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Neural interactions between transspinal evoked potentials and muscle spindle afferents in humans

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ABSTRACT

The objective of this study was to establish neural interactions between transspinal evoked potentials (TEPs) and muscle spindle group Ia afferents in healthy humans. Soleus H-reflexes were assessed following transspinal stimulation at conditioning-test (C-T) intervals that ranged from negative to positive 100 ms. TEPs were recorded from the right and left ankle/knee flexor and extensor muscles, and their amplitude was assessed following stimulation of soleus muscle spindle group Ia afferents at similar C-T intervals. Transspinal conditioning stimulation produced a short-latency, long-lasting soleus H-reflex depression. Excitation of muscle spindle group Ia afferents produced depression of ipsilateral ankle TEPs and medium-latency facilitation of the ipsilateral knee TEPs. At specific C-T intervals, the soleus H-reflex and ipsilateral ankle TEPs were summated based on their relative onset and duration. No changes were observed in the contralateral TEPs. These effects were exerted at both peripheral and spinal levels. Both transspinal and muscle spindle group Ia afferent stimulation produce long-lasting depression of the soleus H-reflex and TEPs, respectively. Transspinal stimulation may promote targeted neuromodulation and can be utilized in upper motoneuron lesions to normalize spinal reflex hyperexcitability and alter excitation thresholds of peripheral nerve axons.

1. Introduction

Transcutaneous spinal cord stimulation over the thoracolumbar region, termed here transspinal stimulation, produces bilateral transspinal evoked potentials (TEPs) along with muscle contractions in ankle and knee muscles (Knikou, 2013a, 2013b). TEPs have distinct neurophysiological characteristics regarding their shape, latency, duration, and propagation of associated volleys (Maruyama et al., 1982; Knikou, 2013a, 2013b, 2014; Einhorn et al., 2013). TEPs are described to be susceptible to spinal inhibitory mechanisms acting at pre- or post-motoneuronal levels, similar to those documented for the Hoffmann (H)-reflex (Knikou, 2008). However, a comprehensive investigation of neural interactions between TEPs and H-reflexes is lacking.

Transspinal stimulation has been associated with orthodromic excitation of motor axons and antidromic excitation of muscle spindle group Ia afferents and their terminal branches, leading to transynaptic excitation of spinal neurons close and far away from the stimulation site (Coburn, 1985; Hunter and Ashby, 1994). TEPs are also thought to be mediated by nonsynaptic activation of motoneurons, transynaptic excitation of descending projections and local spinal interneuron circuits (Maruyama et al., 1982; Maertens de Noordhout et al., 1988; Sharpe and Jackson, 2014; Hunter and Ashby, 1994; Gaunt et al., 2006; Ladenbauer et al., 2010; Knikou, 2013a). These activation sites are supported by the short-latency depression followed by long-latency facilitation of corticospinal excitability in response to transspinal conditioning stimulation (Knikou, 2014). Additionally, the increased spinal output when TEPs and descending motor volleys are synchronized to meet at the spinal cord (Knikou, 2014), and the decreased excitability of monosynaptic reflexes in both arms and legs in response to transspinal conditioning stimulation (Einhorn et al., 2013; Knikou, 2013a) further support the existence of robust neural interactions between TEPs, muscle spindle afferents, and putative motor volleys. It should also be noted that transspinal stimulation affects cortical feedback mechanisms, afferent-mediated facilitation of corticospinal excitability, and descending-mediated tibialis anterior (TA) flexor reflex facilitation (Knikou et al., 2015; Dixon et al., 2016). These findings provide evidence for transspinal stimulation to effectively modulate cortical, corticospinal, and spinal neural excitability.

In an effort to delineate neural interactions between TEPs and muscle spindle group Ia afferents, we studied the amplitude modulation

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profile of the soleus (SOL) TEPs and SOL H-reflexes when transspinal and posterior tibial nerve (PTN) stimulation were delivered at an intensity that both responses were of similar size with respect to the SOL motoneuron pool, as evidenced by the size of the responses relative to the maximal M-wave. SOL H-reflexes were recorded in response to transspinal conditioning stimulation at negative (transspinal delivered after PTN stimulation) and positive (transspinal delivered before PTN stimulation) conditioning-test (C-T) intervals that ranged from −100 to 100 ms. TEPs were recorded bilaterally from ankle and knee muscles in response to right PTN conditioning stimulation at similar negative and positive C-T intervals, but the C-T interval was considered negative when PTN was delivered after transspinal stimulation.

We demonstrate medium and long-latency inhibition of ipsilateral ankle TEPs by muscle spindle group Ia afferent stimulation, and pronounced short-latency, long-lasting SOL H-reflex inhibition by transspinal conditioning stimulation, both depending on the relative onset timing and duration of TEPs and H-reflexes. These results constitute the first evidence for neurophysiological integration of distinct spinal and afferent volleys that can produce targeted neuromodulation in humans.

2. Materials and methods

2.1. Subjects

Twenty three (11 male) healthy adult subjects between the ages of 19 and 39 years (27.4 ± 5.03; mean ± SD) participated in the study. All experimental procedures were conducted in compliance with the Declaration of Helsinki after Institutional Review Board (IRB) approval by the City University of New York (New York, USA). Each subject signed an informed consent form before study enrollment and participation. People with pacemakers, metal implants in the body, medications known to alter central nervous system excitability, and history of neurological, muscular or psychiatric disorders were excluded from the study.

2.2. Surface EMG recordings

Single differential bipolar surface electromyography (EMG) electrodes (MA300-28, Motion Lab Systems Inc., Louisiana, USA) were used to record compound muscle action potentials from the right and left SOL, TA, medial gastrocnemius (MG), peroneus longus (PL), lateral hamstrings (LH), medial hamstrings (MH), gracilis (GRC), and rectus femoris (RF) muscles. The electrodes were maintained in place by Tegaderm transparent films (3 M Healthcare, Minnesota, USA). The EMG signals were filtered using a cut-off frequency of 20–1000 Hz (1401 plus running Spike 2; Cambridge Electronics Design Ltd., Cambridge, UK).

