Neurophysiological Characterization of Transpinal Evoked Potentials in Human Leg Muscles

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The objectives of this study were to characterize the neurophysiological properties of the compound muscle action potentials (CMAPs) evoked by transcutaneous electric stimulation of the spine (tsESS), and the effects of tsESS on the soleus H-reflex in seated and standing healthy human subjects. In seated semi-prone subjects with the trunk semi-flexed, two re-usable self-adhering electrodes (cathode), connected to act as one electrode, were placed bilaterally on the iliac crests. A re-usable pregelled electrode (anode) was placed on the thoracolumbar region at thoracic 10–lumbar 1 and held under constant pressure throughout the experiment. CMAPs were recorded bilaterally from ankle muscles with subjects seated semi-prone at 1.0, 0.3, 0.2, 0.125, and 0.1 Hz following tsESS. The soleus H-reflex, evoked by posterior tibial nerve stimulation via conventional methods, was measured following tsESS at inter-stimulus intervals (ISIs) that ranged from −100 to 100 ms with the subjects seated semi-prone and during standing. The tsESS-induced CMAPs were not decreased at low stimulation frequencies, and the soleus H-reflex excitability was profoundly decreased by tsESS at ISIs that ranged from −5 to 20 ms with the subjects seated semi-prone and during standing. CMAPs induced by tsESS may be utilized to assess spinal-to-muscle conduction time while bypassing spinal motoneuron excitability and tsESS can be used as a modality to decrease spinal reflex hyper-excitability in neurological disorders. Bioelectromagnetics

Key words: soleus H-reflex; low-frequency depression; electric stimulation; spine; multisegmental responses; thoracolumbar region

INTRODUCTION

Transcutaneous electric stimulation of the spine (tsESS) over the cervicothoracic or thoracolumbar region produces compound muscle action potentials (CMAPs) in upper and lower limb muscles in humans while at rest and during walking [Mills and Murray, 1986; Maertens de Noordhout et al., 1988; Courtine et al., 2007; Minassian et al., 2007; Sabbahi and Sengul, 2011]. These CMAPs are described to be susceptible to spinal inhibitory mechanisms acting at a pre- and/or post-alpha motoneuronal level, similar to those reported for the soleus Hoffmann (H)-reflex [Knikou, 2008]. For example, the soleus H-reflex is profoundly depressed following Achilles tendon vibration and activation of antagonist motoneurons in healthy humans [Abbruzzese et al., 2001; Knikou, 2008; Jessop et al., 2013]. Soleus (SOL) muscle voluntary contraction increased the homologous CMAPs induced by tsESS, while voluntary contraction of the antagonistic tibialis anterior (TA) muscle or vibration of the Achilles tendon decreased the SOL CMAPs [Maertens de Noordhout et al., 1988]. Further, during walking the electrically induced CMAPs were increased and/or decreased when the corresponding muscle from which they were recorded was active or relaxed, respectively [Courtine et al., 2007], similar to the soleus H-reflex phase-dependent modulation during walking in healthy humans [Knikou et al., 2009; Knikou et al., 2011].

A distinguished characteristic of the monosynaptic H-reflex is that it is profoundly depressed when

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primary muscle spindle (Ia) afferents are activated at a low frequency [Jessop et al., 2013]. This depression occurs at the synapse between Ia afferents and alpha motoneurons and is ascribed to a presynaptic inhibitory mechanism known as homosynaptic depression [Hultborn et al., 1996]. Sources of this depression include a decrease in the amount of released neurotransmitters [Kuno, 1964], depletion of releasable vesicles, failure of action potential conduction at axonal branches [Brody and Yue, 2000], decrease in presynaptic quantal size [Chen et al., 2004], and adaptation of exocytosis machinery [Hsu et al., 1996]. The low frequency-mediated depression of the Ia excitatory postsynaptic potentials in humans has been attributed to similar neural mechanisms as those documented in animals [Crone and Nielsen, 1989; Hultborn et al., 1996; Kohn et al., 1997], while recent findings suggest that it is prone to descending influences [Raoul et al., 2012].

We have recently shown that the CMAPs recorded from the ankle muscles following transcutaneous magnetic stimulation of the spine (tsMSS) over the thoracolumbar region are not susceptible to homosynaptic depression and that tsMSS depresses soleus H-reflex excitability in semi-prone-seated subjects [Knikou, 2013]. In this study, we examined whether similar effects are produced following transcutaneous electric stimulation of the thoracolumbar region. Accordingly, we established the susceptibility of tsESS-induced CAMPs to low stimulation frequencies, the relationship of their latency and shape to soleus H-reflexes, and the effects of tsESS on the soleus H-reflex in healthy people while seated semi-prone and during standing.

MATERIALS AND METHODS

Subjects

The experimental protocol was approved by the Graduate Center of the City University of New York (NY, USA) Institutional Review Board committee and was conducted in compliance with the Declaration of Helsinki. Each subject signed an informed consent form before participating to the study. Ten adults (seven male and three female) between the ages of 21–42 years (28 $\pm$ 7.4), without neuromuscular or orthopedic disorders, participated in the study. These subjects also formed the subject group in a previous study [Knikou, 2013].

Electromyography Recordings

Following standard skin preparation, single differential bipolar surface electromyography (EMG) electrodes (Bagnoli 8 System, Delsys, Boston, MA) were placed bilaterally on the SOL, medial gastrocnemius (MG), TA, and peroneus longus (PL) muscles, and were secured with 3M Tegaderm transparent film (3M, St. Paul, MN). All EMG signals were filtered with a cut-off frequency of 20–1000 Hz (1401 plus running Spike2; Cambridge Electronic Design, Cambridge, UK) and were sampled at 2000 Hz.

