results of the spinal reflexes for each condition are shown in Figure 3 (ipsilateral leg) and Figure 4 (contralateral leg). The Friedman test showed significant effects

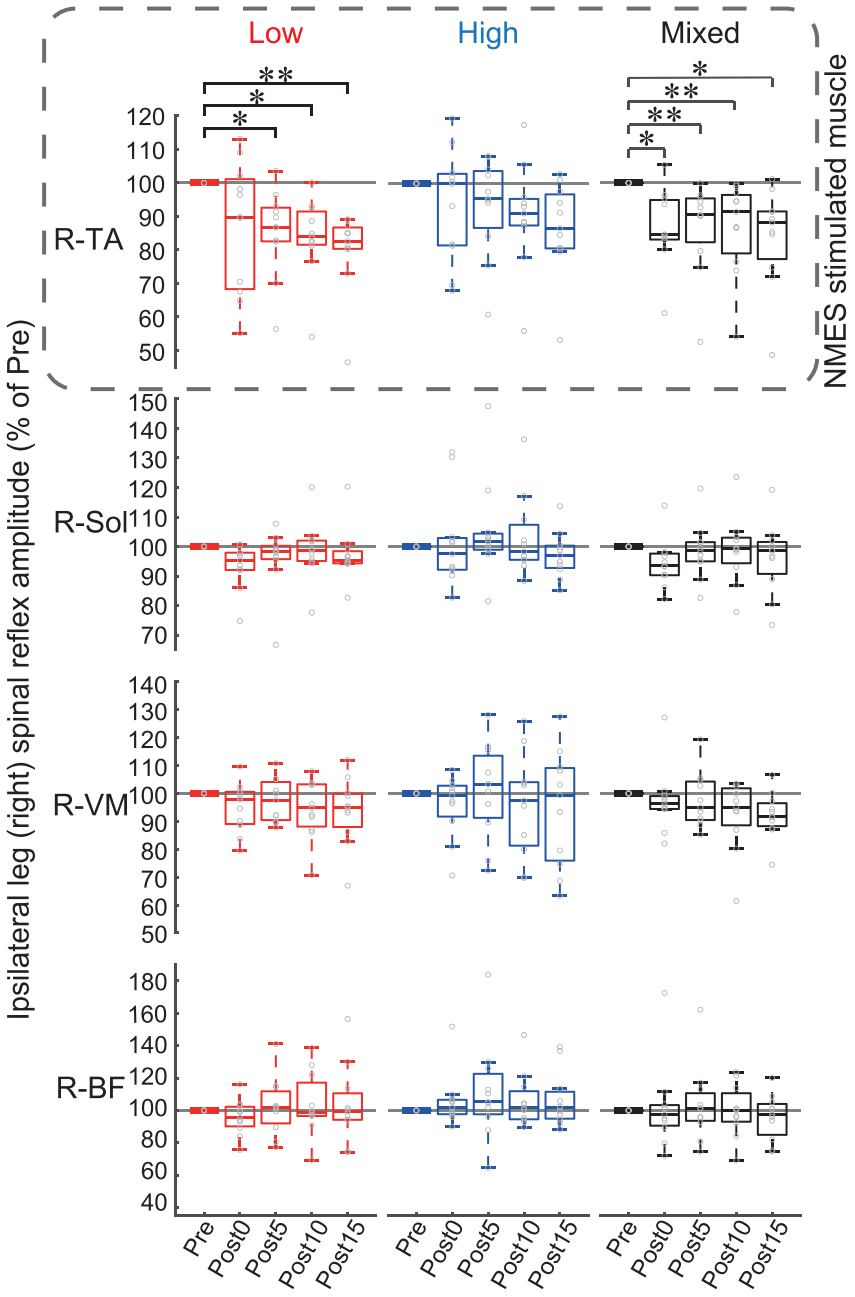

FIGURE 3 Results of the spinal reflex peak-to-peak amplitude normalized as a percentage of the baseline (% of Pre) for the ipsilateral (right) leg muscles (tibialis anterior (R-TA), soleus (R-Sol), vastus medialis (R-VM) and biceps femoris (R-BF)). Responses were measured at the baseline (Pre) and immediately (Post0), 5 min (Post5), 10 min (Post10), and 15 min (Post15) after the intervention and they were expressed as a percentage of the Pre assessment for each muscle and each of the three interventions: (1) low-frequency; (2) high-frequency; (3) mixed-frequency. Note that NMES was applied on the R-TA. Asterisks indicate statistically significant differences compared with Pre. *$p < 0.050$, **$p < 0.010$.