2.3. Experimental procedures

With subjects seated, first we established the optimal stimulation site for the SOL H-reflex in accordance with methods we have previously utilized in human studies (Knikou, 2008, 2013a, 2013b, 2017). Square pulse stimuli of 1-ms duration (DS7A, Digitimer Ltd., Hertfordshire, UK) were triggered by Spike 2 scripts (Cambridge Electronics Design Ltd., Cambridge, UK). A stainless steel plate of 4 cm^2 in size was secured proximal to the patella, while a hand-held monopolar stainless steel head electrode was used as a probe to establish the most optimal stimulation site of the right PTN. The optimal site at the popliteal fossa corresponded to the lowest stimulus intensity at which the SOL H-reflex could be evoked without the presence of an M-wave, and the maximal M-wave had a similar shape to the maximal SOL H-reflex. The hand-held electrode was then replaced by a pre-gelled disposable electrode (4.4 cm diameter, ConMed Suretrace adhesive gel electrode, New York, USA), and was maintained under constant pressure throughout the experiment via a custom-made pressure pad and athletic pre-wrap.

Subjects then transitioned to a treatment table where the optimal stimulation site for transspinal stimulation was established. Identification of the T10 vertebra was performed based on palpation and anatomical landmarks. A single self-adhesive electrode (cathode; 10.2 × 5.1 cm, Uni-Patch™ EP84169, Minnesota, USA) was placed evenly between the left and right paravertebrae sides. Depending on the subject's body height the electrode spanned from T10 to L1-2. The cathode electrode was held under constant pressure via a custom-made pressure pad throughout the experiment and maintained via Tegaderm transparent film. Subjects then laid supine with hips and knees flexed at 30° and both legs in midline, supported as needed by pillows and bolsters. Two self-adhering electrodes (anode; similar type to the cathode), connected to function as a single electrode, were placed on the left and right iliac crests or parallel to the abdominal muscles based on each subject's reported comfort (Knikou, 2014, 2017). The anode and cathode electrodes were connected to a constant current stimulator (DS7A, Digitimer Ltd., Hertfordshire, UK), that was triggered by an analog-to-digital acquisition system with customized scripts written in Spike 2. With single pulses of 1-ms duration the transspinal stimulation intensity increased progressively and the right SOL TEP threshold was determined.

2.4. Experimental protocol for data acquisition

Whilst the subjects laid in supine, the PTN stimulation site was rechecked based on the parameters previously described to ensure that physiological characteristics of the M-wave and H-reflex were not altered. Then, the stimulation intensity was increased progressively to determine the maximal M-wave amplitude. The intensity that evoked a SOL H-reflex, on the ascending limb of the recruitment curve, ranging from 20 to 30% of the maximal M-wave was then determined. Similarly, the transspinal stimulation intensity was increased progressively to determine the intensity that evoked a SOL TEP on the ascending limb of the SOL TEP recruitment curve, ranging from 20 to 30% of the soleus maximal M-wave (Knikou, 2014; Dixon et al., 2016). This was done in order to ensure that similar types and number of alpha motoneurons were excited following transspinal and PTN stimuli. Across subjects, the SOL TEP was 26.21 ± 2.56% and the SOL H-reflex was 28.13 ± 1.8% of the SOL maximal M-wave (paired *t*-test; $p = 0.271$).

SOL H-reflexes were recorded under control conditions, and in response to transspinal conditioning stimulation at negative (transspinal delivered after PTN stimulation) and positive (transspinal delivered before PTN stimulation) C-T intervals that ranged from −100 to 100 ms. Similarly, TEPs from ankle and knee muscles were recorded under control conditions, and following PTN stimulation at negative (PTN delivered after transspinal stimulation) and positive (PTN delivered before transspinal stimulation) C-T intervals ranging from −100 to 100 ms

These C-T intervals were selected in order to establish the onset (i.e. negative) and the duration (i.e. positive) of the conditioning effects in consolidation with the time needed for afferent volleys to reach the spinal cord and their relative duration (Ertekin, 1976a; Delwaide et al., 1985). Due to the long-lasting duration of presynaptic inhibition acting on Ia afferent terminals (Knikou, 2008; Côté et al., 2018), the maximal positive C-T interval was set at 100 ms. Further, we selected to record TEPs from both right and left ankle and knee muscles in response to right soleus muscle spindle group Ia afferent stimulation in order to establish whether this type of conditioning stimulation can affect TEPs recorded in the ipsilateral and contralateral legs. This was based on the well-defined heteronymous Ia afferent connections and interlimb spinal reflex circuits in humans (Bayoumi and Ashby, 1989; Meunier et al., 1990, 1993; see review of Côté et al., 2018). Lastly, TEPs can be easily elicited innocuously with low-intensity stimulation, having similar onset latencies between right and left muscles (Knikou, personal observations). The fact that transspinal stimulation intensity was set based

on the right SOL TEP amplitude as a percentage of the SOL maximal M-wave did not constitute an obstacle to observe TEPs in any of the tested muscles because TEPs can easily be elicited at these intensities (Knikou, personal observations; Sayenko et al., 2015).

At each C-T interval, 15 SOL TEPs and/or H-reflexes along with at least 30 control TEPs and/or H-reflexes were recorded at 0.2 Hz. Conditioned TEPs and/or H-reflexes were randomly recorded across the C-T intervals tested. During the experiment, subjects reported no pain, and blood pressure was not altered.

2.5. Offline data analysis

All compound muscle action potentials (TEPs, maximal M-waves, M-waves, H-reflexes) under control conditions and in response to conditioning stimulation recorded with subjects supine were measured as the area of the full-wave rectified waveform (Spike 2, Cambridge Electronics Design Ltd., Cambridge, UK) for identical time windows.

