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RESEARCH ARTICLE

Changes in corticospinal excitability evoked by common peroneal nerve stimulation depend on stimulation frequency

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Abstract The afferent volley generated during neuromuscular electrical stimulation (NMES) can increase the excitability of the human corticospinal (CS) pathway. This study was designed to determine the effect of different frequencies of NMES applied over the common peroneal nerve on changes in CS excitability for the tibialis anterior (TA) muscle. We hypothesized that higher frequencies of stimulation would produce larger increases in CS excitability than lower frequencies. NMES was applied at 10, 50, 100, or 200 Hz during separate sessions held at least 48 h apart. The stimulation was delivered in a 20 s on, 20 s off cycle for 40 min using a 1 ms pulse width. The intensity of stimulation was set to evoke an M-wave in response to a single pulse that was 15% of the maximal M-wave. CS excitability was evaluated by the amplitude of motor-evoked potentials (MEPs) in TA evoked by transcranial magnetic stimulation. MEPs were recorded immediately before and after the 40 min of NMES and in each 20 s "off" period. For each subject, MEPs recorded during three successive "off" periods were averaged together ($n = 9$ MEPs), providing a temporal resolution of 2 min for assessing changes in CS excitability. When delivering NMES at 100 Hz, MEPs became significantly elevated from those evoked before the stimulation at the 24th min, and there was a twofold increase in MEP amplitude after 40 min. NMES delivered at 10, 50, and 200 Hz did not significantly alter MEP amplitude. The amplitude of MEPs evoked in soleus and vastus medialis followed similar patterns as those evoked simultaneously in TA, but these changes were mostly not of statistical significance. There were no changes in the ratio of maximal H-reflex to maximal M-wave in TA or soleus. These experiments demonstrate a frequency-dependent effect of NMES on CS excitability for TA and show that, under the conditions of the present study, 100-Hz stimulation was more effective than 10, 50, and 200 Hz. This effect of NMES on CS excitability was strongest in the stimulated muscle and may be mediated primarily at a supraspinal level. These results contribute to a growing body of knowledge about how the afferent volley generated during NMES influences the CNS and have implications for identifying optimal NMES parameters to augment CS excitability for rehabilitation of dorsiflexion after CNS injury.

Keywords Neuromuscular electrical stimulation · Motor cortex · Common peroneal nerve · Rehabilitation · Plasticity · Stimulation frequency

Introduction

Trauma to the spinal cord or brain disrupts circuits in the central nervous system (CNS) that control movement. Although some pathways may remain intact, there is a net reduction in activity in neural circuits controlling the affected muscles. Initially, reduced voluntary control after spinal (e.g., spinal cord injury; SCI) or cortical (e.g., stroke) trauma is a direct result of the original injury. However, the CNS has a strong capacity to adapt whereby plasticity in the organization and excitability of synaptic connections between the cortex and muscle occur based on a "use it or lose it" principle (Brasil-Neto et al. 1993; Elbert et al. 1997; Chen et al. 1999). Thus, the prolonged disuse that

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occurs after CNS trauma can lead to maladaptive CNS plasticity that exacerbates the initial functional impairments and can lead to secondary complications.

Plasticity in the CNS is not always maladaptive, but also can be beneficial, such as the expansion of cortical areas and increases in excitability of neural circuits that are associated with the acquisition of new motor skills (Pascual-Leone et al. 1993; Pascual-Leone et al. 1995; Classen et al. 1998; Butefisch et al. 2000; Ziemann et al. 2001). Such plasticity is thought to stem from the simultaneous activity in afferent and efferent pathways that occurs during repeated voluntary movements. Although different from voluntary movement, the sensory feedback generated during neuromuscular electrical stimulation (NMES) also induces plasticity in the CNS (Khaslavskaia et al. 2002; Ridding et al. 2000), particularly when combined with voluntary activation (Kido and Stein 2004; Khaslavskaia and Sinkjaer 2005; Barsi et al. 2008). Herein, we refer to NMES as any prolonged, repetitive, electrical stimulation applied over muscle or peripheral nerve. NMES is often used after SCI or stroke to generate contractions to assist in performing functional movements (Liberson et al. 1961; Prochazka et al. 1997; Chae et al. 2008). From a rehabilitation perspective, it is interesting to note that improvements in function can persist after the stimulation is turned-off, and these improvements are thought to be mediated at least in part by plasticity in CNS circuits (Conforto et al. 2002; Hoffman and Field-Fote 2007; Celnik et al. 2007).