Posterior Tibial Nerve Stimulation (Soleus H-Reflex)

The soleus H-reflex was evoked by stimulation of the right posterior tibial nerve at the popliteal fossa with square pulse stimuli of 1 ms duration delivered by a constant current stimulator (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) [Knikou and Taglianetti, 2006]. The anode (stainless steel electrode 4 cm in diameter) was placed proximal to the patella. A stainless steel, hand-held, monopolar (cathode) electrode was placed at the popliteal fossa and used as a probe to determine the most optimal stimulation site [Knikou, 2008]. This site corresponded to the one during which, at low stimulation intensities, Ia afferents could selectively be excited with absent activation of motor axons (M-wave), and the shape of the M-wave was similar to that of the H-reflex at low and high stimulation intensities. When the optimal stimulation site was identified, the monopolar electrode was replaced by a permanent electrode (N-10-A, Medicotest, Ølstykee, Denmark) that was held under constant pressure throughout the experiment via an athletic wrap. The posterior tibial nerve was stimulated at intensities that ranged from 5.3 to 30.5 mA (17 $\pm$ 2.2) across subjects. These intensities corresponded to soleus M-waves that ranged from 3% to 5% of the maximal M-wave.

Transcutaneous Electric Stimulation of the Spine

Subjects were seated semi-prone with the trunk semi-flexed on a Biodex adjustable chair (Accessory Chair Model 870-170, Biodex Medical Systems, Shirley, NY) with their hips at 110°–120°, knees at 100°–125°, ankles at 90° and both feet and arms supported. All subjects were asked to relax during the experiments, not rotate their head, and not move their arms or legs. Two re-usable self-adhering electrodes of 10.16 $\times$ 5.08 cm (anodes; Model EP84169, Uni-Patch, Wabasha, MN), connected to function as a single electrode, were placed bilaterally on the iliac crests (Fig. 1A). The thoracic 10 vertebras were identified through palpation, and a monopolar, stainless steel, circular, handheld electrode (anode) was used to establish the most optimal stimulation site.

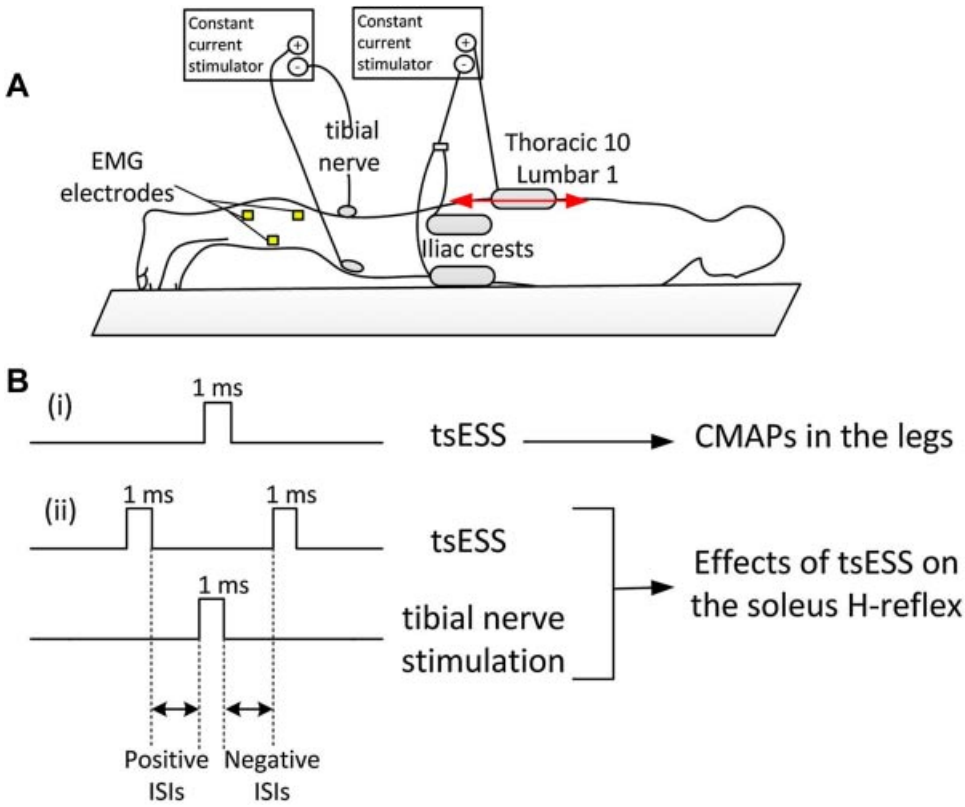

Fig. 1. **A**: Position of the anode electrode placed over the thoracolumbar region and cathodes placed bilaterally on the iliac crests. Transcutaneous electric stimulation of the spine (tsESS) was delivered either alone or in combination with posterior tibial nerve stimulation. Double arrow on the anode electrode indicates the possible traveling directions of impulses induced by tsESS. **B**: Schematic illustration of the stimulation protocols utilized in this study. In Graph ii, the timing of tsESS and posterior tibial nerve stimulation is indicated. Both compound muscle action potentials (CMAPs) and soleus H-reflexes were evoked by single square pulses 1 ms in duration. EMG, electromyograms; ISIs, inter-stimulus intervals.