The latencies of the SOL H-reflex and ipsilateral ankle TEPs recorded under control conditions were estimated based on the cumulative sum technique on the rectified waveform average (Ellaway, 1978). The group latency of the right SOL H-reflex and right SOL, TA, MG, and PL TEPs was 31.53 ± 1.86, 19.96 ± 2.02, 19.16 ± 5.19, 18.3 ± 4.33, and 18.8 ± 2.67 ms (mean ± SD), respectively. These latencies are similar to those we have previously reported (Knikou, 2013a). Based on the latency and duration of the right SOL H-reflexes and ipsilateral ankle TEPs, as well as the time needed for afferent volleys to reach the spinal cord (~10 ms), at specific C-T intervals summation of TEPs and H-reflexes was evident. This occurred for the negative C-T intervals (transspinal delivered after PTN stimulation) ranging from -19 to -4 ms when the test response was the SOL H-reflex. This was also the case for the positive C-T intervals (PTN delivered before transspinal stimulation) ranging from 4 to 25 ms when the test response was the ankle TEPs. An example of this summation from two subjects is shown in Fig. 1. Note that the right SOL H-reflex and the right SOL TEP cannot be separated on the surface EMG at the negative C-T intervals of 19, 16, 13, 10 and 4 ms (Fig. 1C-G). This is consistent to the temporal summation observed between TA motor evoked potentials (MEPs) and TA TEPs when transspinal conditioning stimulation was delivered after cortical test stimulation (Knikou, 2014). Further, in some cases a small M-wave was present along with the SOL H-reflex. In these cases, summation between SOL M-waves and TEPs occurred in the surface EMG, as illustrated in Fig. 1H. This was evident for the C-T intervals ranging from 7 to 25 ms when PTN conditioning was delivered after transspinal test stimulation.

To counteract these neuronal phenomena and establish the net effects of transspinal conditioning stimulation on SOL H-reflexes, the average unconditioned SOL TEP value was subtracted from the conditioned SOL H-reflex values at the negative C-T intervals that summation was evident. Similarly, in order to establish the net effect of muscle spindle group Ia afferent stimulation on TEPs, the average unconditioned SOL H-reflex value was subtracted from the conditioned TEP values at the positive C-T intervals that summation was evident. Further, the average M-wave value recorded under control conditions (whenever present) was subtracted from the right ankle TEPs for C-T intervals during which a summation between M-waves and TEPs was evident. For all cases, the resultant values were expressed as a percentage of the mean amplitude of the associated unconditioned (or control) SOL H-reflex or TEP. This analysis was done only for the SOL H-reflex and ankle TEPs recorded from the right leg. The TEPs recorded from the right and left knee muscles (LH, MH, GRC and RF), and from the left ankle muscles in response to muscle spindle group Ia afferent conditioning stimulation were expressed as a percentage of the mean amplitude of the associated unconditioned TEP.

2.6. Statistics

All data were subjected to the Shapiro-Wilk test for normal distribution. The mean amplitude of the normalized conditioned SOL H-reflex from each subject was grouped based on the C-T interval. One-way analysis of variance (ANOVA) was applied to the data to establish significant differences between control and conditioned H-reflexes, followed by post hoc Bonferroni t-tests for multiple comparisons. Similarly, the mean amplitude of the normalized TEPs from each subject was grouped based on the C-T interval, muscle, and leg side (right/left). One-way ANOVA was applied to establish significant differences between control and conditioned TEP, followed by post hoc Bonferroni t-tests for multiple comparisons. This analysis was conducted separately for TEPs recorded from different muscles. Significance was set at $p < 0.05$. Mean and standard error (SEM) are indicated, unless otherwise stated.

3. Results

3.1. Effects of transspinal stimulation on soleus H-reflex

In Fig. 2A-B, waveform averages of SOL H-reflexes recorded from the right leg under control conditions (gray lines) and following transspinal conditioning stimulation (black lines) of the thoracolumbar region at negative and positive C-T intervals are indicated for two subjects. Note that a negative C-T interval corresponds to transspinal stimulation being delivered after PTN stimulus. SOL H-reflex waveform averages are shown as depicted on the surface EMG, without the TEPs being subtracted from SOL H-reflexes for the C-T intervals that summation was evident. For each subject, the overall amplitude of the normalized subtracted SOL H-reflex is indicated in Fig. 2C and 2D, respectively. It is apparent that transspinal stimulation significantly reduced the SOL H-reflex amplitude from C-T interval of −22 ms until 25 ms (Fig. 2C) based on multiple comparisons versus control H-reflex ($F_{19} = 301.53$, $p < 0.001$). A long-lasting soleus H-reflex depression was also evident in the other subject (Fig. 2D; $F_{16} = 223.58$, $p < 0.001$).

The normalized conditioned SOL H-reflex amplitude recorded from all 23 subjects following transspinal stimulation is depicted in Fig. 3. The C-T interval is denoted on the abscissa and the conditioned SOL H-reflexes are presented as a percentage of the unconditioned reflex values. One-way ANOVA along with post hoc Bonferroni *t*-tests showed that the SOL H-reflexes varied significantly across the C-T intervals tested. The SOL H-reflexes at negative C-T intervals beyond 19 ms and at all positive C-T intervals were significantly different from control H-reflex values ($F_{23} = 126.33$, $p < 0.001$). It is evident that transspinal stimulation resulted in substantial early-latency, long-lasting depression of the SOL H-reflex.

3.2. Effects of muscle spindle group Ia afferent stimulation on TEPs

Waveform averages of TEPs recorded from the right and left ankle muscles under control conditions and following muscle spindle group Ia afferents conditioning stimulation are indicated for one subject in Fig. 4A and B, respectively. Note that negative C-T intervals correspond to PTN being delivered after transspinal stimuli. TEP waveform averages are shown as depicted on the surface EMG without H-reflexes being subtracted for the C-T intervals that summation was evident. Muscle spindle group Ia afferent stimulation produced a significant depression, even at the long C-T intervals of 50 and 100 ms, in the ipsilateral but not in the contralateral ankle flexor/extensor TEPs. The amplitude modulation profile of TEPs in the right ankle muscles was polyphasic with abolishment of inhibition at the interval that summation occurred, followed thereafter by depression and full recovery at the negative C-T interval of 7 ms (Fig. 4C). No significant changes were noted in TEPs recorded from the contralateral ankle muscles (Fig. 4D).