for R-TA in low-frequency [$\chi^2(4) = 20.945$, $p < 0.001$], high-frequency [$\chi^2(4) = 11.782$, $p = 0.019$] and mixed-frequency [$\chi^2(4) = 19.055$, $p < 0.001$] conditions, R-Sol in mixed-frequency condition [$\chi^2(4) = 11.127$, $p = 0.025$] and L-Sol in high-frequency condition [$\chi^2(4) = 13.745$, $p = 0.008$], while the results were not significantly different for other recorded muscles in each condition (all $p > 0.050$). Post-hoc testing corrected for multiple comparisons showed that the spinal reflex responses in R-TA muscle during low-frequency condition were significantly inhibited in Post5 ($p = 0.020$), Post10 ($p = 0.020$), and Post15 ($p < 0.010$) time point, compared with Pre. Similarly, post hoc testing showed that the spinal reflex responses in R-TA muscles during mixed-frequency condition were significantly inhibited in Post0 ($p = 0.049$), Post5 ($p < 0.010$), Post10 ($p < 0.010$), and Post15 ($p = 0.020$) time point, compared with Pre.

3.1.2 | Maximum motor response (M_{max})

Results of the M_{max} are shown in Figure 5. The Friedman test showed that M_{max} amplitude in the R-TA (NMES stimulated muscle) was not significantly different between each time point for each intervention (all $p > 0.050$).

