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RESEARCH ARTICLE

Short-term inhibition of spinal reflexes in multiple lower limb muscles after neuromuscular electrical stimulation of ankle plantar flexors

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Abstract

Neuromuscular electrical stimulation (NMES) of lower limbs elicits muscle contractions through the activation of efferent fibers and concomitant recruitment of afferent fibers, which can modulate excitability of the central nervous system. However, neural mechanisms of NMES and how unilateral stimulation of the soleus affects spinal reflexes in multiple lower limb muscles bilaterally remains unknown. Twelve able-bodied participants were recruited, and spinal reflex excitability changes were tested after four interventions, each applied for 60 s, on the right plantar flexors: (1) motor-level NMES; (2) sensory-level NMES; (3) voluntary contraction; (4) rest. Spinal reflexes were elicited using single-pulse transcutaneous spinal cord stimulation applied on the lumbar level of the spinal cord to evoke bilateral responses in multiple lower limb muscles, while maximum motor response (M_{max}) was tested in the soleus by stimulating the posterior tibial nerve. Spinal reflexes and M_{max} before each intervention were compared to immediately after and every 5 min subsequently, for 15 min. Results showed that motor-level NMES inhibited spinal reflexes of the soleus and other studied muscles of the ipsilateral leg, but not the contralateral leg (except vastus medialis) for 15 min, while not affecting soleus muscle properties (M_{max}). Voluntary contraction effect lasted less than 5 min, while sensory-level NMES and rest did not produce an effect. Short-term spinal reflex excitability was likely affected because antidromic impulses during motor-level NMES coincided in the spinal cord with afferent inputs to induce spinal neuroplasticity, whereas afferent input alone did not produce short-term effects. Such activation of muscles with NMES could reduce spasticity in individuals with neurological impairments.

Keywords Neuromuscular electrical stimulation · Spinal reflex · Neuroplasticity · Soleus · Rehabilitation

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Abbreviations

ANOVA	Analysis of variance
BF	Biceps femoris
FES	Functional electrical stimulation
H-reflex	Hoffmann reflex
M_{max}	Maximum motor response
NMES	Neuromuscular electrical stimulation
PPS	Paired-pulse stimulus
TA	Tibialis anterior
tSCS	Transcutaneous spinal cord stimulation
$tSCS_{amp}$	Peak-to-peak amplitude of the spinal reflexes
VM	Vastus medialis
Sol	Soleus

Introduction

Induction of activity-dependent neuroplasticity can improve walking and balance of individuals with neurological injury (Dietz et al. 1995; Wolpaw 2007). Moreover, neuromuscular

electrical stimulation (NMES), a technique that activates individual muscles to produce single joint movements, and functional electrical stimulation (FES), which uses patterned and temporally programmed electrical stimulation to generate coordinated limb movements, can guide cortical and spinal neuroplasticity in the central nervous system (Bergquist et al. 2011; Milosevic et al. 2017; Popovic et al. 2001). Such techniques can be used to regain motor function in the lower limbs of individuals with neurological impairments. Specifically, it has been demonstrated that patterned FES of lower limb flexors and extensors during treadmill training improves voluntary walking and balance in individuals with incomplete spinal cord injury (Kapadia et al. 2014). Moreover, long-term use of foot-drop stimulator, which applies stimulation to the common peroneal nerve to activate ankle dorsiflexors [i.e., tibialis anterior (TA) muscle], improves walking capacity in individuals with stroke and multiple sclerosis (Everaert et al. 2010; Stein et al. 2010). Although evidence supports clinical effectiveness of these interventions, optimal stimulation methods to guide neurophysiological effects are still not fully established.

When electrodes are placed on the skin over the targeted muscle, the applied electrical stimulation depolarizes motor axons, causing direct contraction of muscles. The afferent pathways are also co-stimulated during NMES and FES applications, either directly via the stimulating pulse, or as reafferent inflow to the spinal cord at the onset of relaxation secondary to the stimulation-induced muscle contraction. On the other hand, when electrodes are placed on the common peroneal nerve, stimulation can activate the TA muscle directly through motor axons, or through the flexor reflex. Regardless, afferent input is critical to produce central (i.e., spinal) effects and modulate the central nervous system circuitry (Bergquist et al. 2011). Therapeutic changes after the use of foot-drop stimulator are typically accompanied with increased corticospinal excitability between the motor cortex and the lower limb muscles (Everaert et al. 2010; Stein et al. 2010; Thompson et al. 2004, 2011). Functional magnetic resonance imaging studies have demonstrated that at least 4 weeks of intensive gait training with a foot-drop stimulator are required to alter the supplementary motor area neural activations (Gandolla et al. 2016). Together, these studies suggest that cortical changes are involved in long-term carryover effects induced by electrical stimulation of the TA muscle. Stimulation of the common peroneal nerve, which innervates the TA, alters the spinal mechanisms of reciprocal Ia inhibition (Obata et al. 2018; Perez et al. 2003), while it does not always affect H reflex (Hoffmann reflex) excitability in the TA (Obata et al. 2015; Thompson et al. 2011). On the other hand, stimulation of the soleus muscle (i.e., ankle extensor) does not induce changes in the corticospinal circuits (Kitago et al. 2004; Lagerquist et al. 2012), while it was shown to modulate soleus H-reflex responses (Egawa et al. 2013; Jimenez et al. 2018; Kitago et al. 2004; Lagerquist et al. 2012). These results are unsurprising given differential control in the corticospinal and spinal connections to the TA and soleus (Morita et al. 2000). While the TA muscle is known to have a stronger corticospinal input, the soleus has stronger spinal connections (Lagerquist et al. 2012; Morita et al. 2000; Taborikova and Sax 1968). However, it is still not fully clear how unilateral NMES of the soleus modulates spinal reflex excitability and how this may affect other non-stimulated muscles.