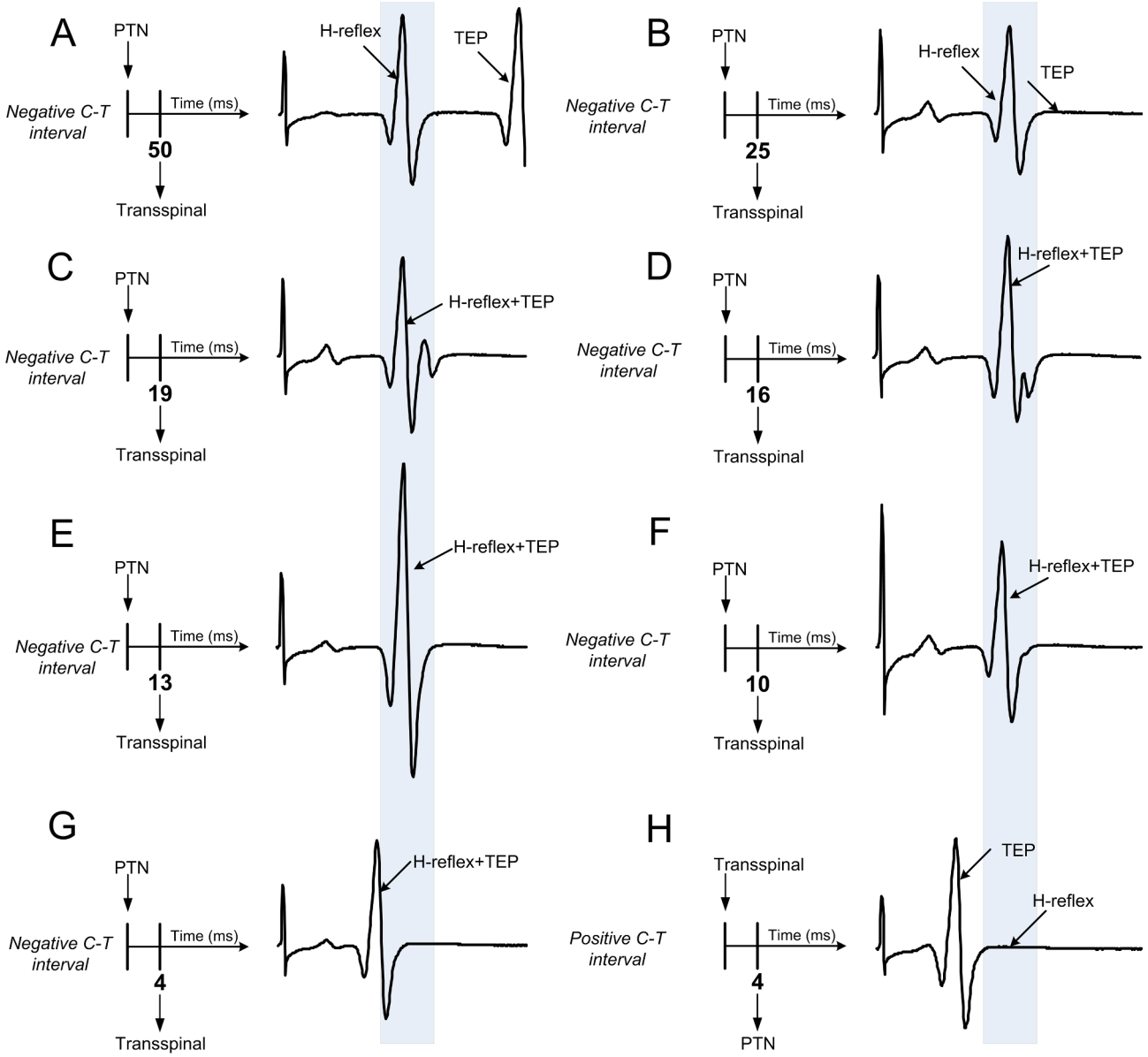

Fig. 1. Summation of H-reflexes and transspinal evoked potentials (TEPs) on surface EMG. Amplitude of the right soleus (SOL) EMG following transspinal stimulation over the thoracolumbar region and posterior tibial nerve (PTN) stimulation are shown. In all paradigms PTN stimulation is the test stimuli and transspinal stimulation is the conditioning stimuli, and waveform averages were computed based on the test stimuli. At the negative conditioning-test (C-T) intervals of 50 and 25 ms, the SOL TEP can be easily separated from the SOL H-reflex based on latency and duration (A, B). However, at the negative C-T intervals of 19, 16, 13, 10 and 4 ms, the SOL H-reflex and TEP do not occlude each other but are summated (C-G), and thus cannot be separated based on latency and duration. To counteract this neuronal phenomenon and establish the net effect of the conditioning stimulus, the control H-reflex values were subtracted from the conditioned TEP values and the control TEP values were subtracted from the conditioned H-reflex values. Note that a negative C-T interval denotes that transspinal was delivered after PTN stimulation.

The normalized subtracted amplitude of TEPs recorded from each muscle and all 23 subjects following conditioning stimulation of group Ia afferents is depicted in Fig. 5. The C-T interval is denoted on the abscissa and TEPs are presented as a percentage of the associated unconditioned TEP values. The right SOL TEPs were decreased at C-T intervals ranging from 4 to 100 ms ($F_{23} = 128.2$, $p < 0.001$), with right SOL TEPs reaching control values at C-T intervals ranging from negative 100 to 0 ms. A similar result was also found for the right MG and PL TEPs (Fig. 5B, 5C), supporting that muscle spindle group Ia afferent conditioning stimulation can affect the amplitude of TEPs recorded from homonymous and heteronymous ipsilateral ankle muscles. A similar behavior was also observed for the antagonistic TA muscle, during which TEPs exhibited depression at the C-T intervals ranging from 7 to 100 ms (Fig. 5D; $F_{23} = 135.2$, $p < 0.001$). For TEPs recorded from the left ankle or knee muscles, two peaks of facilitation were evident in the right MH and LH TEP (Fig. 5E, 5F) at the negative C-T interval of 7 ms and at the positive C-T interval of 13 ms (right MH TEP: $F_{19} = 101.72$, $p < 0.001$; right LH TEP: $F_{19} = 98.61$, $p < 0.001$), while no significant differences were found for the remaining left ankle/knee TEPs ($p > 0.05$ for all). It is apparent that stimulation of muscle spindle group Ia afferents produces a significant short-latency, long-lasting depression of TEPs recorded from the ipsilateral ankle muscles, and facilitation of TEPs recorded from the ipsilateral knee flexor muscles.