NMES-induced plasticity in the CNS manifests as changes in synaptic organization and excitability. Such plasticity can be detected experimentally as changes in the size of motor-evoked potentials (MEPs) evoked by transcranial magnetic stimulation (TMS). Increases in MEP amplitude induced by NMES have been shown for a range of muscles and have been evoked using a variety of stimulation parameters (intensity, frequency, pulse width, pattern, and duration). One approach has been to deliver NMES at intensities near motor threshold to activate primarily sensory axons (often called somatosensory stimulation). For increasing the excitability of corticospinal (CS) pathways to hand muscles, NMES has typically been delivered at these low intensities and at frequencies between 3 and 30 Hz (Ridding et al. 2000; McKay et al. 2002a, b; Pitcher et al. 2003). This type of stimulation is designed to "prime" CNS circuits and enhance sensory feedback for rehabilitation training sessions (Hoffman and Field-Fote 2007). Another approach is to deliver NMES at intensities above motor threshold to activate both sensory and motor fibers and generate functional contractions. This approach has been used to increase the excitability of the CS pathway to tibialis anterior (TA) by delivering NMES at intensities ranging from two times motor threshold (Khaslavskaia and Sinkjaer 2005) to intensities that evoked an M-wave that was 50% of maximal (M_{max}) (Knash et al. 2003; Kido and Stein 2004). While stimulating at these intensities, frequencies have ranged from 25 Hz (Knash et al. 2003) to 200 Hz (Khaslavskaia et al. 2002). This type of NMES is intended to be delivered during rehabilitation sessions to generate and assist with functional movements. The advantages of this approach are that higher intensity stimulation will produce a larger afferent volley, the NMES may have beneficial effects at the level of the muscle, and the influences on the CNS are enhanced when NMES is combined with voluntary movements (Kido and Stein 2004; Khaslavskaia and Sinkjaer 2005; Barsi et al. 2008). Unfortunately, the wide range of NMES parameters and muscles tested between different studies makes it difficult to identify the optimal parameters for augmenting CS excitability.

The influence of NMES frequency on CS excitability is not yet well defined. Research with stimulation of the pharynx has explored a range of combinations of stimulation intensity, frequency, and duration on changes in CS excitability for swallowing muscles. Excitability increased the most with stimulation applied at 75% of the maximum tolerated intensity, a frequency of 5 Hz, and a duration of 10 min, suggesting that NMES-driven cortical plasticity is dependent on stimulation parameters (Fraser et al. 2002) and the effect is most likely related to the strength of the afferent volley sent to the CNS. To our knowledge, only one study has investigated how changes in NMES frequency affect CS excitability for limb muscles. For the first dorsal interosseus muscle of the hand, CS excitability was depressed by 3-Hz NMES and enhanced by 30-Hz NMES (Pitcher et al. 2003). To date, no studies have explored the effect of NMES frequency on CS excitability for muscles of the lower limb. We have shown previously that high-frequency (100 Hz) NMES that increases the afferent volley to the spinal cord can enhance the central or "reflexive" contribution to electrically evoked contractions of ankle musculature compared to NMES at lower frequencies (Collins et al. 2002; Klakowicz et al. 2006; Dean et al. 2007). The present experiments are based on the rationale that higher frequencies of stimulation would also enhance afferent input sent to the brain and thus be optimal for increasing CS excitability.

The purpose of this study was to quantify the effect of frequency of common peroneal (CP) nerve stimulation (10, 50, 100, 200 Hz) on CS excitability for TA. An additional goal was to characterize the time course of changes in CS excitability during the stimulation with a higher temporal resolution than in previous studies. The TA muscle was chosen because reduced function in the ankle dorsiflexors is common following CNS trauma and NMES is often used for rehabilitation of that muscle (Liberson et al. 1961; Merletti et al. 1978; Chae et al. 2008). We delivered NMES in a 20 s on, 20 s off cycle for 40 min to evoke the repetitive

type of movements that would be suitable for rehabilitation. In past studies, CS excitability has been quantified in a minimum of 10- or 15-min increments (Khaslavskaia et al. 2002; McKay et al. 2002a; Knash et al. 2003). In the present study, changes in CS excitability during the stimulation were quantified in 2-min intervals. We hypothesized that higher frequencies of stimulation would increase TA MEPs to a greater extent than lower frequencies. MEPs evoked concurrently in soleus (Sol) and vastus medialis (VM) were analyzed to determine whether changes in CS excitability were specific to the homonymous muscle (TA) or were more generalizable to heteronymous muscles. To discern whether changes in spinal excitability were induced by NMES, the ratios of maximal H-reflex (H_{max}) to M_{max} were determined for Sol and TA before and after stimulation. The results of this study provide insight into the optimal NMES frequency for increasing CS excitability for rehabilitation of impaired dorsiflexion after CNS injury.

Methods

Participants

Six men and two women ranging in age from 22 to 46 with no known neurological disorders volunteered for this study. All subjects gave written, informed consent prior to testing. The experiments were conducted according to the Human Research Ethics Committee at the University of Alberta. Subjects were seated with their backs and necks supported, and hip, knee, and ankle angles at ~110°, 100°, and 90°, respectively. Padded restraints were secured around the right foot to minimize movement, and the left foot was placed on a foot rest. Subjects were instructed to not consume caffeine within 12 h prior to experimental sessions or during a session to eliminate the influence of caffeine on CNS excitability (Walton et al. 2003) and to refrain from intense physical activity within 12 h before the testing sessions.

Experimental procedure

All subjects participated in three 2- to 3-h testing sessions at least 48 h apart in which NMES frequencies of 10, 50, and 100 Hz were tested on different days. The order in which the different frequencies were tested was randomized for each subject. Six subjects returned, and two were unavailable, for a fourth session during which NMES was applied at 200 Hz. The time of day of each session was the same for each subject to reduce the potential confounding effect of diurnal changes in CNS excitability (Lagerquist et al. 2006; Tamm et al. 2009).