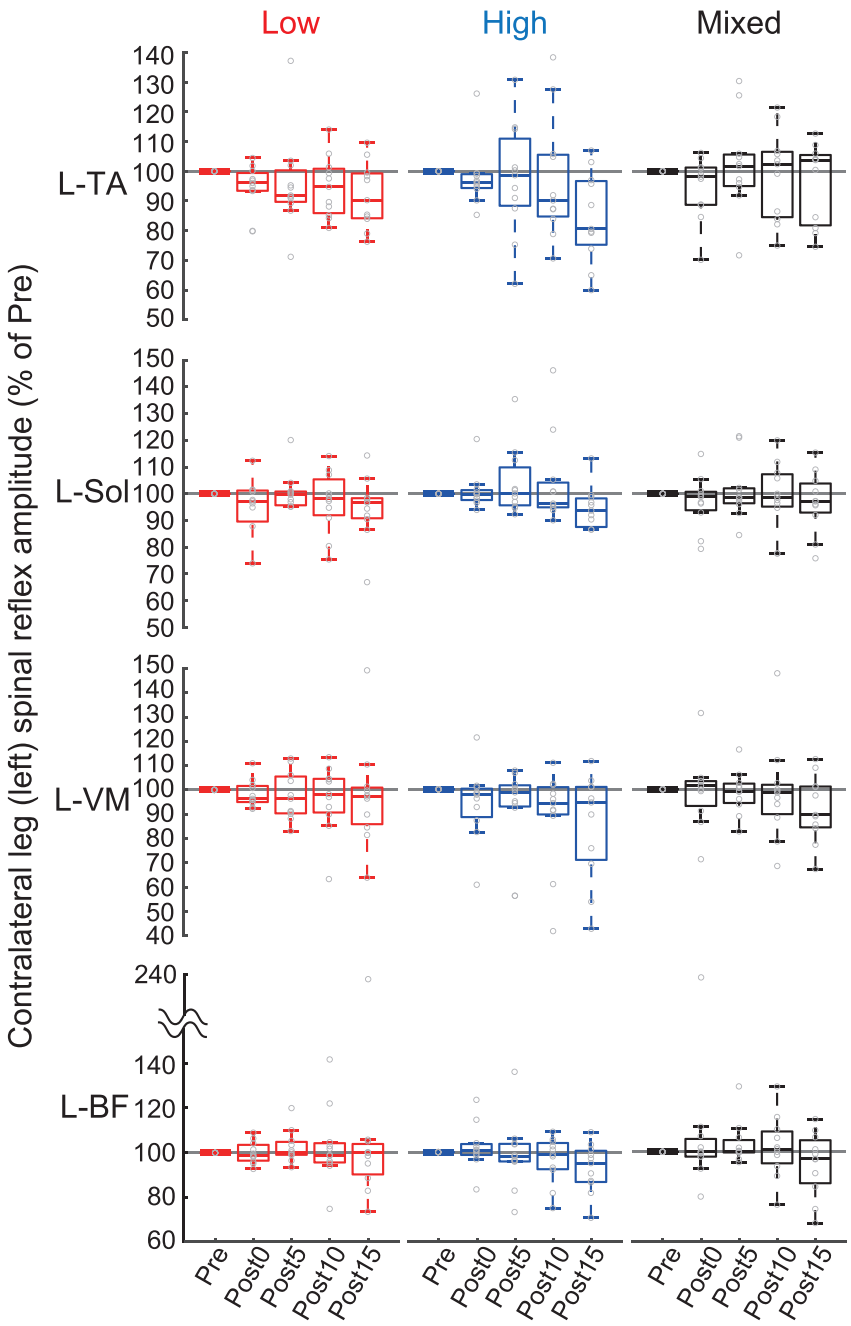

FIGURE 4 Results of the spinal reflex peak-to-peak amplitude normalized as a percentage of the baseline (% of Pre) for the contralateral (left) muscles (tibialis anterior (L-TA), soleus (L-Sol), vastus medialis (L-VM) and biceps femoris (L-BF)). Responses were measured at the baseline (Pre) and immediately (Post0), 5 min (Post5), 10 min (Post10), and 15 min (Post15) after the intervention and they were expressed as a percentage of the pre-assessment for each muscle and each of the three interventions: (1) low-frequency; (2) high-frequency; (3) mixed-frequency.

4 | DISCUSSION

We investigated frequency-dependent effects of TA NMES on spinal reflex circuits in multiple lower-limb muscles. Our results showed that low-frequency and mixed-frequency NMES intervention on the TA muscle inhibited the spinal excitability of the stimulated muscle for at least 10 and 15 min, respectively, while nonstimulated muscles on the ipsilateral and contralateral sides were not affected. On the other hand, high-frequency NMES intervention did not affect the spinal excitability of any lower-limb muscles. Overall, our results indicate that short-duration (1 min) TA NMES intervention could inhibit spinal excitability in the stimulated TA muscle, but not the other nonstimulated muscles and that these effects depend on the stimulation frequency.

4.1 | Effect of NMES with different frequencies

Neurophysiological effects of NMES on spinal excitability were suggested to depend on the stimulation frequency (see Table 2 for a summary of the previous studies investigating the effects of lower-limb NMES without voluntary contraction on corticospinal and spinal excitability). For instance, studies investigating the effects of low-frequency (≤50 Hz) NMES showed that 40 Hz continuous

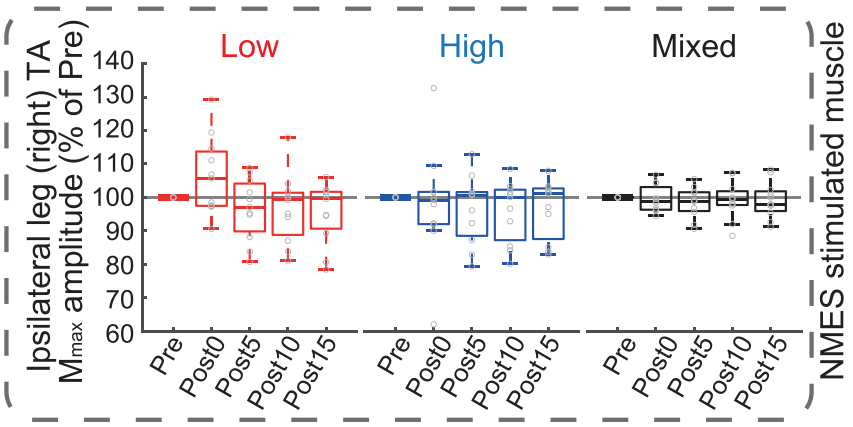

FIGURE 5 Results of the maximum motor response (M_{max}) peak-to-peak amplitude normalized as a percentage of the baseline (% of Pre) for the ipsilateral (right) tibialis anterior (R-TA) muscle. Responses were measured at the baseline (Pre) and immediately (Post0), 5 min (Post5), 10 min (Post10), and 15 min (Post15) after the intervention and they were expressed as a percentage of the pre-assessment for each of the three interventions: (1) low-frequency; (2) high-frequency; (3) mixed-frequency.

TABLE 2 Summary of previous studies investigating the effects of lower-limb NMES without voluntary contraction on the corticospinal and spinal excitability.