Transcutaneous spinal cord stimulation (tSCS) is a technique that can be used to evoke spinal reflexes in multiple lower limb muscles by delivering a single electrical stimulus on the lumbar level of the spinal cord to stimulate the dorsal roots of the spinal nerves (Courtine et al. 2007; Masugi et al. 2016, 2017; Minassian et al. 2007). It was previously demonstrated that the response to a second tSCS stimulus, 50 ms after the first, can be suppressed or completely abolished. The afferent volley induced by the first pulse can result in the reduction of the second reflex output due to post-activation depression or presynaptic inhibition, which provides evidence that the evoked responses are of sensory origin (i.e., activate the dorsal roots) (Courtine et al. 2007; Minassian et al. 2007). The tSCS-evoked spinal reflexes share common features as the H-reflex, with the advantage of being able to induce responses bilaterally and in multiple muscles simultaneously. These responses have been referred to as multisegmental monosynaptic responses (Courtine et al. 2007), posterior root-muscle reflexes (Minassian et al. 2007), root-evoked potentials (Roy et al. 2012), and spinal reflexes (Masugi et al. 2016, 2017). Hereafter, tSCS-evoked responses will be referred to as spinal reflexes. It has previously been demonstrated that unilateral passive muscle stretching, which provides afferent input from ankle plantar flexors, modulates spinal reflexes of ipsilateral, but not the contralateral, lower limb muscles (Masugi et al. 2017). However, effects of unilateral NMES on spinal reflexes of ipsilateral and contralateral non-stimulated muscles remain unknown. Therefore, the objectives of this study were to induce spinal reflexes in multiple lower limb muscles bilaterally using tSCS to study how NMES of ankle plantar flexors (i.e., soleus) affects spinal reflex excitability. We hypothesized that application of unilateral NMES on ankle plantar flexors, which provides a strong afferent input to the central nervous system as well as depolarizes motor axons, will modulate spinal reflex excitability (tested by tSCS) of the soleus as well as other ipsilateral lower limb muscles, but not the contralateral muscles.

Methods

Participants

Twelve able-bodied individuals participated in the study (Table 1). None of the participants had a history of

neurological and sensory impairments, or musculoskeletal injury that could affect their participation in the study. Informed consent was obtained from all individual participants included in the study in accordance with the principles of the Declaration of Helsinki. The experimental procedures were approved by the local institutional ethics committee at The University of Tokyo (44-112-533-2).

Study protocol

During the study, participants remained in the supine position (Fig. 1a). Four different conditions, each lasting 60 s, were tested: (1) $NMES_{motor}$:NMES was applied on the right soleus unilaterally at the motor stimulation level, which was set at 1.5× motor threshold amplitude, such that it produced an isometric plantarflexion force without exerting any voluntary effort (see NMES section and Table 1); (2) $NMES_{sensory}$: NMES was applied on the right soleus unilaterally at the

Table 1 Participant age, weight, height, neuromuscular electrical stimulation (NMES) amplitude for sensory ($NMES_{sensory}$) and motor ($NMES_{motor}$) stimulation, as well as transcutaneous spinal cord stimulation (tSCS) amplitude and vertebral level of the cathode

Participant	Age (years)	Weight (kg)	Height (cm)	NMES (mA)		tSCS	
				$NMES_{sensory}$	$NMES_{motor}$	Amp (mA)	Level
1	23	80	180	18	36	50	L1–L2
2	24	73	173	15	33	70	L1–L2
3	27	71	181	13	32	–	L2–L3
4	26	73	179	14	34	55	L1–L2
5	32	58	166	15	33	55	L1–L2
6	25	60	168	11	36	70	T12–L1
7	22	60	182	8	21	45	L1–L2
8	26	63	173	12	24	55	L1–L2
9	30	72	175	8	30	55	L1–L2
10	27	60	169	10	24	50	L1–L2
11	30	80	180	8	30	45	L1–L2
12	34	82	175	17	33	70	L2–L3
Mean	27.2	69.3	175.1	12.4	30.5	56.4	
SD	3.7	8.8	5.4	3.5	4.9	9.5	