Electromyography (EMG)

EMG was recorded from TA, Sol, and VM of the right leg using bipolar (2.25 cm^2) surface-recording electrodes (Vermed Medical, Bellow Falls, Vermont). EMG signals were pre-amplified (1,000×) and band pass filtered at 30–1,000 Hz (NeuroLog system; Digitimer, Welwyn Garden City, Hertfordshire, England). Data were sampled at 2,000 Hz for maximal voluntary isometric contractions and 5,000 Hz for all evoked potentials with a 12-bit A/D converter (National Instruments, Austin, Texas). During the collection of MEPs, data were recorded in 450-ms sweeps from 100 ms before to 350 ms after stimulus delivery.

NMES

NMES was applied over the right CP nerve using bipolar (2.25 cm^2) surface electrodes (Vermed Medical Inc.) placed near the fibular head at the site that evoked a response (M-wave or H-reflex) in TA at the lowest stimulation intensity. Rectangular pulses of 1-ms duration were delivered from a Digitimer (DS7A, Hertfordshire, England) constant current stimulator at an intensity at which a single stimulus evoked an M-wave that was ~15% of M_{max} in TA. The stimulation was delivered for 40 min at either 10, 50, 100, or 200 Hz in a 20 s on, 20 s off cycle.

TMS

To test the excitability of the CS pathway, MEPs were evoked in the right TA using TMS (Magpro R30; Medtronic Inc., Minneapolis, Minnesota) applied using a figure of eight coil (Medtronic MC-B70, Minneapolis, Minnesota). All MEPs were evoked while subjects remained relaxed. MEPs were recorded before (control), during, and after each 40-min period of NMES, as depicted in Fig. 1. The optimal stimulation site for right TA was found by moving the coil over the left motor cortex to find the site that elicited the largest amplitude MEP in TA at the lowest intensity of stimulation. Using a Brainsight image-guided stimulation system (Rogue Research, Montreal, Quebec), this site was recorded and the coil was manually held in place to maintain position and orientation (precision: ±3 mm) for all trials. MEP threshold for TA was then determined by finding the lowest intensity that produced MEPs of at least 50 uV in 4 out of 8 trials. The intensity of TMS was then set at 120% of this threshold for the remainder of the experiment. Six MEPs were recorded immediately before and after each 40-min bout of NMES at an inter-stimulus interval that varied randomly between 5 and 8 s. To quantify the time course of any changes in CS excitability during the 40 min of NMES, 3 MEPs were evoked

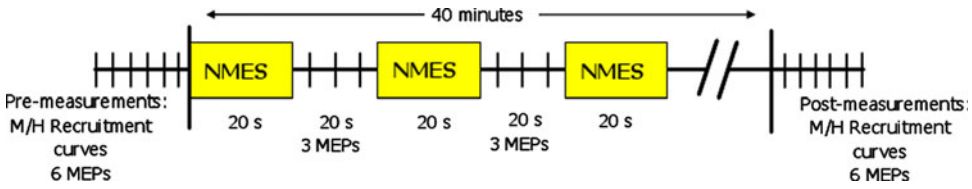

Fig. 1 Schematic of timeline for one experimental session. NMES was delivered at 10, 50, 100, or 200 Hz over the CP nerve on separate days. Each *vertical line* represents the timing of delivery of the TMS. MEPs were evoked by TMS delivered at 120% of resting MEP threshold determined before each 40-min period of NMES

in each 20 s "off" period of the stimulation at an inter-stimulus interval of 8 s (see Fig. 1).

M-wave/H-reflex (M/H) recruitment curves

To assess changes in spinal excitability, we calculated the ratio of H_{max}: M_{max} using data from M/H recruitment curves collected before and after the NMES from TA in all eight subjects and Sol in six subjects (see Fig. 1). The right CP and tibial nerves were stimulated (Digitimer, DS7A, Hertfordshire, England; 1 ms pulse width) using bipolar (2.25 cm^2) surface electrodes (Vermed Medical Inc.) placed near the fibular head and over the popliteal fossa, respectively. In many subjects, it was difficult to evoke consistent H-reflexes in the TA muscle at rest, therefore all M/H recruitment curves for TA were collected while subjects held a background contraction of 5% maximal EMG output using visual feedback of TA EMG low-pass filtered at 1 Hz. Sol M/H recruitment curves were collected at rest. Each recruitment curve was constructed from responses to 40 stimuli delivered with an inter-stimulus interval that varied randomly between 3 and 5 s. Stimulation intensity was varied pseudo-randomly from below M-wave and H-reflex threshold to 1.5–2 times the minimum current required to evoke M_{max}.

Data analyses

MEPs recorded from TA were measured peak-to-peak and normalized to M_{max}. M_{max} was calculated as the largest M-wave in TA from each M/H recruitment curve. All MEP data (TA, Sol, VM) were visually inspected *post hoc* and responses evoked when there was background EMG activity prior to the stimulation were removed from the analysis. MEPs were discarded if the EMG during the 1 s prior to the TMS stimuli exceeded two standard deviations of the average baseline signal recorded at rest before the stimulation. Of the 16,200 MEPs that were evoked from eight subjects, 86 MEPs (<1% of total responses) were removed from the analyses based on this criteria.