This site corresponded to the one that transpinal evoked potentials (TEPs) in most ankle muscles could be evoked at low stimulation intensities. The monopolar, hand-held, stainless steel electrode was then replaced by a self-adhering electrode of 10.16 $\times$ 5.08 cm, and held under constant pressure throughout the experiment via an athletic wrap (Fig. 1A). This electrode (anode) was placed on top of the spine, equally between the left and right paravertebrae sides. Because of its size, the stimulating electrode covered the area up to the lumbar 1 vertebrae. The stimulator for tsESS (DS7A) was triggered by an analog-to-digital acquisition system with customized scripts written in Spike2 with single square pulses of 1 ms duration. The stimulation intensity during which CMAPs in the leg muscles were first noted on the oscilloscope (TDS 2014, Tektronix, Beaverton, OR) was termed the TEP threshold. The stimulation intensities ranged from 61.3 to 92.3 mA (78.78 $\pm$ 10.61) across subjects and corresponded to 1.1–1.2 times the TEP threshold intensity. At these stimulation intensities, subjects reported no pain or discomfort.

Experimental Protocol

With the subjects seated semi-prone and having established the most optimal stimulation sites, CMAPs were recorded randomly at 1.0, 0.3, 0.2, 0.125, and 0.1 Hz in order to establish their susceptibility to low-frequency stimulation. At each stimulation frequency, 10 CMAPs were recorded. When the susceptibility of CMAPs to low-frequency stimulation was not examined, tsESS was always delivered at 0.2 Hz (Fig. 1Bi) with a single square pulse of 1 ms duration. The maximal M-wave following posterior tibial nerve stimulation was then evoked and measured as the peak-to-peak amplitude on the oscilloscope, and saved for offline analysis. The stimulation intensity was

adjusted to evoke control H-reflexes on the ascending limb of the recruitment curve that ranged from 15% to 25% of the maximal M-wave. tsESS preceded the soleus H-reflex at inter-stimulus intervals (ISIs) between the two pulses that ranged from −100 (−100, −50, −20, −10, and −5 ms) to 100 ms (0, 5, 10, 20, and 100 ms) with subjects seated semi-prone and during standing (Fig. 1Bii). Negative ISIs denoted that tsESS was delivered after posterior tibial nerve stimulation. During standing, the soleus H-reflex was depressed compared to that recorded with the subjects seated [Knikou and Rymer, 2003]. The soleus H-reflex depression during standing was counteracted by appropriate adjustments of the stimulation intensity in order to assess the true conditioning reflex effects of tsESS. Conditioned and control H-reflexes were randomly recorded. At each tested ISI, 20 H-reflexes at 0.2 Hz were recorded for all subjects.

Data Analysis

All recorded potentials were measured as the area under the full-wave rectified waveform. The latency of the CMAPs was measured from the start of the single pulse to the first positive or negative deflection from the baseline. The CMAPs that manifested as a prolonged stimulus artifact (usually in the left leg) were excluded from this analysis. The latency was grouped across subjects based on the muscle, and a Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks was conducted to establish statistically significant differences of the CMAPs' latency across muscles. A Student's *t*-test was also used to establish statistically significant differences between the left and right CMAPs.

For each subject, the CMAPs evoked at 1.0, 0.3, 0.2, and 0.125 Hz were expressed as a percentage of the mean amplitude of the associated CMAP evoked at 0.1 Hz. The CMAPs evoked by tsESS were grouped across subjects based on frequency and muscle, and an ANOVA for repeated measures at 32 levels (4 frequencies × 8 muscles) was conducted to establish statistically significant differences of the CMAPs as a function of frequency and muscle.

The soleus H-reflex, recorded when tsESS was delivered at specific ISIs, was expressed as a percentage of the mean amplitude of the control reflex values. The mean amplitude of the soleus H-reflex from each subject was grouped based on the ISI tested, and a one-way ANOVA was conducted to establish statistically significant differences. This analysis was conducted separately for H-reflexes recorded with the subjects seated semi-prone and during standing, as well as for the M-waves that were expressed as a percentage of the maximal M-wave. A two-way ANOVA at 20 levels (2 positions × 10 ISIs) was conducted to establish statistically significant differences between the soleus H-reflexes recorded with the subjects seated semi-prone and during standing. All statistical tests were conducted at the 95% confidence interval. Mean amplitudes are reported along with the standard error of means (SEM). Standard deviation (SD) of means is reported only for the latency of the CMAPs and H-reflexes.

RESULTS

In Figure 2, non-rectified waveform averages ($n = 10$ evoked at 0.2 Hz) of CMAPs following tsESS of the thoracolumbar region recorded from three subjects while seated semi-prone are indicated. Note that the shape of CMAPs was not similar across muscles (Fig. 2), consistent with our recent observations [Knikou, 2013]. CMAPs of ankle extensors manifested as a biphasic and/or a triphasic waveform, while CMAPs of ankle flexors manifested mostly as a polyphasic waveform (see right (R) TA in subject 3 and left (L) TA in subjects 5 and 7 in Fig. 2). Last, the CMAPs of the ankle flexor muscles were larger in amplitude compared to those observed in ankle extensors.