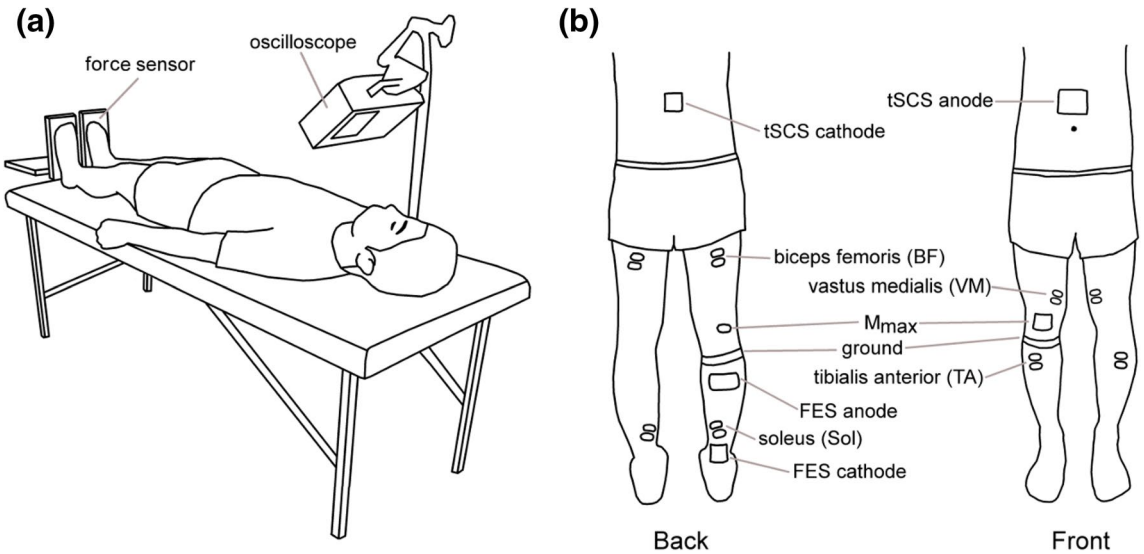

Fig. 1 Experimental setup showing: (**a**) posture of the participants during the experiment; and (**b**) recording electrode locations placed bilaterally on the: soleus (Sol), tibialis anterior (TA), vastus medialis (VM), and biceps femoris (BF) muscles as well as the ground electrode; stimulating transcutaneous spinal cord stimulation (tSCS) electrodes on the thoracic spine for inducing spinal reflexes; stimulating neuromuscular electrical stimulation (NMES) electrode location for stimulating the Sol muscle unilaterally; the electrode locations on the posterior tibial nerve to induce the maximum motor response (M_{max}) of the Sol muscle unilaterally

sensory stimulation level, which was set below the motor threshold amplitude, such that it did not produce muscle contractions and/or plantarflexion force (see NMES section and Table 1); (3) Voluntary: participants were asked to exert a voluntary isometric plantarflexion force with the right leg, which was set at same target force level as $NMES_{motor}$ stimulation (see text below); (4) Rest: participants remained at rest for duration of the assessment. The force produced during ankle plantarflexion was measured using two strain gauge sensors (LCB03K025L, A&D Company Limited, Japan), which were fixed on separate metal frames under each foot (Fig. 1a). The force produced by the right leg (intervention side) was displayed on an oscilloscope (TDS2012C, Tektronix Inc., USA) (Fig. 1a) to ensure that force levels during $NMES_{motor}$ and Voluntary conditions were matched. Target force level was determined for each participant prior to start of the experiment during NMES setup (see the NMES section) and it was set to the isometric force produced when the right leg was stimulated at the $NMES_{motor}$ amplitude. During the experiment, participants were asked to match the force target level by plantarflexing their ankle for the Voluntary intervention, which was the same force produced during the $NMES_{motor}$ intervention. The sensor measurements on the left side were used to ensure that the left leg did not move during any of the conditions. The order of all experimental conditions was randomized between participants and a break of at least 5 min between conditions was administered.

Assessments to evaluate spinal reflexes and maximum motor response (M_{max}) were performed in the following time intervals for each experimental condition: (i) Pre: before; (ii) Post0: immediately after; (iii) Post5: 5 min after; (iv) Post10: 10 min after; (v) Post15: 15 min after the intervention.

Neuromuscular electrical stimulation (NMES)

A portable electrical stimulation system Complex Motion (Compex, Switzerland) was used to deliver NMES unilaterally to the right soleus (rSol) muscle (Fig. 1b) for a period of 60 s by applying rectangular, biphasic, asymmetric charge balanced stimulation pulses with a 300 µs pulse width and 40 Hz frequency via surface electrodes (10 × 5 cm on the anode and 5 × 5 cm on the cathode) (Popovic et al. 2001). First, the experimenter identified the motor threshold by gradually increasing the stimulation amplitude with 1 mA increments by checking for palpable contractions. The sensory stimulation amplitude ($NMES_{sensory}$) was set at the level below the motor threshold, such as not to produce muscle contractions/plantarflexion force, but above the perceptual threshold (Table 1). The motor stimulation amplitude ($NMES_{motor}$) was set to 1.5× the motor threshold amplitude, or the highest tolerable amplitude, which was higher than the motor threshold (Table 1).