Changes in CS excitability during the 40 min of NMES were quantified by averaging MEPs over 2-min intervals. In this way, the 3 MEPs evoked in each of 3 successive "off" periods were averaged together ($n = 9$). A two-way repeated measures analysis of variance (ANOVA) was used to compare the effect of different frequencies of NMES on the MEP amplitude for TA. Using the data available for all eight subjects, the factors for the ANOVA were "Frequency" (three levels: 10, 50, 100 Hz) and "Time" (22 levels: pre-NMES, post-NMES, each 2-min interval during NMES). The same analysis was used to compare changes in MEP amplitude for TA evoked by 100- and 200-Hz stimulation for the six subjects who received the NMES at 200 Hz, but with only two levels of "Frequency." Because our main interest was in the "Frequency × Time" interaction, main effects of "Frequency" and "Time" are only reported when the interaction was not significant.

MEPs were also evoked in Sol and VM each time a TA MEP was elicited. Sol and VM MEPs were measured peak-to-peak as described earlier for TA. However, because we did not measure M_{max} in these muscles in all subjects, Sol and VM MEP measurements were not converted to a percentage of M_{max} and were left in mV. Because the amplitude of a MEP as measured in mV could differ between days due to changes at the recording site, comparisons between data collected on separate days (i.e., at different frequencies) were not appropriate for analyzing these data. Thus, separate one-way repeated measures ANOVAs with 22 levels of time were used to test the influence of each NMES frequency on MEPs recorded from Sol and VM. This analysis enabled us to evaluate the effect of time of stimulation, but not the effect of frequency, on changes in MEP amplitude for these heteronymous, non-stimulated muscles.

Our measure of spinal excitability was the ratio of H_{max}:M_{max}. H_{max} and M_{max} were measured from peak-to-peak, and their ratio was calculated from the average of the three largest H-reflexes and the single largest M-wave from each M/H recruitment curve for both TA and Sol muscles. A two-way repeated measures ANOVA with "Frequency" (3 levels: 10, 50, 100 Hz) and "Time" (2 levels: pre-NMES, post-NMES) as factors was used to analyze changes in H_{max}:M_{max} ratios. The same analysis was used to compare changes in H_{max}:M_{max} ratios evoked by 100- and 200-Hz NMES for the six subjects who participated in both sessions, but with only two levels of "Frequency."

For all tests, the significance level was set at $p < 0.05$. *Post hoc* analyses (Tukey HSD tests) were performed when appropriate. All descriptive statistics are reported as mean ± standard error.

Results

Significant increases in TA MEPs were induced by NMES delivered at 100 Hz but not 10, 50, or 200 Hz. MEPs became significantly larger than control (pre) values at the 24th min of 100-Hz NMES and remained elevated throughout and immediately following the 40-min stimulation period. Changes in MEPs of other, non-stimulated, leg muscles (Sol, VM) followed similar patterns as those observed for TA, but these changes were mostly not of statistical significance. There were no statistically significant changes in H_{max}:M_{max} ratios in either TA or Sol for any of the NMES protocols.

MEPs in TA

Figure 2 shows data recorded from one subject before, during, and after 40 min of NMES of the CP nerve at 10, 50, 100, and 200 Hz. Panel A shows MEPs recorded before and after each NMES protocol. In this individual MEPs increased by 20% after 40 min of 10-Hz NMES, decreased by 9% after NMES at 50 Hz, increased by 169% after NMES at 100 Hz, and increased by 38% after 200-Hz NMES; however, changes in MEP amplitude for individual subjects were not tested for statistical significance. Panel B shows that MEPs evoked throughout 40 min of NMES at 100 Hz were consistently larger than those evoked during NMES at the other three frequencies.

The amplitude of MEPs averaged across all subjects ($n = 8$) who received the 10-, 50-, and 100-Hz NMES are shown in Fig. 3. The data in panel A show that MEPs evoked during the 100-Hz NMES became larger than those evoked during NMES at the other frequencies approximately halfway through the stimulation. The ANOVA analyses identified a significant interaction between "Frequency" and "Time" [$F_{(42,\ 294)} = 2.25$, $p < 0.01$]. *Post hoc* comparisons showed that 40 min of NMES at 100 Hz, but not 10 or 50 Hz, increased TA MEPs significantly from control. After the 100-Hz stimulation, MEPs were significantly elevated by 101% while MEPs evoked after the 10-Hz (27% increase) and 50-Hz (54% increase) stimulation were not significantly different from control. The mean amplitude of MEPs recorded before and after each NMES protocol for each subject is shown in Panel B. The significant increases in MEP amplitude from control during NMES at 100 Hz began 24 min into the stimulation and MEPs remained elevated