4. Discussion

This work demonstrated for the first time that TEPs and H-reflexes summate at specific times based on the timing of interaction between transspinal and peripheral nerve stimuli, and have a similar amplitude

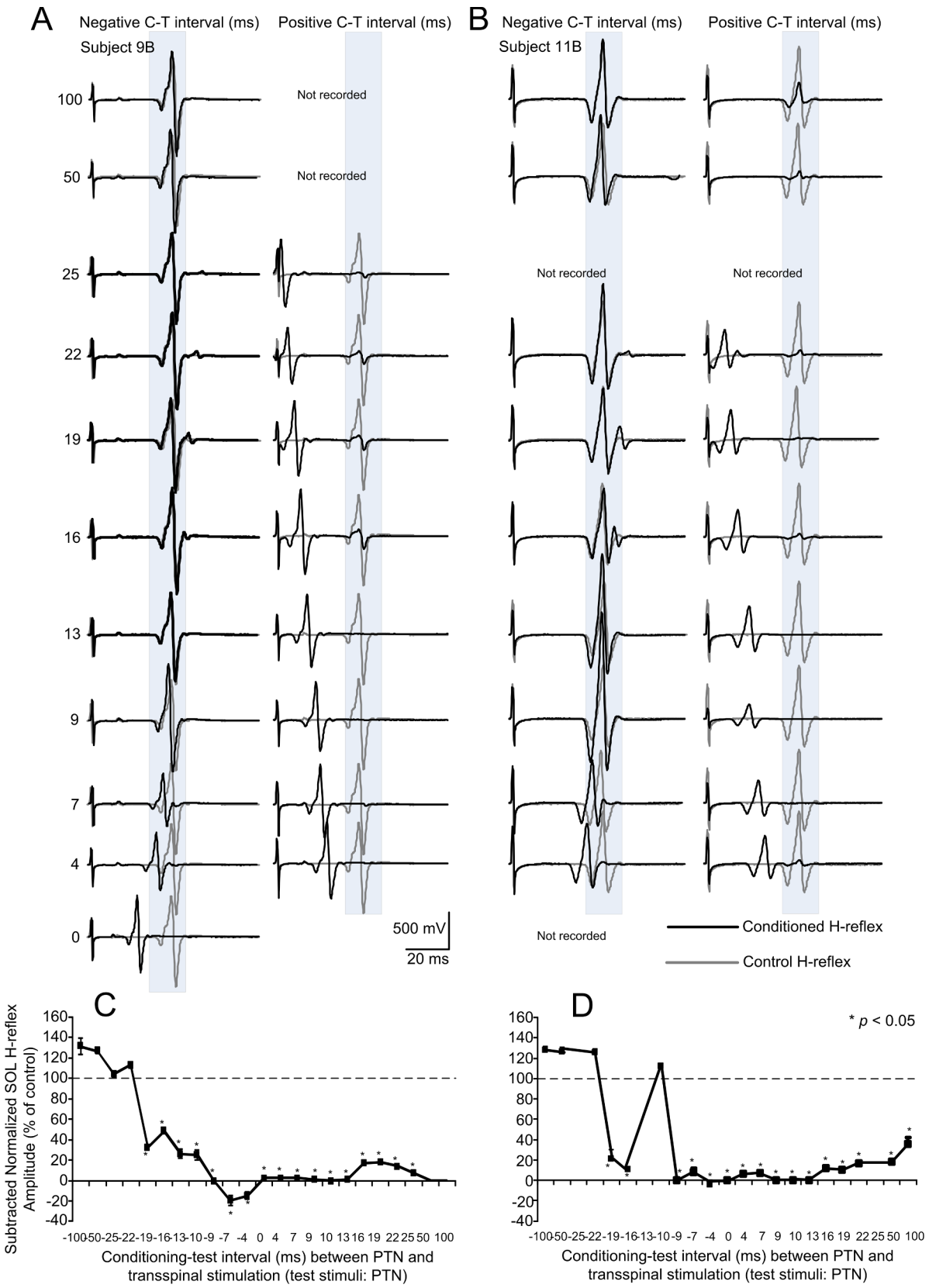

Fig. 2. Soleus (SOL) H-reflexes following transspinal stimulation. (A, B) Waveform averages of the right SOL H-reflex from two representative subjects under control conditions (gray lines) and following transspinal stimulation (black lines) for all conditioning-test (C-T) intervals tested. All EMGs are shown as captured, without SOL transspinal evoked potentials (TEPs) being subtracted from the SOL H-reflexes to counteract summation of these responses. A negative C-T interval denotes that transspinal stimulation was delivered after posterior tibial stimulation (PTN). (C, D) Overall mean amplitude of the conditioned right SOL H-reflexes for the same subjects, in which the net conditioning stimulus effect (i.e., the TEPs induced by transspinal conditioning stimulation were subtracted) is indicated. Asterisks indicate statistically significant differences of conditioned SOL H-reflexes from control values ($p < 0.05$; one-way ANOVA). Error bars denote the SEM.

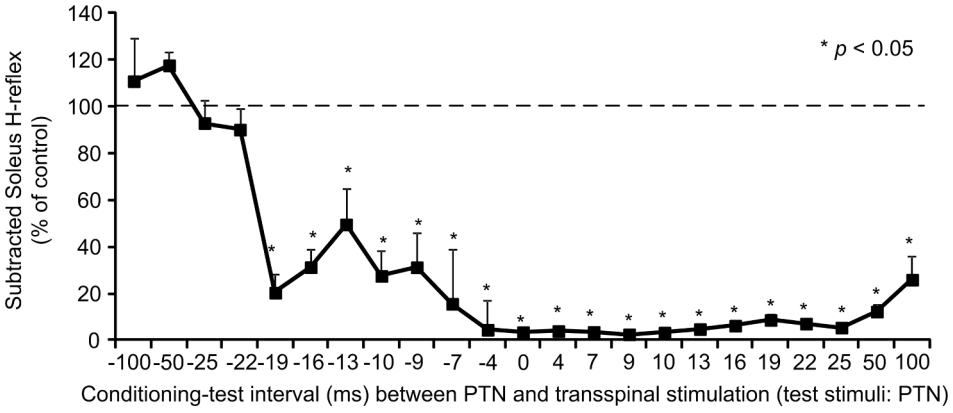

Fig. 3. Effects of noninvasive transspinal stimulation on soleus (SOL) H-reflex. Amplitude of SOL H-reflexes following transspinal stimulation over the thoracolumbar region from 23 subjects. On the abscissa the conditioning-test (C-T) interval (ms) tested is indicated. A negative C-T interval denotes that transspinal stimulation was delivered after posterior tibial nerve (PTN) stimulation. Asterisks indicate significant differences of conditioned SOL H-reflexes from control reflex values ($p < 0.05$; one-way ANOVA). Error bars denote the SEM.