Spinal reflex excitability

Spinal reflexes were evoked simultaneously in multiple muscles using tSCS (Courtine et al. 2007; Minassian et al. 2007) on: (a) soleus (Sol); (b) tibialis anterior (TA); (c) vastus medialis (VM); (d) biceps femoris (BF) muscles bilaterally (right: rSol, rTA, rVM and rBF; left: lSol, lTA, lVM, and lBF) using surface electromyography electrodes (Ag/AgCl; Vitrode F-150S, Nihon Kohden, Japan). A reference electrode was placed around the knee (Fig. 1b). A constant current electrical stimulator (DS7A, Digitimer Ltd., UK) was used to apply a single monophasic square pulse, with a 1 ms pulse duration, and a stimulation amplitude between 0 and 100 mA. The stimulator was externally triggered by an analog output from a data acquisition system (USB-6259 BNC, National Instruments, USA), which was controlled by custom written program (LabVIEW, National Instruments, USA). The stimulus was delivered on the spinal cord at the lumbar level to simultaneously evoke responses in multiple muscles (Masugi et al. 2016, 2017). The anode electrode (7.5 × 10 cm) was placed on the trunk above the umbilicus, and the cathode electrode (5 × 5 cm) was positioned on the spine between L1–L2 lumbar spine process. After evaluating the responses, the cathode was moved and response tested at the L2–L3 and T12–L1 levels at the same stimulus amplitude to determine the optimal stimulation location (Roy et al. 2012). The location that induced largest responses in all the tested muscles was chosen as the stimulation site (Table 1). Next, the recruitment curve of responses in all the muscles was obtained for each participant, by gradually increasing the tSCS stimulation amplitude from 0 to 100 mA in 10 mA increments. The response in the right soleus at the maximal stimulation amplitude was limited to approximately 5 mV, similar to what has been reported by Courtine et al. (2007). To eliminate the ceiling effect of the evoked responses, stimulus amplitude was adjusted to obtain responses on the ascending part of the recruitment curve in all muscles and it was kept constant for the duration of the experiment (Table 1). During the experiment, 12 single-pulse stimuli were elicited at each time interval and their responses were averaged.

Paired-pulse stimulation (PPS)

Prior to starting the experiment, a paired-pulse stimulation (PPS) paradigm was implemented by delivering two stimulation pulses with a 50 ms inter-stimulus-interval based on the previous studies (Courtine et al. 2007; Hofstoetter et al. 2014; Masugi et al. 2016, 2017; Minassian et al. 2007). During the PPS paradigm, 12 paired-pulse stimuli were elicited for each participant and their responses were averaged.

Maximum motor response (M_{max})

In addition to the spinal reflexes, in 10 out of 12 participants, M_{max} was induced in the right Sol (rSol) muscle (intervention side) unilaterally. M_{max} was elicited by stimulating the posterior tibial nerve using an electrical stimulator to apply a single monophasic square pulse, with a 1 ms pulse duration (SEN-7203, Nihon Kohden, Japan) via surface electrodes placed on the popliteal fossa (cathode; 1.8 × 3.6 cm) and over the patella (anode; 5 × 5 cm) (Fig. 1b) (Crone et al. 1999; Obata et al. 2015). In total, three stimuli were elicited at each time interval and their responses were averaged.

Data acquisition and processing

All responses (i.e., spinal reflexes and M_{max}) were band-pass filtered (15−3,000 Hz) and amplified (×1000) using a biosignal amplifier (MEG-6108, Nihon Kohden, Japan). All data were digitized at sampling frequency of 10,000 Hz using an analog-to-digital converter (Powerlab/16SP, AD Instruments, Australia) and saved on the computer for offline analysis. Peak-to-peak amplitude of the spinal reflexes ($tSCS_{amp}$) and M_{max} were computed offline in Matlab (Mathworks Inc., USA) without any additional processing (Masugi et al. 2016, 2017). Prior to statistical analysis, responses after the intervention (Post0, Post5, Post10, and Post15) were expressed as a percent of that before (Pre) each intervention.

Statistics

Comparisons of the $tSCS_{amp}$ and M_{max} between the time intervals for each experimental intervention were performed using the Friedman test, a non-parametric equivalent for repeated-measure analysis of variance (ANOVA). Significant results were followed up with multiple comparisons using the Wilcoxon signed-rank test to compare baseline (Pre) and post assessment (Post0, Post5, Post10, and Post15) responses. Moreover, Wilcoxon signed-rank test was used to compare the first and second responses of the PPS protocol. Non-parametric tests were chosen, because the Shapiro–Wilk test indicated that all identified measures were not normally distributed. Prior to the analysis, a two-way repeated-measure ANOVA confirmed interactions between experimental interventions and time intervals. Significance level was set to $p < 0.05$.

Results

Paired-pulse stimulation (PPS)

The results of the PPS paradigm are summarized in Fig. 2. Comparison of the first and second $tSCS_{amp}$ during the PPS protocol, suggested significant suppression of the second response for all recorded muscles (Fig. 2). In addition, comparisons of the first and second $tSCS_{amp}$ during the PPS paradigm for the 12 repeated trials for each participant confirmed a significant suppression of the second response for each participant in all recorded muscles. These results suggest that tSCS stimulation induced spinal reflexes simultaneously in the four identified muscle groups bilaterally (Courtine et al. 2007; Minassian et al. 2007).