	NMES target	NMES frequency	Neurophysiological assessment
Mang et al.[5]	Common peroneal nerve	10, 50, 100, and 200 Hz	Facilitation of corticospinal excitability in the TA muscle, while no changes in spinal excitability when delivering NMES at 100 Hz
Grosprêtre et al.[12]	Triceps surae muscle belly	20 and 100 Hz	Inhibition of H-reflex in the Sol muscle after both 20 and 100 Hz NMES, but to a greater extent following 100 Hz
Milosevic et al.[13]	Sol muscle belly	40 Hz	Inhibition of tSCS-elicited spinal reflexes of the Sol muscle and other ipsilateral muscles
Jimenez et al.[17]	Tibial nerve	15 Hz and theta burst	Inhibition of H-reflex of the Sol muscle after theta burst stimulation
Borzuola et al.[26]	Plantar flexor muscle belly	50 Hz	Inhibition of H-reflex of the Sol muscle
Lagerquist et al.[27]	Tibial nerve	100 Hz	No change in corticospinal excitability and H-reflex in the Sol muscle
Khaslavskaia et al.[29]	Common perineal nerve	30 Hz	Facilitation of corticospinal excitability in the TA muscle
Wegrzyk et al.[31]	Triceps surae muscle belly	25 and 100 Hz	Inhibition of H-reflex in the Sol muscle after 100 Hz NMES
Borzuola et al.[32]	Triceps surae muscle belly	50 Hz	Inhibition of H-reflex in the Sol muscle

Abbreviations: NMES, neuromuscular electrical stimulation; Sol, soleus; TA, tibialis anterior; tSCS, transcutaneous spinal cord stimulation.

NMES applied to the Sol muscle for 1 min inhibited spinal reflexes in the Sol muscle for at least 15 min.[13] Similarly, Borzuola et al. showed that 50 Hz NMES to the triceps surae muscle for 3 min in 6 s on/6 s off duty cycle inhibited spinal reflexes in the Sol muscle.[26] These results suggest that low-frequency (≤50 Hz) NMES has an inhibitory effect on spinal reflexes, which is consistent with our current result even though the TA muscle was stimulated.

Other studies examining the effect of high-frequency (>50 Hz) NMES showed that 100 Hz NMES to the plantar flexor muscle belly (gastrocnemius and Sol muscles) involving approximately 40 contractions (6 s on/6 s off) inhibited spinal reflexes in the Sol muscle.[12] Conversely, Mang et al. applied NMES over the common peroneal nerve at 100 Hz for 40 min (20 s on/20 s off) to elicit facilitation of corticospinal excitability in the TA muscle, while no changes in spinal excitability were observed.[5] Lagerquist et al. also showed

that stimulation of the tibial nerve at 100 Hz for 40 min (5 s on and 5 s off) resulted in no change in spinal excitability of the Sol muscle.[27] Taken together, these studies suggest that high-frequency NMES applied to the muscle belly could inhibit spinal reflex excitability, while the stimulation of nerves does not alter spinal reflex excitability. However, our current study demonstrated that high-frequency NMES did not modulate spinal reflex circuits, although the muscle belly was stimulated. This could potentially be attributed to sensory axon hyperpolarization during continuous stimulation,[28] which may not have occurred during intermitted (on/off) stimulation used in a previous study by Grosprêtre et al., who demonstrated modulation of spinal excitability.[12]

In the mixed-frequency condition, the low-frequency and high-frequency NMES were switched every second, so in addition to the inhibitory effect of the low-frequency stimulation on spinal excitability, the intermittent application of the high-frequency may have superimposed the inhibitory effect of the low-frequency and high-frequency NMES. It was suggested that afferent input has an important role in the suppression of spinal reflexes.[13,20] The mixed-frequency NMES intervention in this study also suggests that the reduction of sensory axon hyperpolarization in intermittent high-frequency NMES allowed more afferent inputs to be transmitted to the spinal cord, leading to more rapid suppression of spinal reflex circuits. Moreover, it is known that low-frequency and high-frequency NMES respectively recruit different motor unit populations.[1,10,11] Specifically, low-frequency NMES activates large-diameter motor axons innervating fast-twitch, fatigable muscle fibers, while high-frequency NMES recruits smaller spinal motor neurons with lower voluntary threshold.[10] Therefore, their mixed activation could have induced more diverse afferent feedback which may contributed to the effective neuromodulation in our current study.

Our results also showed that M_{max} responses in the stimulated TA muscle were not significantly different between each time point for all intervention conditions, indicating that muscle fatigue was not induced by the interventions. This may be because a 1 min intervention was too short to induce fatigue. Therefore, future studies are warranted to examine longer-duration interventions with various frequencies NMES to examine possible effects on muscle fatigue.