modulation profile. Furthermore, transspinal stimulation produced a short-latency, long-lasting SOL H-reflex depression, and excitation of muscle spindle group Ia afferents depressed TEPs of the ipsilateral ankle flexors and extensors in healthy humans. Excitation of muscle spindle group Ia afferents produced facilitation of the ipsilateral knee flexor TEPs, while no effects were noted in the contralateral TEPs recorded from ankle or knee muscles.

4.1. Spinal reflex excitability amplitude modulation following transspinal stimulation

Transspinal stimulation produced a profound short-latency, long-lasting SOL H-reflex depression, consistent with our previous observations (Knikou, 2013a; Einhorn et al., 2013) that might have been exerted at either pre- or post-synaptic levels to soleus alpha motoneurons, simultaneously at both synaptic levels, or in the peripheral sensory-motor axon. Low threshold stimulation of a mixed peripheral nerve produces several intrathecally recorded segmental potentials in the human spinal cord (Ertekin, 1976a). The first potential with a conduction velocity of 37 m/s is fast, early and sharp and is related to activation of the ascending dorsal funiculus fibers. In contrast, the second component is a triphasic compound action potential of very high amplitude and longer duration and has been related to activation of spinal roots (Ertekin, 1976a, 1976b). Furthermore, the first potential has 10–12 ms latency and 5–8 ms duration, while the second potential has 9–23 ms duration (Ertekin, 1976a). Given these latency and duration ranges, and that monosynaptic Ia afferent transmission at the sacral spinal cord is approximately 0.4 ms (Ertekin et al., 1996), soleus alpha motoneurons had already been depolarized by muscle spindle group Ia afferents when transspinal stimulation was delivered 10–19 ms after PTN stimulation (i.e. negative C-T interval). Thus, the SOL H-reflex depression was likely exerted at peripheral sensory-motor axons for these C-T intervals (Fig. 3). This action site is supported by the repeatedly observed summation of SOL H-reflex and SOL TEP in the surface EMG (Fig. 1). Possible mechanisms include potentiation of group Ia afferent hyperpolarization, and changes in the concentration of ion channels at nerve terminals (Hunter and Ashby, 1994; Tsentsevitsky et al., 2017).

The long-lasting SOL H-reflex depression when transspinal was delivered before PTN stimulation (i.e. positive C-T interval) is likely related to spinal interneuronal circuits activated indirectly by transspinal stimulation. The Ia afferent mediated action potentials run dorsally and perpendicular to the surface of the spinal cord, representing activity of dorsal roots traveling rostrally and ventral roots traveling caudally (Yiannikas and Shahani, 1988). These action potentials are known to produce depolarization of primary afferent terminals responsible for presynaptic inhibition, and thus can initiate interneuronal activity (Wall, 1958). Non-reciprocal group I inhibition and recurrent inhibition, which are known to last up to 10 ms, likely did not contribute to SOL H-reflex depression because PTN stimulation was delivered at intensities that the H-reflex is monosynaptic (Knikou, 2008). Presynaptic inhibition of SOL Ia afferents could potentially account for the depression observed from 10 to 100 ms involving polysynaptic spinal reflex pathways (Ugawa et al., 1995; Knikou, 2008; Côté et al., 2018). However, we cannot exclude the possibility that at long C-T intervals, orthodromic transmission of impulses in dorsal column fibers may have modulated supraspinal activity of the somatosensory system. Dorsal column axons conduction velocity of 68 ± 5 m/s and their long lasting duration (30 ms) (Wall, 1958; Ugawa et al., 1995), allow sufficient time for supraspinal activity to affect spinal reflex circuits, as verified by the altered corticospinal excitability following transspinal stimulation (Knikou, 2014).

4.2. Amplitude modulation of TEPs following Ia afferent stimulation

Stimulation of muscle spindle group Ia afferents produced (1) a profound long-lasting depression of TEPs recorded from ipsilateral ankle flexor/extensor muscles, (2) facilitation of ipsilateral TEPs recorded from knee flexors (MH, LH), and (3) no significant effects in the contralateral TEPs recorded either from ankle or knee muscles (Fig. 5). TEPs had a similar amplitude modulation profile to that of the SOL H-reflex, with the exception that amplitude modulation was observed at later times and only at positive C-T intervals (compare Figs. 3 and 5).

These effects are likely related to the relative timing and interaction of action potentials generated by transspinal and PTN stimuli. Following collision experiments, involving paired shocks delivered at the wrist and Erb's point or the wrist and cervical column, recovery from blocking was evident at longer interstimulus intervals as it was possible to detect F-waves from the proximal stimulus (Mills and Murray, 1986). Because the latency of arm TEPs was identical to those evoked from a needle stimulus near the C8 root, it was concluded that electrical stimuli applied over the vertebrae in the midline excited the motor roots at their exit from the spinal canal (Mills and Murray, 1986). Similar results were found upon magnetic stimulation over the cervical and/or lumbar spinal enlargements, during which the evoked responses appeared at latencies corresponding to (F + M − 1)/2 and to stimulation of the roots near to their exit from the spinal column (Ugawa et al., 1989). Additional collision experiments and simulation studies verified that activation occurs at the root exit site in the vertebral foramina, while large diameter proprioceptive afferent fibers are also activated (Maertens de Noordhout et al., 1988; Danner et al., 2011). The excitation site being distal to the anterior horn cells was supported by