Spinal reflexes ($tSCS_{amp}$)

The results of the spinal reflexes are summarized in Fig. 3. On the ipsilateral (right) leg, rSol (intervention muscle) $tSCS_{amp}$ was not affected by Rest or $NMES_{sensory}$ interventions, but there was a significant suppression immediately after the Voluntary intervention, and a significant suppression that lasted for 15 min after the $NMES_{motor}$ intervention (Fig. 3). Similarly, the rTA and rBF $tSCS_{amp}$ was not affected by Rest or $NMES_{sensory}$ interventions, while there was a significant suppression immediately after the Voluntary intervention, and a significant suppression that lasted for 15 min after the $NMES_{motor}$ intervention (Fig. 3). Finally, rVM $tSCS_{amp}$ was not affected by Rest or $NMES_{sensory}$ interventions, while both Voluntary and $NMES_{motor}$ interventions had a small, but statistically significant suppression that lasted for 15 min (Fig. 3).

On the contralateral (left) leg, lSol, lTA, and lBF $tSCS_{amp}$ were not affected by Rest, $NMES_{sensory}$, Voluntary, or $NMES_{motor}$ interventions. On the other hand, lVM $tSCS_{amp}$ was not affected by Rest or $NMES_{sensory}$ interventions, while Voluntary and $NMES_{motor}$ interventions had a small, but statistically significant suppression that lasted for 15 min (Fig. 3).

Maximum motor response (M_{max})

The results of the maximum motor response (M_{max}) are summarized in Fig. 4. The rSol (stimulated muscle) M_{max} was not affected by Rest (NOTE: no significant post-hoc), $NMES_{sensory}$, or $NMES_{motor}$ interventions, while there was a very small, but statistically significant facilitation after the Voluntary intervention that lasted for 15 min (Fig. 4).

Discussion

Short-term inhibition of the spinal reflexes after NMES

Our results demonstrated that 60 s of NMES of ankle plantar flexors unilaterally (rSol) at motor contraction level