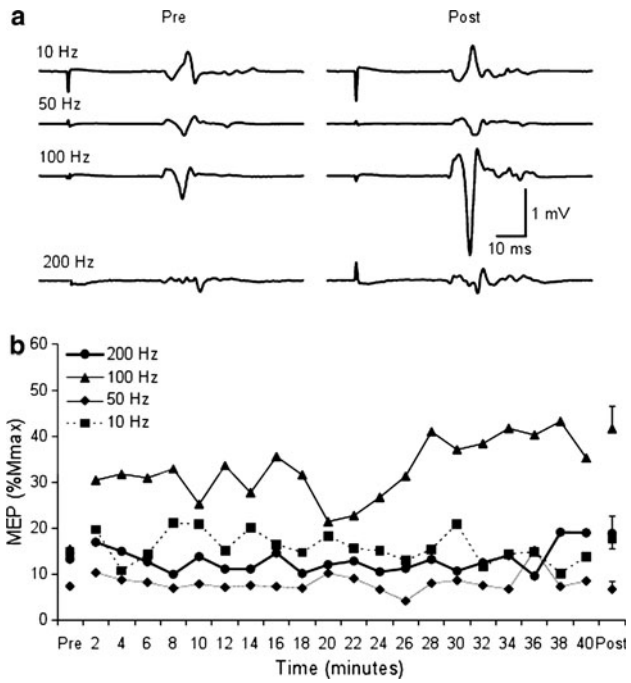

Fig. 2 Changes in TA MEP amplitude induced by NMES delivered at four frequencies in a single subject. *Panel A* shows the mean waveforms of MEPs ($n = 6$) evoked before (*control*; left panel) and after (*post*; right panel) each frequency. *Panel B* shows the mean amplitude of MEPs recorded before (*pre*), during (2–40), and after (*post*) 40 min of NMES at each frequency. Data collected during the stimulation are an average of 9 MEPs. *Error bars* represent one standard error

during and after the 100-Hz stimulation, as shown by the open triangles in Panel A. MEPs recorded before the stimulation (i.e., control) were not significantly different between frequencies. In contrast, starting 28 min into the stimulation, MEPs evoked during the 100-Hz NMES became significantly elevated from MEPS recorded at the same time point during NMES at 10 and 50 Hz as shown by the asterisks. There were no significant increases in TA MEPs from control at any time during or after the 10- or 50-Hz stimulation.

Mean data averaged over the 6 participants who received both 100- and 200-Hz NMES are shown in Fig. 4. There was a significant interaction between "Frequency" and "Time" [$F_{(21,\ 105)} = 2.24$, $p < 0.01$]. *Post hoc* comparisons showed that 40 min of 200-Hz NMES did not significantly alter TA MEPs whereas 100-Hz NMES increased TA MEPs significantly from control. The open symbols in Fig. 4a show that MEPs started to become significantly elevated from control at the 28th min of NMES. The asterisks in Fig. 4a indicate significant differences between MEPs evoked at the same time point during 100- and 200-Hz NMES. Mean pre-NMES and post-NMES MEP amplitudes for each subject are shown in Fig. 4b.

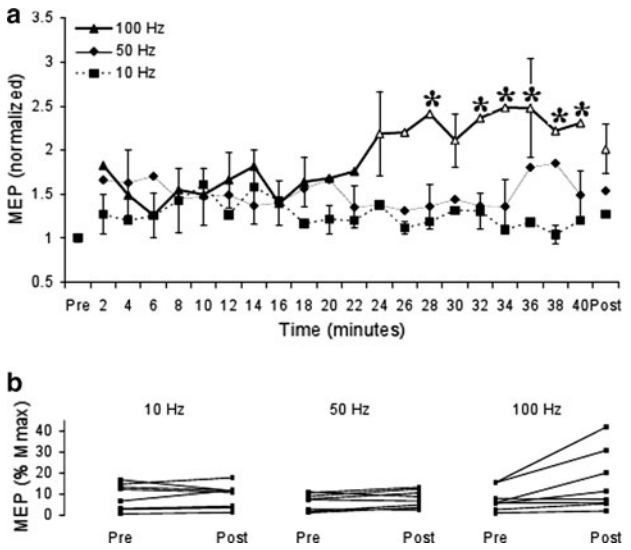

Fig. 3 Amplitude of MEPs recorded from TA averaged across the group ($n = 8$) before (*pre*), during (2–40), and after (*post*) 10-, 50-, and 100-Hz NMES. All data were normalized to control MEP amplitude recorded before the NMES. *Open data symbols* in *Panel A* indicate significant differences from control (*pre*). *Asterisks* indicate significant differences from MEPs recorded at the same time point during 10- and 50-Hz NMES. *Error bars* represent one standard error. For clarity, error bars have been staggered and are shown for only one, rather than all three data points, at each time point. *Panel B* shows mean MEP amplitude recorded before (*pre*) and after (*post*) each stimulation frequency for each subject

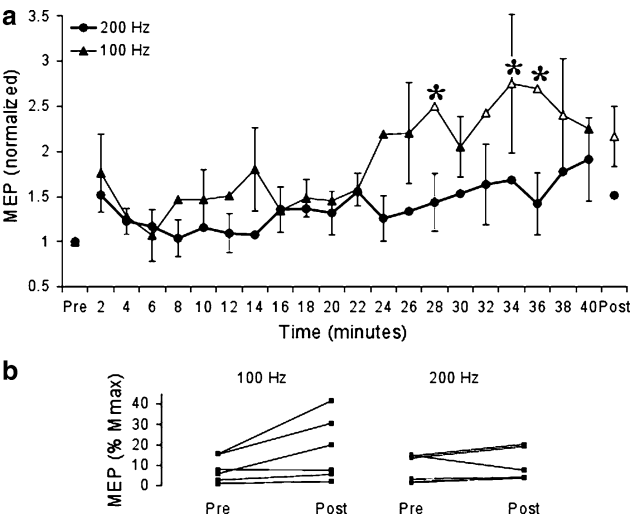

Fig. 4 Amplitude of MEPs recorded from TA averaged across the group ($n = 6$) before (*pre*), during (2–40), and after (*post*) 100- and 200-Hz NMES. All data were normalized to MEP amplitude recorded before the NMES. *Open data symbols* in *Panel A* indicate significant differences from control (*pre*). *Asterisks* indicate significant differences from MEPs recorded at the same time point during 200-Hz NMES. *Error bars* represent one standard error. For clarity, error bars have been staggered and are shown for only one, rather than both data points, at each time point. *Panel B* shows mean MEP amplitude recorded before (*pre*) and after (*post*) stimulation at 100 and 200 Hz for each subject