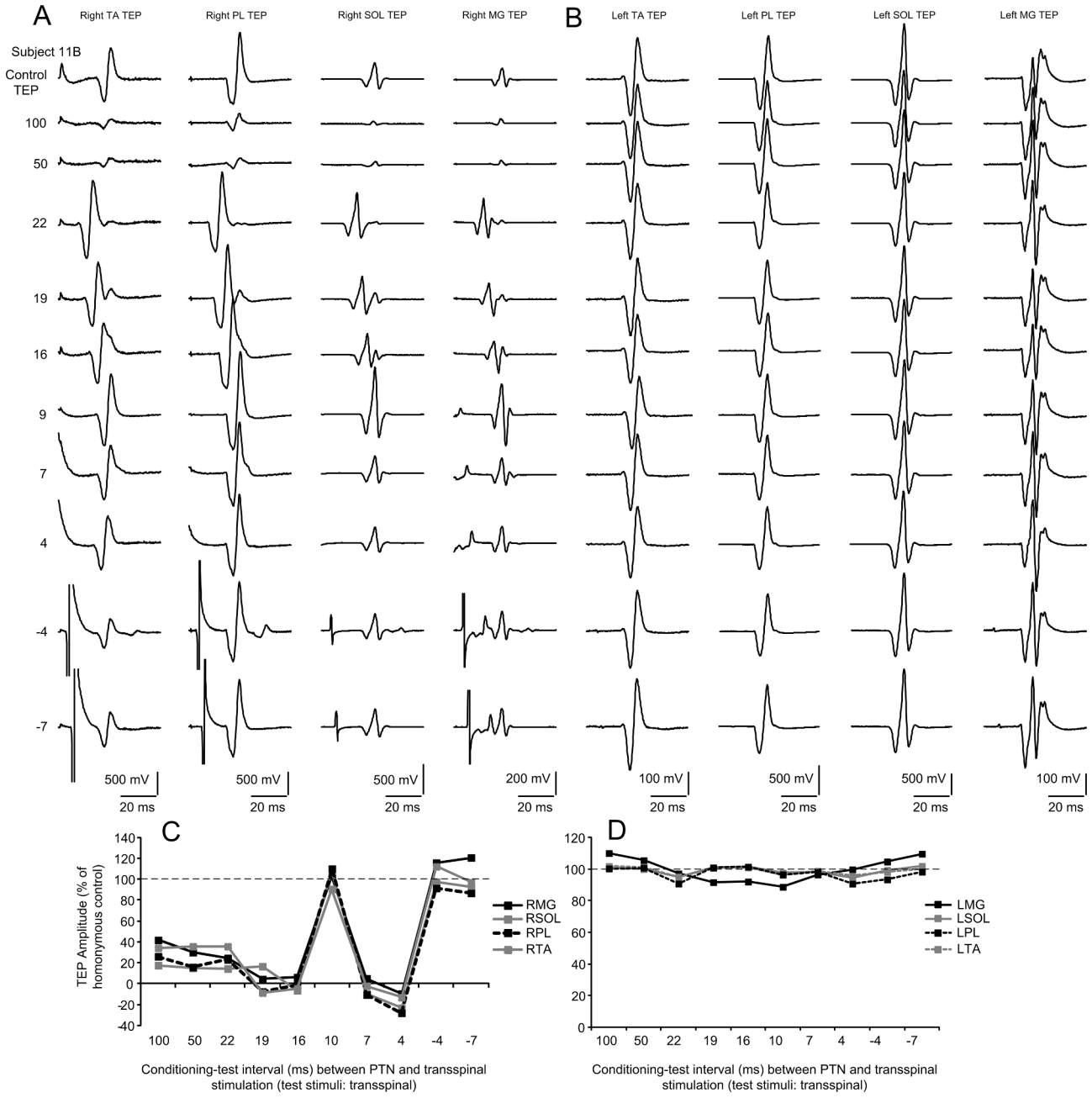

Fig. 4. Effects of stimulation of muscle spindle group Ia afferents on transspinal evoked potentials (TEPs). (A, B) Waveform averages of the right and left tibialis anterior (TA), peroneus longus (PL), soleus (SOL), and medialis gastrocnemius (MG) TEPs from one subject under control conditions and following stimulation of muscle spindle group Ia afferents for the conditioning-test (C-T) intervals that significant effects were observed. A negative C-T interval denotes that transspinal stimulation was delivered before posterior tibial nerve (PTN) stimulation. (C, D) Overall mean amplitude of the conditioned TEPs for the same subject. Asterisks indicate significant differences of conditioned TEPs from control values ($p < 0.05$; one-way ANOVA).

computed tomography scan measurements of the distance between the dura and intervertebral foramina (Epstein et al., 1991). Last, computer simulation of the electrical field produced by transspinal stimulation showed that impulses are generated in the dorsal column fibers, descending fibers of the lateral corticospinal tract, and that their impulses travel along the posterior and anterior root fibers exciting the fibers at the spinal cord entry or at their exit from the spinal canal (Coburn, 1985; Ladenbauer et al., 2010). However, modeling studies showed that muscle spindle group Ia afferents in dorsal root fibers have significantly lower excitation thresholds compared with ventral root fibers and dorsal column fibers, with the latter requiring triple the stimulation intensity (Rattay et al., 2000; Danner et al., 2011). Based on the stimulation we used, it is unlikely that transspinal stimulation in this study excited dorsal column fibers (Danner et al., 2014).