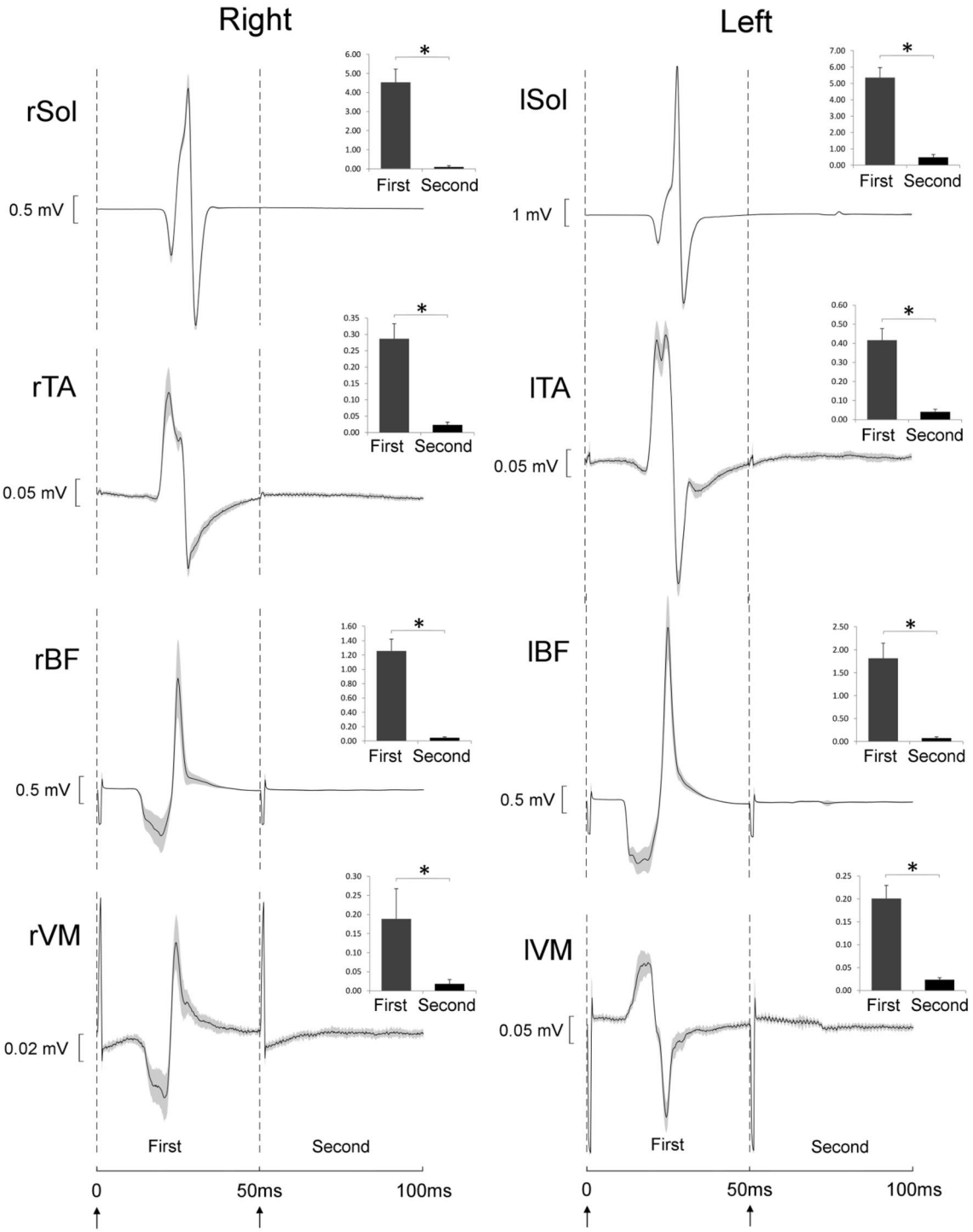

Fig. 2 Responses elicited by paired-pulse stimulation (PPS) protocol. Time-series plots are showing the mean and standard deviation (SD) responses of the 12 repeated trials for one representative participant. Bar graphs represent peak-to-peak amplitude mean and standard error (SE) of all participants. Shown are the first and second stimulus response, which were separated by 50 ms, for the: right and left soleus (rSol and lSol), tibialis anterior (rTA and lTA), vastus medialis (rVM and lVM), and biceps femoris (rBF and lBF). Legend: $*p < 0.01$; $**p < 0.05$

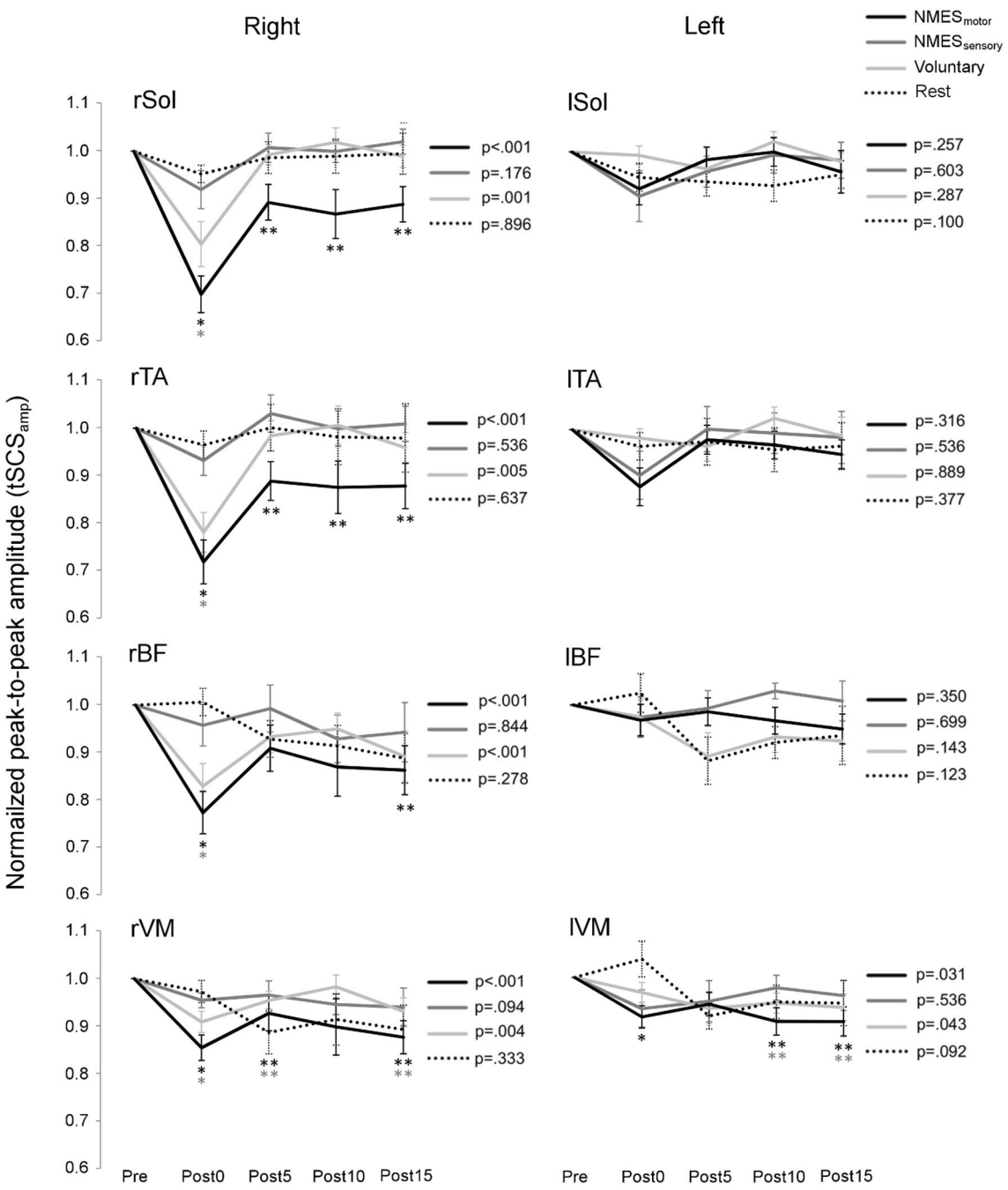

Fig. 3 Results of the spinal reflexes (tSCSamp) peak-to-peak amplitude mean and standard error (SE) for the right and left soleus (rSol and lSol), tibialis anterior (rTA and lTA), vastus medialis (rVM and lVM) and biceps femoris (rBF and lBF). Responses were measured for 15 min, in 5 min intervals, starting immediately after the intervention (Post0, Post5, Post10, and Post 15) and they were expressed as a percentage of baseline assessment (Pre) for each muscle and each of the four interventions: (1) NMES$_{motor}$; (2) NMES$_{sensory}$; (3) Voluntary; and (4) Rest. Note that NMES was applied on the rSol during NMES$_{motor}$ and NMES$_{sensory}$ interventions. Legend: $*p<0.01$; $**p<0.05$, where statistically significant results, where NMES$_{motor}$ is shown in black and Voluntary in gray