In Figure 3, non-rectified waveform averages of the maximal M-wave, H-reflex (∼20% of the maximal M-wave), and CMAPs of the SOL muscle recorded from two subjects are indicated. In subject 3, the soleus H-reflex latency was prolonged by 13 ms compared to the SOL CMAP (Fig. 3A), while in subject 7 the soleus H-reflex latency was prolonged by 15 ms compared to the SOL CMAP (Fig. 3B). The mean latencies of the soleus H-reflex and the CMAPs recorded from all muscles and subjects are shown in Figure 3C. The soleus H-reflex latency (30.71 ± 1.49; mean ± SD) was 1.85 times the SOL CMAPs latency (16.57 ± 2.14; mean ± SD), while the SOL CMAPs latency was prolonged by ∼1.2 ms compared to the half latency for the soleus H-reflex. A Kruskal–Wallis one-way ANOVA on ranks showed that the latency of the CMAPs was statistically significant from the soleus H-reflex latency ($F = 31.24$, $P < 0.001$), while a Student's *t*-test conducted separately for the pairs of the R and L SOL, TA, MG, and PL CMAPs showed that the latencies of CMAPs recorded from both legs were not statistically significant ($P > 0.05$).

The overall amplitude of the CMAPs evoked by tsESS and recorded from the right and left SOL, MG, TA, and PL muscles at 1.0, 0.3, 0.2, and 0.125 Hz, respectively, is shown in Figure 4. CMAPs are presented as a percentage of the mean amplitude of the associated CMAP evoked at 0.1 Hz. The right

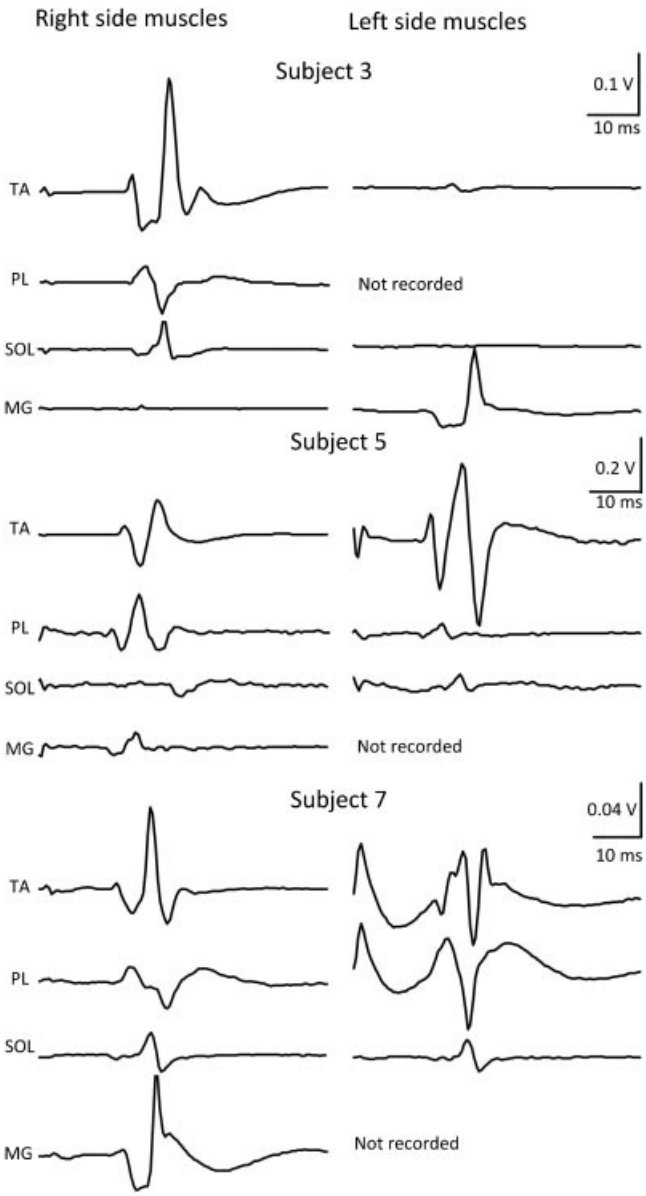

Fig. 2. Transpinal evoked potentials. Representative examples of non-rectified waveform averages ($n = 10$, elicited at 0.2 Hz) of CMAPs recorded from the tibialis anterior (TA), peroneus longus (PL), soleus (SOL), and medialis gastrocnemius (MG) muscles form both legs following tsESS of the spine over the thoracolumbar region.

SOL CMAPs did not vary at different stimulation frequencies ($F = 0.067$, $P = 0.99$). The same result was also found for the CMAPs recorded from the R and L MG, TA, and PL muscles. An ANOVA for repeated measures at 32 levels (8 muscles $\times$ 4 frequencies) showed that the amplitude of the CMAPs did not vary with changes in stimulation frequencies ($F = 0.088$, $P = 0.96$).

The effects of tsESS of the thoracolumbar region at ISIs of -5 and $+5$ ms on the average soleus H-reflex recorded from two subjects seated semi-prone is shown in Figure 5A. In both examples, the magnitude of the conditioned soleus H-reflex was significantly reduced compared to control reflex values, and the soleus H-reflex depression occurred with stable soleus M-waves under control conditions and during tsESS. The amplitude of the conditioned soleus H-reflex from all subjects and ISIs tested is shown in Figure 5B. The soleus H-reflex varied significantly across the ISIs tested ($F = 6.86$, $P < 0.001$), while the differences in the soleus H-reflexes at ISIs ranging from -5 to $+20$ ms were statistically significant from the other ISIs and from control reflex values ($P < 0.05$). The soleus H-reflex depression coincided with stable M-waves ($F = 0.153$, $P = 0.998$) that ranged from $2.3 \pm 1.1\%$ to $3.5 \pm 0.63\%$ of the maximal M-wave across subjects (Fig. 5C).