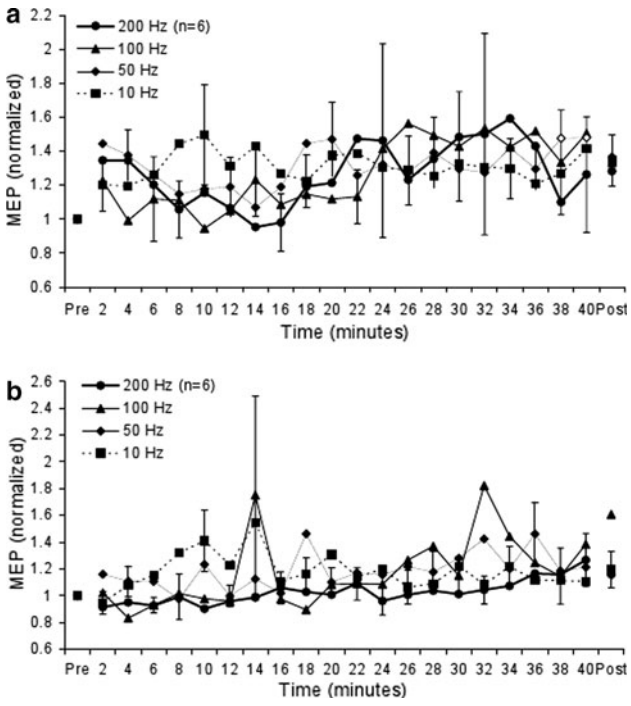

Fig. 5 Time course of changes in MEP amplitude for Sol (*panel A*) and VM (*panel B*) averaged across the group during 10-, 50-, 100-, and 200-Hz NMES. *Open data points* indicate significant differences from control (*pre*). *Error bars* represent one standard error. For clarity, error bars have been staggered and are shown for only one, rather than all four data points, at each time point

MEPs in Sol and VM

MEPs were also evoked in Sol and VM by the same stimulus that evoked MEPs in TA. There was no significant main effect of "Time" of stimulation for Sol MEPs recorded during 10-Hz [$F_{(21,\ 147)} = 1.22$, $p = 0.24$], 100-Hz [$F_{(21,\ 147)} = 1.05$, $p = 0.41$], or 200-Hz NMES [$F_{(21,\ 105)} = 0.60$, $p = 0.91$], but there was a significant main effect of "Time" during 50-Hz stimulation [$F_{(21,\ 147)} = 1.78$, $p = 0.03$]. *Post hoc* comparisons showed that Sol MEP amplitude was significantly elevated from control at the 38th and 40th min during the 50-Hz stimulation as denoted by the open symbols in Fig. 5a but were not significantly elevated immediately after the NMES. There was no significant effect of "Time" of stimulation for VM MEP amplitude during 10-Hz [$F_{(21,\ 147)} = 1.51$, $p = 0.08$], 50-Hz [$F_{(21,147)} = 1.27$, $p = 0.21$], 100-Hz [$F_{(21,\ 147)} = 1.13$, $p = 0.32$] or 200-Hz [$F_{(21,\ 105)} = 1.09$, $p = 0.37$] NMES.

M/H recruitment curves

Stimulation applied at 10, 50, 100, and 200 Hz produced no significant changes in H_{max}:M_{max} ratio for TA or Sol. The 4 two-way repeated measures ANOVAs used to analyze

these data showed that all main effects and interaction effects had p-values greater than 0.2. Averaged across the four stimulation frequencies, H_{max}:M_{max} ratios for TA were 9 ± 4 before and 9 ± 5 after stimulation and for Sol were 55 ± 24 before and 50 ± 22 after stimulation.

Discussion

The present experiments were designed to test the hypothesis that higher frequencies of CP nerve stimulation would increase CS excitability for TA to a greater extent than lower frequencies of stimulation. Our main finding was that 100-Hz NMES applied over the CP nerve was more effective than 10-, 50-, and 200-Hz NMES at increasing CS excitability for TA. Changes in CS excitability for heteronymous non-stimulated muscles (Sol, VM) were mostly not of statistical significance, suggesting that the effect of NMES on CS excitability was strongest in the stimulated muscle. The H_{max}:M_{max} ratio in TA and Sol was not altered by NMES at any frequency, suggesting that there were no changes in spinal excitability for either muscle.