4.2 | Effect of NMES on the nonstimulated muscles

In our current study, NMES was applied to the right TA muscle and significantly suppressed spinal reflexes in the stimulated TA muscle but not in the other nonstimulated muscles both in the ipsilateral and contralateral leg. Mang et al. stimulated the common peroneal nerve for 40 min and observed an increase in corticospinal excitability in the TA muscle, but no effect was observed in the ipsilateral Sol and VM muscles.[5] Khaslavskaia et al. also stimulated the common peroneal nerve for 30 min and observed an effect of increasing corticospinal excitability in the TA muscle, but not in the Sol muscle, the antagonist muscle.[29] On the other hand, it was reported that NMES applied on the Sol muscle could inhibit spinal reflexes in the nonstimulated muscles including ipsilateral TA, VM, and BF muscle as well as stimulated Sol muscle.[13] Therefore, these studies suggest that effects on nonstimulated muscles may depend on whether NMES is applied to the TA or Sol muscles. Because our current study also applied NMES on the TA muscle, modulation of nonstimulated other muscles could not be observed, consistent with previous studies.[5,29] The differential effects on other nonstimulated muscles when the TA and Sol muscles are stimulated may result from the differences in physiological muscle compositions and functions of the muscles. The TA muscle has stronger direct coupling between corticospinal tracts and spinal alpha motoneurons and greater involvement of the motor cortex.[30] In contrast, the Sol muscle has weaker cortical control than the TA and has motor functions that work in coordination with other muscles, such as postural maintenance.[27] Based on this knowledge and the results of this study, it is possible that the TA and Sol muscles have different neural mechanisms, and that stimulation of the TA muscle may have resulted in an inhibitory effect in the heteronymous muscles, but not as strong as when the Sol muscle was stimulated. The fact that the effects were different when NMES was given to the TA muscle and when it was given to the Sol muscle expanded the knowledge on the muscle-dependent effect of NMES on spinal reflex circuits.

4.3 | Possible mechanisms of inhibition at the spinal level after NMES

Overall, our results showed that low-frequency and mixed-frequency NMES intervention to TA muscle inhibited the spinal reflexes of the stimulated muscle for at least 10 min. Considering that there was no change in M_{max} under any condition, it could be interpreted that the suppression of spinal reflexes in this study is related to the mechanism of the central (spinal) system rather than peripheral mechanisms. Inhibition of spinal reflex elicited by tSCS in our current study is consistent with the previous studies reporting inhibition of H-reflex amplitude after NMES.[12,31,32] It was suggested that the possible reason for reduced spinal reflexes is a presynaptic inhibitory mechanism involving homosynaptic

post-activation depression of Ia terminals and primary afferent depolarization.[12] Moreover, another possible reason was suggested that repetitive electrical stimulation raises the excitability thresholds of sensory and motor axons of the stimulated muscle.[33] Martin et al. suggested that repeated stimulation of the Ia afferents could lead to hyperpolarization of its axonal branches, thereby raising their threshold for excitability and reducing their responsiveness to stimuli of the same intensity.[34] Because it is known that spinal reflex elicited by tSCS has characteristics similar to the H-reflex responses,[15,16] the inhibition of spinal reflex in our current study could also be induced by these mechanisms such as those considered in H-reflex study. However, to elucidate detailed mechanisms of spinal reflex inhibition induced by NMES, further studies are warranted.

4.4 | Limitations

While the sample size ($n = 11$) was comparable to previous similar studies investigating the effects of NMES intervention on neural excitability in the TA muscle,[5,29] we did not calculate the sample size prior to the study. Therefore, it is important to acknowledge the possibility that our current study may be underpowered. Indeed, it should be noted that a qualitative observation suggests inhibition in spinal reflexes in the R-TA muscle of most participants during the high-frequency condition in the post assessment (Figure 3), although this effect did not reach statistical significance.

5 | CONCLUSION

We investigated the effect of TA NMES interventions with different frequencies on spinal excitability in multiple lower-limb muscles. Our results showed that spinal reflexes in the stimulated TA muscle was significantly inhibited immediately after mixed-frequency NMES intervention, which lasted for at least 15 min. Moreover, low-frequency NMES inhibited spinal reflexes 5 min after the intervention, though not immediately after the intervention. On the other hand, high-frequency NMES did not show significant effects on spinal reflexes. These results suggest that even a very short-duration NMES intervention using low- and mixed-frequency could inhibit spinal excitability after the intervention, potentially attributed to their ability to recruit more diverse afferent inputs.

AUTHOR CONTRIBUTIONS

S.A., A.S., and M.M. designed the study. S.A., A.S., S.T., and M.M. collected the data. S.A. and A.S. analyzed the data. S.A., A.S., T.N., and M.M. interpreted the results of the experiment. S.A. and A.S. prepared the figures and drafted the manuscript. All authors have revised the article for the intellectual content and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest, either financial or otherwise.

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