A representative example of the effects of tsESS on the soleus H-reflex with subjects standing is shown in Figure 6A for ISIs of $+5$ and -5 ms. The soleus H-reflex varied significantly across the ISIs tested ($F = 8.32$, $P < 0.001$; one-way ANOVA), while post hoc Bonferroni tests for multiple comparisons showed that the differences in the soleus H-reflexes at ISIs ranging from -5 to $+20$ ms were statistically significant from the other ISIs and from control reflex values ($P < 0.05$; Fig. 6B). A two-way ANOVA at 20 levels (2 positions $\times$ 10 ISIs) showed that the H-reflex amplitude varied significantly as a function of the body position ($F = 16.88$, $P < 0.001$). The soleus H-reflex depression following tsESS with the subjects standing coincided with stable M-waves ($F = 0.143$, $P = 0.998$) that ranged from $2.3 \pm 0.02\%$ to $3.5 \pm 1.1\%$ of the maximal M-wave across subjects (Fig. 6C), signifying that the same motoneurons were activated under control conditions and during reflex conditioning.

DISCUSSION

This study demonstrated for the first time that transcutaneous electric stimulation of the thoracolumbar region significantly attenuates soleus H-reflex excitability in seated and standing healthy human subjects, and that CMAPs evoked by tsESS are not susceptible to low frequency depression. These findings are consistent with those we have recently reported following magnetic stimulation of the human spine [Knikou, 2013].

The CMAPs recorded from the ankle extensor muscles had mainly a diphasic or triphasic shape, while the CMAPs of ankle flexors had a polyphasic

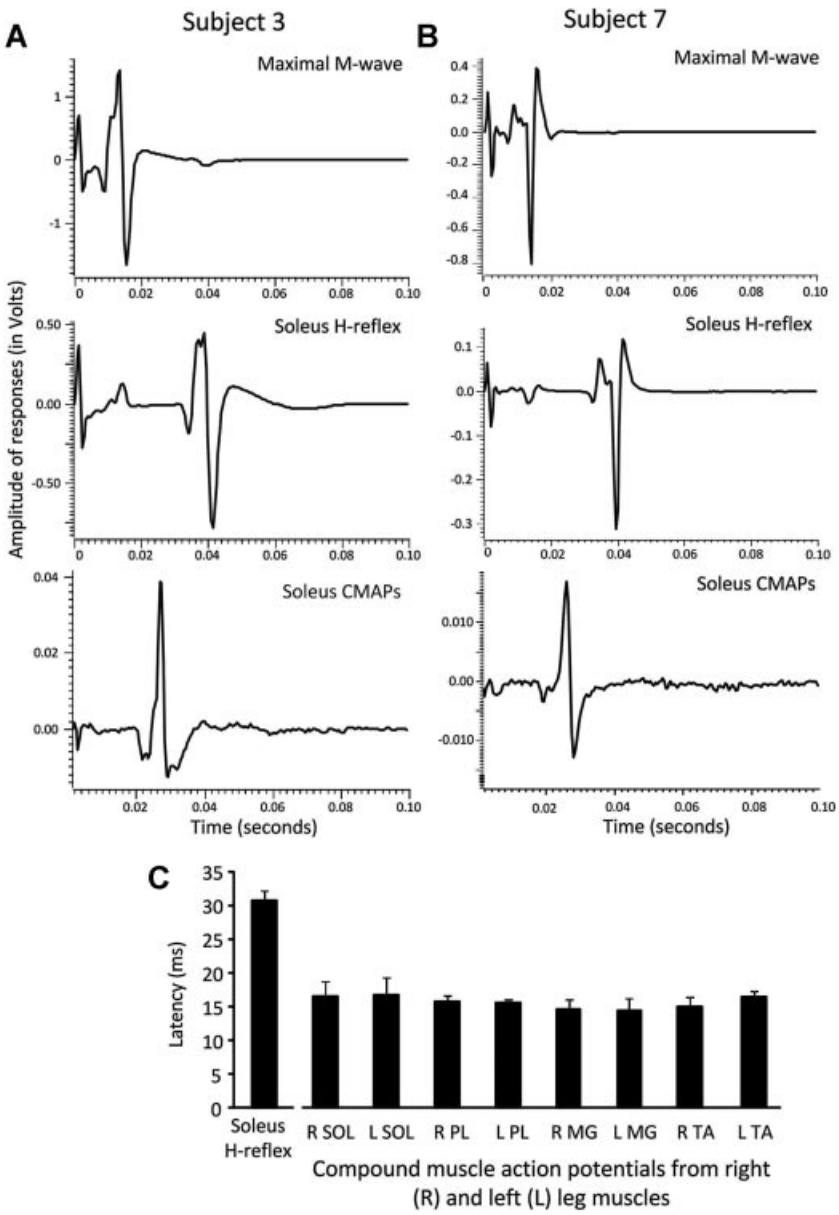

Fig. 3. Latency of transpinal evoked potentials. **A** and **B**: Non rectified waveform averages (evoked at 0.2 Hz) of soleus maximal M wave and soleus H reflex following posterior tibial nerve stimulation, and soleus (SOL) CMAPs. **C**: Overall average latency (in ms) of the soleus H reflex and CMAPs recorded from the right (R) and left (L) SOL, peroneus longus (PL), medialis gastrocnemius (MG), and tibialis anterior (TA) muscles. Error bars denote the SEM.