(NMES$_{motor}$) inhibited spinal reflexes in the soleus muscle for at least 15 min after the stimulation (Fig. 3 - rSol). Voluntary plantar flexion also inhibited the spinal reflexes of the soleus muscle, but the responses returned to baseline within 5 min. Overall, these results confirm our hypothesis that motor-level NMES activation of the soleus can modulate spinal reflexes induced by tSCS, which is consistent with reports that showed reduced H-reflex responses (Egawa et al. 2013; Jimenez et al. 2018). However, some research also reported enhanced H-reflex excitability after NMES of

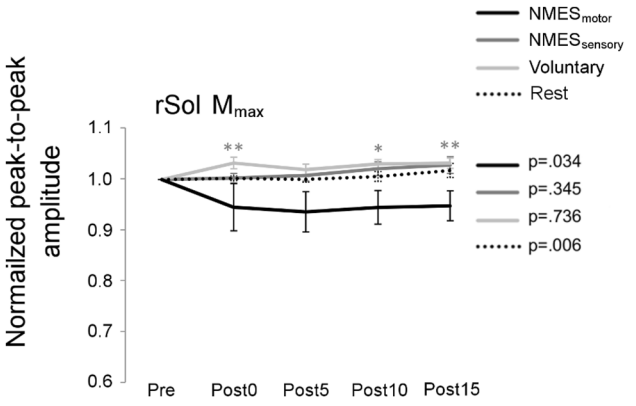

Fig. 4 Results of the maximum motor response (M_{max}) peak-to-peak amplitude mean and standard error (SE) for the right soleus (rSol). Responses were measured for 15 min, in 5 min intervals, starting immediately after the intervention (Post0, Post5, Post10, and Post 15) and they were expressed as a percentage of baseline assessment (Pre) for each muscle and each of the four interventions: (1) $NMES_{motor}$; (2) $NMES_{sensory}$; (3) Voluntary; and (4) Rest. Note that NMES was applied on the rSol during $NMES_{motor}$ and $NMES_{sensory}$ interventions. Legend: $^{*}p<0.01$; $^{**}p<0.05$, where statistically significant results, where $NMES_{motor}$ is shown in black and Voluntary in gray

the soleus (Kitago et al. 2004; Lagerquist et al. 2012). Intermittent (i.e., ON/OFF) motor cortex stimulation was shown to induce long-term potentiation of the spinal and corticospinal circuits, while continuous stimulation protocols induced long-term depression (Huang et al. 2005; Suppa et al. 2016). Our current study and a study by Jimenez et al. (2018) utilized continuous stimulation protocols and demonstrated inhibition of spinal excitability, while others showing facilitation utilized intermittent protocols (Kitago et al. 2004; Lagerquist et al. 2012). Similarly, it has been demonstrated that longer training with NMES (10 days) eventually decreased H-reflex excitability of the soleus muscle (Egawa et al. 2013). Therefore, our results suggest that NMES can drive inhibition of spinal circuitry in lower limb muscles.

Our current study also showed that motor-level NMES ($NMES_{motor}$) did not significantly change M_{max} amplitude (Fig. 4). M_{max}, the maximum amplitude of the motor response, which represents the response of the whole motoneurone pool (Crone et al. 1999), has been used to assess the effects of fatigue (Lentz and Nielsen 2002; Obata et al. 2015). It has been demonstrated that M_{max} amplitude is decreased as a result of muscle fatigue (peripheral muscle-membrane changes) (Crone et al. 1999; Lentz and Nielsen 2002; Stutzig and Siebert 2015). However, it took on average about 11 min of NMES at higher intensities (average amplitude: 43.5 mA with 400 µs pulse width) compared to our study which delivered NMES for a period of 60 s (average amplitude: 30.5 mA with 300 µs pulse width; Table 1) to induce fatigue (Stutzig and Siebert 2015). Despite reduction in the soleus H-reflex after NMES-induced fatigue, it was also shown that H-reflex of other synergistic lower limb muscles of the triceps surae group remained unaffected (Stutzig and Siebert 2015). In our current study, NMES had an inhibitory effect on the spinal reflexes of various synergistic and antagonistic non-stimulated ankle, hamstrings, and quadriceps muscles (rTA, rBF, and rVM, respectively) (Fig. 3). Therefore, spinal mechanisms, not muscle fatigue, were affected by NMES in our current study.