Frequency-dependent changes in CS excitability

Increases in CS excitability can be evoked by NMES, and the electrically evoked afferent drive transmitted to the cortex is crucial for inducing these changes (Ridding et al. 2000, 2001; Khaslavskaia et al. 2002; McKay et al. 2002b; Knash et al. 2003; Kido and Stein 2004; Khaslavskaia and Sinkjaer 2005). Stimulating the pharynx at a fairly high intensity, Fraser and colleagues (2002) found that 5-Hz NMES was most effective for increasing CS excitability. Stimulating muscles of the hand at a fairly low intensity, 3-Hz NMES depressed CS excitability and 30-Hz stimulation facilitated CS excitability (Pitcher et al. 2003). Despite the difference in stimulation intensity between these studies, they suggest that changes in CS excitability driven by NMES are dependent on stimulation frequency and that the relationship between NMES frequency and CS excitability changes may be different for different muscle groups. The present study is the first to investigate the relationship between NMES frequency and CS excitability for muscles of the lower limb. Consistent with our hypothesis, 100-Hz stimulation was more effective for increasing CS excitability than lower frequencies (10 and 50 Hz). CS excitability increased significantly (a twofold increase) following 100-Hz NMES, but the changes were smaller and not significant after 10- and 50-Hz NMES (27 and 54% increases, respectively). Contrary to our hypothesis, 200-Hz NMES was less effective than 100 Hz for increasing CS excitability and did not significantly alter MEP amplitudes from control. By the 28th min of stimulation, CS excitability for TA during 100-Hz CP nerve stimulation was significantly higher than CS excitability for TA at the same time point of the 10-, 50-, and 200-Hz stimulation protocols. As shown in Figs. 3b and 4b, some subjects had particularly robust increases in CS excitability following 100-Hz NMES while others did not. Similar inter-subject variability was found for the effect of NMES on CS excitability for hand muscles (Kaelin-Lang et al. 2002), suggesting that the sensory volley evoked by NMES has a greater effect on CS excitability in some individuals than in others. Nevertheless, our data show a frequency-dependent effect of NMES for increasing CS excitability for TA and show that when NMES is delivered under the conditions of the present study (i.e., stimulus intensity, pulse width, pattern, and duration), 100-Hz stimulation was more effective than 10, 50, or 200 Hz. Whether this effect of frequency would remain for different combinations of stimulation parameters is presently unknown and is beyond the scope of the present study.

The observed changes in CS excitability may depend on the number of pulses delivered rather than the frequency of NMES. Longer periods of 10- and 50-Hz stimulation might produce similar changes in CS excitability to those observed during 100-Hz stimulation. However, if CS excitability depended only on the number of pulses, it would have increased the most during our 200-Hz stimulation and this did not occur. When using NMES for rehabilitation, determining the number of pulses needed to evoke changes in CS excitability is less important than determining which NMES frequency induces the changes to the greatest extent and the fastest. Hence, our aim was to determine an effect of frequency on inducing CS excitability changes rather than exploring the effect of number of pulses.

Contrary to our results, past research has shown significant increases in CS excitability for limb muscles following frequencies of NMES $\leq$30 Hz. In the upper limb, ulnar nerve stimulation at 10 Hz increased CS excitability for the first dorsal interrosseus and abductor digiti minimi muscles after 45 min of stimulation (Ridding et al. 2000; McKay et al. 2002a). However, this is outside the range of our 40-min stimulation protocol, stimulation intensities were lower than those used in the present study, and differences in cortical organization between the upper and lower limbs (Kurusu and Kitamura 1999) could influence how NMES affects CS excitability. In the lower limb, the significant increases in CS excitability reported by Khaslavskaia and Sinkjaer (2005) and Knash, and colleagues (2003) of 38 and 50%, respectively, following 25–30-Hz NMES are comparable to the non-significant changes in MEPs evoked by 10- and 50-Hz stimulation in the present study (27 and 54% increases). Hence, it is important to note that we do not propose that lower frequency NMES does not affect CS excitability, but rather that NMES at 100 Hz has a stronger effect on CS excitability than NMES at lower frequencies.

NMES at 200 Hz did not significantly increase CS excitability in the present study. The lack of change in CS excitability when NMES was applied at 200 Hz may be due to the hyperpolarization of sensory axons beneath the stimulating electrodes. Immediately after an action potential travels along a human axon, fluctuations in axonal excitability include a period of hyperpolarization (Burke et al. 2001). Prolonged trains of NMES with short inter-stimulus intervals, such as our 20 s trains of 200-Hz stimulation, result in deeper and longer periods of hyperpolarization (Burke et al. 2001). When axons are hyperpolarized, they will be more difficult to recruit with NMES, hence decreasing the afferent volley transmitted to the CNS. Khaslavskaia and colleagues (2002) used 200-Hz stimulation of the CP nerve and found significant increases in TA MEP amplitude; however, their stimulation was delivered for 20 ms (i.e., 5 pulses) once every second for 30 min, rather than the 20 s trains used in the present study. Hyperpolarization is maximal with a train of 10–20 impulses, and the delivery of more pulses lengthens the sub-normal excitability period, but if the stimulation stops when the axon first reaches maximal hyperpolarization, then resting state is re-established in ~100 ms (Burke et al. 2001). Thus, the pattern of stimulation utilized by Khaslavskaia and colleagues (2002) may have resulted in less hyperpolarization than the pattern used in this study. Nevertheless, our results suggest that prolonged stimulation trains delivered in an on–off cycle, similar to when NMES is used for rehabilitation, are more effective for increasing CS excitability when delivered at 100 Hz than at 10, 50, or 200 Hz.