phase (Fig. 2). The non-uniform shape of ankle flexor and extensor CMAPs in the same subject and across subjects may be due to excitation of different types of fibers (afferents vs. motor) with diverse conduction velocities, and to the different spinal segments that innervate these muscles. For example, epidural stimulation in normal awake rats at lumbar two induces middle and late responses but not early responses as observed following stimulation at sacral 1 [Gerasimenko et al., 2006]. In humans, a polyphasic phase has been reported for CMAPs recorded from the quadriceps femoris, SOL, biceps, and deltoid muscles [Maertens de Noordhout et al., 1988; Ugawa et al., 1989; Chokroverty et al., 1991]. In the same subjects, the latency of the CMAPs recorded from the SOL and TA muscles was prolonged by approximately 4.5 ms following tsMSS [Knikou, 2013], consistent with the increased latency of evoked motor potentials by transcranial magnetic stimulation compared to transcranial electric stimulation [Nielsen et al., 1995]. Across the

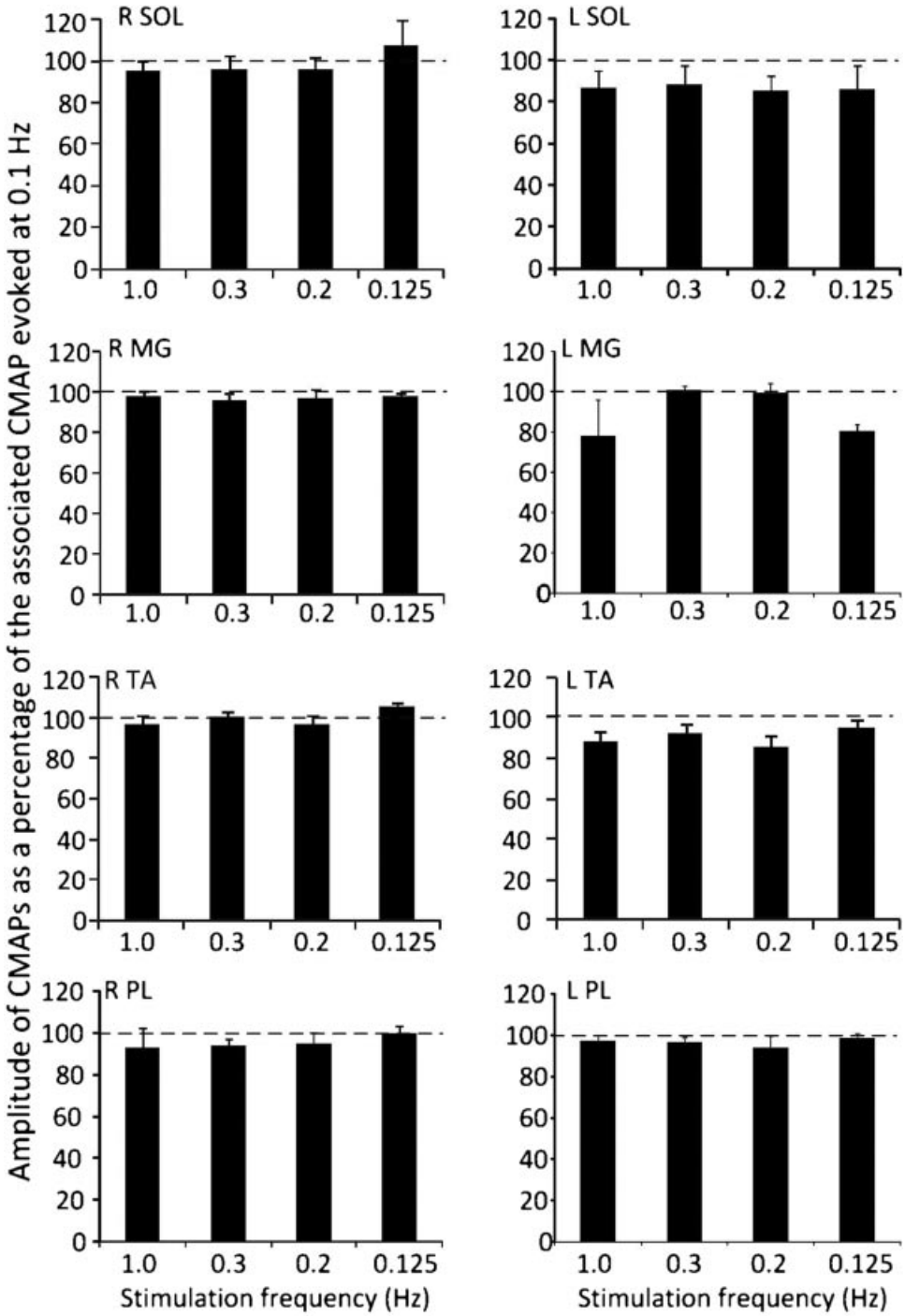

Fig. 4. Susceptibility of tsESS-induced CMAPs to low frequency stimulation. Overall mean amplitude of the compound muscle action potentials (CMAPs) recorded bilaterally from the right (R) and left (L) soleus (SOL), medialis gastrocnemius (MG), tibialis anterior (TA), and peroneus longus (PL) muscles are indicated. CMAPs evoked at 1.0, 0.3, 0.2, and 0.125 Hz are presented as a percentage of the mean amplitude of the associated CMAP evoked at 0.1 Hz. Error bars represent the SEM.