Inhibitory effects of $NMES_{motor}$ were demonstrated not only in the spinal reflexes of the stimulated muscle (rSol), but also in the other non-stimulated muscles of the ipsilateral leg (rTA, rBF, and rVM) (Fig. 3). On the other hand, responses of the contralateral homologous muscles (lSol, lTA, and lBF) were not affected (except for lVM; Fig. 3), which is consistent with our hypothesis. Crossed effects in the homologous contralateral muscles have been reported after prolonged periods of voluntary unilateral training, and it has been suggested that its mechanisms are neural, rather than peripheral (Carroll et al. 2006). Moreover, it was demonstrated that training with NMES can induce larger crossed effects compared to voluntary training alone (Hortobagyi et al. 1999). However, these studies utilized longer training periods, requiring 4–6 weeks to demonstrate cross-education effects. In the upper limb muscles, it was shown that 5 s of NMES can induce crossed effects on the contralateral arm (Hortobagyi et al. 2003). However, our results suggest that brief activation of the lower limb plantar flexors (i.e., soleus) using NMES does not regulate spinal mechanisms in the contralateral leg, but it can affect non-stimulated muscles in the ipsilateral leg.

Potential mechanisms of spinal neuroplasticity after NMES

Overall, as illustrated in Fig. 3, our results demonstrated that motor-level NMES ($NMES_{motor}$) inhibited spinal circuits for at least 15 min, whereas sensory-level stimulation ($NMES_{sensory}$) and the control intervention (Rest) did not affect spinal excitability. Moreover, voluntary contraction of muscles (Voluntary) also inhibited spinal excitability, although the response returned to baseline within 5 min. Electrical stimulation of muscles and nerves has been reported to activate both Ia afferents and α-motoneuron pathways (Bergquist et al. 2011; Lagerquist et al. 2006; Milosevic et al. 2017). Involvement of the Ia afferent input, via the reflex loop, is thought to play an important role for inducing neuroplasticity (Bergquist et al. 2011). Ia afferents contain not only homonymous connections, but also heteronymous connections (Meunier et al. 1993). The latter is a direct projection of Ia afferents to various motoneurons, including trans-joint connection between the ankle and leg muscles. In humans, heteronymous connections have been shown from the soleus to other lower limb muscles but not from

the soleus to the TA (Pierrot-Deseilligny and Burke 2005). It is possible that $NMES_{motor}$ activated both homonymous and heteronymous Ia pathways, which may have induced synaptic plasticity between the Ia afferents from the activated muscle and motoneurons innervating other non-activated muscles, except for the TA. On the other hand, sensory-level stimulation ($NMES_{sensory}$) amplitude might have been too low, thereby only involving cutaneous/skin afferents. Another possible explanation of our results is neuroplasticity of inhibitory pathway to the spinal reflex. Previously, it has been demonstrated that passive stretching of ankle plantar flexors for a period of 60 s inhibited spinal reflexes in ipsilateral lower limb muscles while the ankle remained stretched, but the responses returned to baseline immediately after (Masugi et al. 2017). These results indicate inhibitory effects of the afferent input (likely from muscle spindle and Golgi tendon organ) of distal muscles on the ipsilateral proximal muscles (Masugi et al. 2017). Our current results during isometric plantarflexion (Voluntary) are consistent with those during passive muscle stretching, since muscle contractions also evoke rich afferent feedback, causing acute suppression of spinal reflex excitability which returned to baseline within 5 min. However, since it is expected that $NMES_{motor}$ can activate afferents more efficiently compared to muscle stretching or voluntary plantarflexion (Voluntary), it may have also induced prolonged inhibition of the spinal reflex pathway.

In addition to the Ia afferent pathway, it is possible that the efferent pathway affected the spinal circuits of the stimulated muscle. When muscles or nerves are stimulated at $NMES_{motor}$ level, the efferent pathway is activated both orthodromically, along the axon towards the muscle to generate muscle contractions, as well as antidromically, toward the cell body in the spinal cord (Bergquist et al. 2011; Milosevic et al. 2017; Rushton 2003). This bidirectional propagation is unique to electrical stimulation, and does not occur during voluntary activation of muscles. The antidromic volley depolarizes the α-motoneuron cell bodies within the anterior horn, which have axons that project to skeletal muscles (McLeod and Wray 1966; Rushton 2003). It is possible that antidromic impulse also activated Renshaw cell interneurons, causing changes in heteronymous recurrent inhibition from soleus to other non-stimulated muscles to occur (Mazzocchio et al. 1994; Pierrot-Deseilligny and Burke 2005). Previously, heteronymous recurrent inhibition has been demonstrated from quadriceps to the ankle muscles (Barbeau et al. 2000) as well as from soleus to the quadriceps (Iles et al. 2000). Thus, repetitive activation, accompanied by coincidence of the afferent and efferent input in the spinal cord during $NMES_{motor}$, may induce Hebbian plasticity in the spinal circuits (Hebb 1949; Lagerquist et al. 2006), resulting in short-term effects after the stimulation. Overall, our results suggest that motor-level activation is necessary to induce short-term effects in the spinal circuits, possibly via antidromic activation. Such activation of muscles using NMES could be applied in rehabilitation to reduce spasticity in individuals with neurological impairments such as stroke and spinal cord injury.

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Compliance with ethical standards

Conflict of interest M.R.P. is a shareholder in company MyndTec Inc. The remaining authors have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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