Time course of CS excitability increases

CS excitability has been shown to remain elevated after 30 min of NMES in leg muscles (Khaslavskaia et al. 2002) and 45 min in arm muscles (McKay et al. 2002a). Although CP nerve stimulation transiently increased CS excitability for TA after just 10 min, the increase did not persist to the 20th min and only by the 30th min was a lasting increase observed (Knash et al. 2003). The present study charts the time course of MEP changes during NMES with a higher resolution (2 min) than has previously been documented and shows that sustained increases in CS excitability can occur after 24 min during NMES of the lower limb, somewhat earlier than the 30 min that has been reported previously. Although this time course provides no direct evidence for mechanisms underlying CS excitability changes evoked by NMES, it is consistent with past research suggesting that mechanisms such as long-term potentiation may play a role (Hess and Donoghue 1994; Butefisch et al. 2000; Kaelin-Lang et al. 2002; McKay et al. 2002a).

Generalizability of the effects of NMES

Stimulating the ulnar nerve in the upper limb, Ridding and colleagues (2000) found significant changes in cortical excitability in muscles innervated by the ulnar nerve, but not in a heteronymous non-stimulated muscle. Khaslavskaia and colleagues (2002) showed that NMES of the CP nerve increased TA MEP amplitude twofold, but did not alter MEPs of the antagonist muscle (Sol). These observations suggest that CS excitability changes are specific to the muscles innervated by the stimulated nerve. Similarly, we observed changes in CS excitability that were primarily restricted to TA. Somewhat surprisingly, 50-Hz NMES did not induce significant changes in TA MEPs, but increased MEPs in Sol at the 38th and 40th min (but not immediately thereafter). Research exploring the effect of a single electrical pulse to the tibial nerve shows that MEPs were altered in Sol and TA (the heteronymous muscle) and thus supports a more global effect of afferent input on CS excitability (Roy and Gorassini 2008). Thus, NMES applied to the leg may have an effect on CS excitability for heteronymous muscles, but our data suggest that the effects are not as strong as for the stimulated muscles. This study and past studies have evaluated CS excitability changes in non-stimulated muscles by measuring MEP responses of these muscles when evoked by TMS at the optimal location for the stimulated muscle, rather than the optimal location for the heteronymous muscles. If there are smaller effects of NMES on CS excitability for heteronymous muscles, then perhaps more specific measures are necessary to detect these changes. Further research focusing specifically on the effects of NMES on CS excitability for non-stimulated muscles might clarify the extent to which such changes occur.

Location of excitability changes

Increases in TMS-evoked MEPs could be a result of changes in neural excitability in the brain or spinal cord. Because of their different sites of activation, transcranial electrical stimulation (TES), brainstem electrical stimulation (BES), and F-wave measures have been utilized to differentiate the location of the excitability changes. Many studies show increases in TMS-evoked MEPs following NMES without concomitant changes in TES or BES-evoked MEPs, or F-wave amplitude, and therefore conclude that excitability changes occur in the cortex and not in the spinal cord (Stefan et al. 2000; Ridding et al. 2000; Stefan et al. 2002). Conversely, Khaslavskaia and colleagues (2002) show changes in TMS-evoked MEPs and smaller changes in TES-evoked MEPs following CP nerve stimulation, suggesting that excitability changes do occur in the spinal cord. Nevertheless, the same motor neurons may

not be activated by TMS as are activated by TES, BES, and F-waves, and therefore these data should be interpreted with caution. We measured spinal excitability for TA and Sol with the ratio of H_{max}:M_{max}, whereby increases in the ratio suggest increases in spinal excitability. There was no change in this ratio for either TA or Sol from before to after stimulation, suggesting that changes in MEPs were of cortical origin.

Implications

NMES is commonly used to treat impaired ankle dorsiflexion that often develops after CNS trauma (Liberson et al. 1961; Merletti et al. 1978; Chae et al. 2008). In addition to the obvious improvements in dorsiflexion that occur during the stimulation, it became evident early on that benefits can persist even after the stimulation was turned-off (Liberson et al. 1961). We now know that these persistent benefits include improvements in walking speed (Ladouceur and Barbeau 2000), reduced spasticity (Stefanovska et al. 1989), and increased dorsiflexor strength (Merletti et al. 1978) and these occur concomitantly with increased CS excitability (Knash et al. 2003; Kido and Stein 2004). Persistent increases in hand muscle strength have been induced by peripheral nerve stimulation applied at a low intensity (no visible twitch) to preferentially activate sensory fibers (Conforto et al. 2002). Studies using similar electrical stimulation protocols to Conforto and colleagues have found increases in CS excitability (Ridding et al. 2000; Mckay et al. 2002a, b; Pitcher et al. 2003), suggesting that the functional improvements in the upper limb (Conforto et al. 2002) also coincide with increases in CS excitability. Moreover, increases in MEPs of the biceps muscle by combined motor practice and increased sensory input have been associated with improvements in elbow flexion (Ziemann et al. 2001), and cortical plasticity evoked during motor skill acquisition has been related to marked improvements for skill performance and further skill learning (Pascual-Leone et al. 1995). These results suggest that increased CS excitability is involved in lasting functional improvements. If enhancing CS excitability leads to functional improvements, then development of methods to further enhance CS excitability will be important for maximizing the efficacy of NMES therapies. However, inter-subject variability in the responses to NMES suggests that increases in CS excitability induced by NMES, and any associated benefits for rehabilitation, may be greater in some individuals than others. Nonetheless, our data show that NMES applied at 100 Hz is more effective than NMES at 10, 50, and 200 Hz for increasing CS excitability of the dorsiflexors.

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