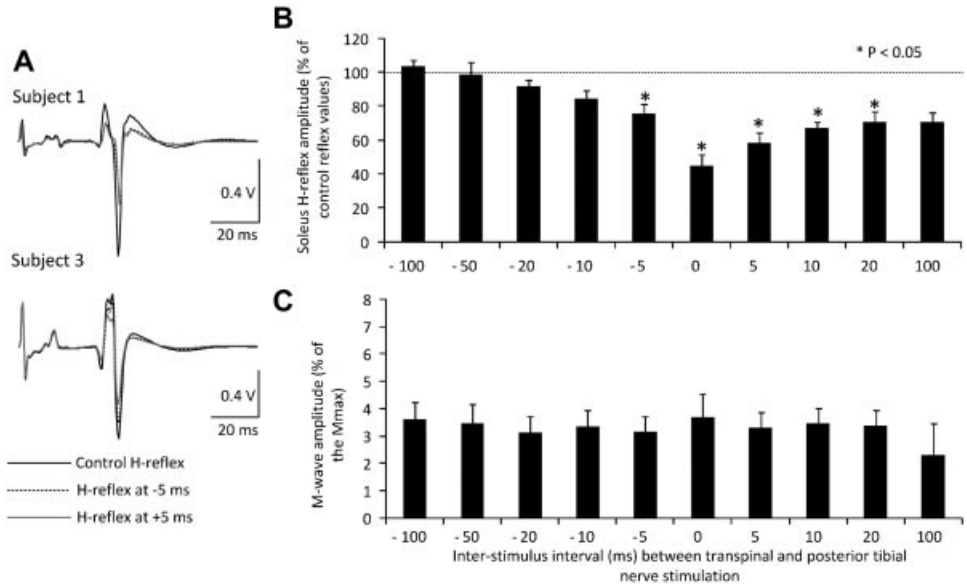

Fig. 5. Effects of tsESS on the soleus H-reflex in semi-prone seated subjects. **A**: Non-rectified waveform averages of the soleus H-reflex in two subjects (S1 and S3) under control conditions and following tsESS at inter-stimulus intervals of +5 and −5 ms. Note that the soleus H-reflex depression occurs with stable M-waves. **B**: Overall average amplitude of the soleus H-reflex conditioned by tsESS as a percentage of the control H-reflex. **C**: Overall average amplitude of the soleus M-wave. Asterisks indicate statistically significant differences between the conditioned and control soleus H-reflex. Error bars indicate the SEM.

studies reported in the literature, the latency of the SOL CMAPs ranged from 15.4 to 27.1 ms [Courtine et al., 2007; Kitano and Koceja, 2009; Sabbahi and Sengul, 2011]. Differences in the latencies and shapes of CMAPs reported in the literature may be related to the placement of the electrode, size of the electrode, and therefore to the density of the electrically induced potential field, resistive and capacitive properties of the electrodes, and thickness of the underlying tissue [Maertens de Noordhout et al., 1988; Butson and McIntyre, 2005; Kuhn et al., 2009].

CMAPs evoked by tsESS were not susceptible to low frequency depression (Fig. 4), an inhibitory neuronal mechanism clearly documented for the soleus H-reflex and attributed to the decreased amount of neurotransmitters from the previously activated afferent fibers at the presynaptic Ia afferent terminals [Hultborn et al., 1996; Knikou, 2008]. This finding is consistent with our recent observations on the CMAPs induced by magnetic stimulation of the spine in semi-prone, seated, healthy subjects [Knikou, 2013]. These data indicate that absent depression of CMAPs at low frequencies is likely related to the excited structures following tsESS.

tsESS generates impulses that travel along the posterior and anterior root fibers exciting the fibers at the spinal cord entry or at their exit from the spinal canal [Ladenbauer et al., 2010]. Excitation of dorsal column fibers, motor axons, and antidromic activation of primary muscle spindle afferents (Ia) in the dorsal column may contribute to the generation of these CMAPs [Coburn, 1985; Maertens de Noordhout et al., 1988; Bayoumi and Ashby, 1989; Hunter and Ashby, 1994; Ladenbauer et al., 2010]. Collision experiments showed that F-waves could be recorded upon concomitant supramaximal peripheral nerve and spinal stimulation, and appear 2.2 ms after the CMAPs [Mills and Murray, 1986; Maertens de Noordhout et al., 1988; Ugawa et al., 1989]. This is consistent with the proposed excitation site that is distal to the anterior horn cells, based on differences between indirect (spinal stimulation) and direct (F-wave) latencies and computed tomography (CT) scan measurements of the distance between the dura and intervertebral foramina [Ugawa et al., 1989; Chokroverty et al., 1991; Epstein et al., 1991]. This means that the nerve roots are excited near their exit from the spinal column or near the emergence of the axons from the anterior horn cells [Mills and Murray, 1986; Cros et al., 1990]. This stimulation site is further supported by results obtained through a recent simulation study that showed that action potentials are initiated along the posterior and anterior root fibers, exciting the fibers at the spinal cord entry or at their exit from the spinal

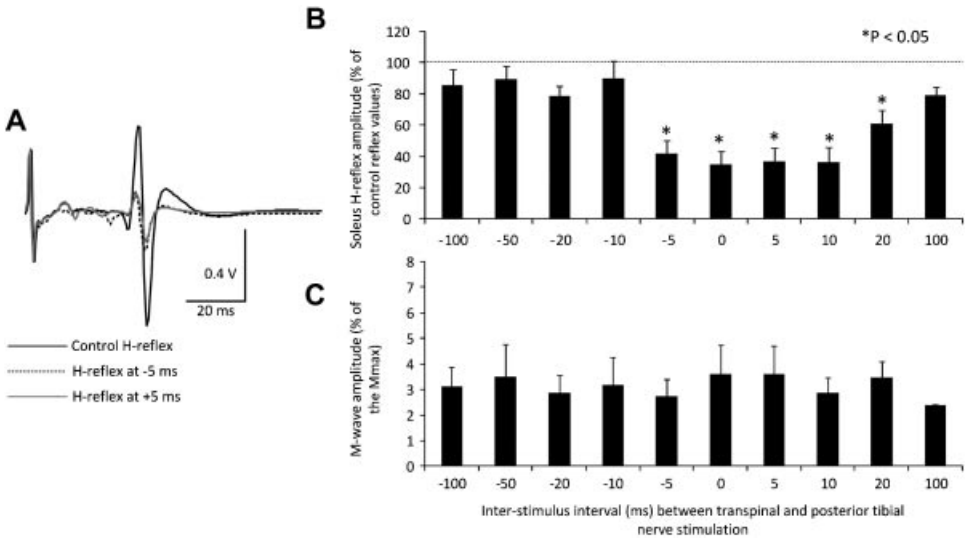

Fig. 6. Effects of tsESS on the soleus H-reflex during standing. **A**: Non-rectified waveform averages of the soleus H-reflex in one subject (S3) under control conditions and following tsESS at inter-stimulus intervals of $+5$ and -5 ms. **B**: Overall average amplitude of the soleus H-reflex conditioned by tsESS as a percentage of the control H-reflex. **C**: Overall average amplitude of the soleus M-wave. Asterisks indicate statistically significant differences between the conditioned and control soleus H-reflex. Error bars indicate the SEM.

canal [Ladenbauer et al., 2010]. Based on our results and on these studies, CMAPs are not depressed when stimulation is delivered at low frequencies because they are not due to synchronized Ia afferent volleys but rather represent composite excitatory potentials of different types of afferents as well as efferents. Our thesis is supported by the postulated co-activation of large and small posterior and anterior root fibers following transcutaneous lumbar spinal cord stimulation [Danner et al., 2011].

One of the important findings of this study is that tsESS over the thoracolumbar region depressed the soleus H-reflex with subjects seated semi-prone and during standing (Figs. 5B and 6B). Because the potentiation of soleus H-reflex depression during standing was counteracted, similar neuronal mechanisms (or circuits) mediated the soleus H-reflex depression in both body positions. Soleus H-reflex depression might have been mediated at a presynaptic or postsynaptic level or simultaneously at both synaptic levels. The soleus H-reflex depression at short ISIs may be due to the potentiation of Ia afferent hyperpolarization by antidromic excitation of Group Ia afferents in the dorsal columns [Hunter and Ashby, 1994], and to repetitive volleys produced by activation of the lowest threshold and fastest conducting afferents acting at a postsynaptic level [Halonen et al., 1989]. Because the latency of the CMAPs recorded from the right and left SOL muscles was prolonged by $\sim$1.2 ms compared to the half latency of the soleus H-reflex, polysynaptic spinal reflex pathways had ample time to affect alpha motoneurons [Ugawa et al., 1995]. At longer ISIs, orthodromic transmission of impulses in dorsal column fibers may have modulated supraspinal activity of the somatosensory system, where with a conduction velocity of 68 $\pm$ 5 m/s [Ugawa et al., 1995], there is sufficient time to affect spinal reflex circuits. This is supported by the altered motor cortex-induced muscle contraction and thalamo-cortical activity following transpinal stimulation in anaesthetized rats and mice [Aguilar et al., 2011; Ahmed, 2011].

Stimulation of the posterior tibial nerve produces action potentials that run dorsally and perpendicular to the surface of the cord, representing activity of dorsal roots traveling rostrally and ventral roots traveling caudally [Yiannikas and Shahani, 1988]. Because these spinal potentials produce depolarization of terminals of primary afferents responsible for presynaptic inhibition, and thus represent interneuronal activity [Wall, 1958], the soleus H-reflex depression is likely related not only to the timing of spinal potentials but also to spinal interneuronal circuits activated indirectly by these spinal potentials.

Spinal somatosensory-evoked potentials following supramaximal or low threshold peripheral nerve stimulation have a latency of 8–15 ms and duration of $\sim$20 ms [Ertekin, 1976; Delwaide et al., 1985], while excitability of dorsal column axons lasts $\sim$30 ms

[Wall, 1958]. With a latency of ∼15 ms for the spinal stimulation-induced CMAPs, synchronized activity at the spinal level is expected to occur between 0 and 30 ms ISIs but this latency analysis is approximate and the neuronal pathways/circuits that are channeled in the spinal cord over multiple spinal segments following tsESS remains to be determined.

CONCLUSION

CMAPs evoked by tsESS represent composite excitatory potentials of motor nerve fibers excited orthodromically with different types of afferents excited antidromically. It is clear that in order to outline in detail the neurophysiological properties of these CMAPs, single motor unit studies and methods exploiting inhibitory and facilitatory neural events are needed. Nonetheless, CMAPs induced by tsESS can provide information about the spinal-to-muscle conduction time that does not depend on excitability of alpha motoneurons in central nervous system disorders. Further, because electric stimulation of the thoracolumbar region depressed soleus H-reflex excitability in subjects seated semi-prone and during standing, it could potentially be utilized as a modality to normalize reflex hyper-excitability in upper motor neuron lesions. These effects as well as parameters of stimulation in neurological disorders warrant further investigation.

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