It is apparent that transspinal stimulation excites several neural elements of the spinal cord and thus direct methods are needed to delineate the exact site of activation taking into account the spinal cord anisotropy (Coburn and Sin, 1985). However, based on the available discussed evidence, transspinal stimulation evokes transynaptic activation of spinal neurons and descending fibers of the corticospinal tract based on summation of TEPs and MEPs in surface EMG and increased corticospinal excitability following transspinal conditioning stimulation

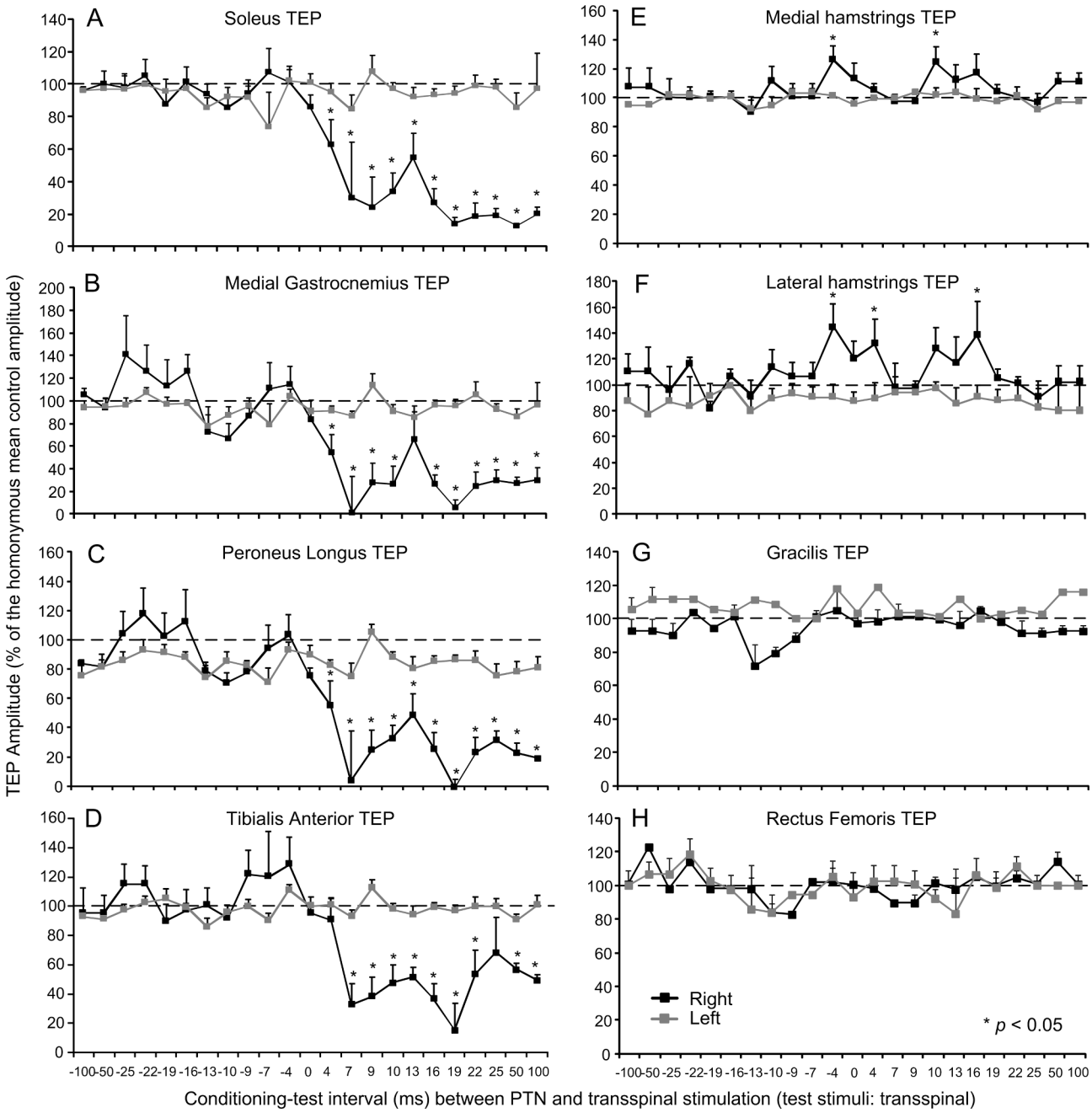

Fig. 5. Modulation of transspinal evoked potentials (TEPs) by excitation of muscle spindle group Ia afferents. Overall mean amplitude of TEPs recorded from the right (black lines) and left (gray lines) soleus (A), medial gastrocnemius (B), peroneus longus (C), tibialis anterior (D), medial hamstrings (E), lateral hamstrings (F), gracilis (G), and rectus femoris (H) muscles following stimulation of soleus group Ia afferents. On the abscissa the conditioning-test (C-T) interval (ms) is indicated. A negative C-T interval denotes that transspinal stimulation was delivered before posterior tibial nerve (PTN) stimulation. Asterisks indicate significant differences of conditioned TEPs from control values ($p < 0.05$; one-way ANOVA). Error bars denote the SEM.

(Mills and Murray, 1986; Ugawa et al., 1989; Chokroverty et al., 1991; Gaunt et al., 2006; Capogrosso et al., 2013; Knikou, 2014; Knikou et al., 2015).

For the TEPs recorded from the ipsilateral ankle muscles, depression started when stimulation of muscle spindle group Ia afferents was delivered 4 or 7 ms before transspinal stimulation (Fig. 5), and not at negative C-T intervals as was the case for the SOL H-reflex. Based on the conduction velocity of group Ia afferents and the time needed for afferent volleys to reach the spinal cord, at the short C-T intervals of 4 or 7 ms interaction likely occurred at the peripheral nerve axons because Ia afferent volleys had not reached the spinal cord. Thus, TEP depression may involve similar neural mechanisms to the soleus H-reflex depression at similar C-T intervals, and be the result of occlusion between the two propagated potentials at the peripheral mixed nerve fibers, hyperpolarization of muscle afferents or changes in the concentration of ion channels at nerve terminals (Tsentsevitsky et al., 2017).

In contrast, at the C-T interval of 10 ms and beyond, Ia afferent volleys had ample time to reach the spinal cord and affect the amplitude of TEPs via monosynaptic activation of alpha motoneurons. It is also important to note that neural interaction between Ia afferent volleys and TEPs was restricted to the ipsilateral ankle muscles, while in

the knee flexor muscles inhibition was replaced by facilitation likely mediated by excitatory spinal interneurons that warrants further investigation. Lack of effects in the contralateral TEPs suggests that TEPs are not prone to inputs from contralateral group Ia afferents, as is the case for the SOL H-reflex during which stimulation of contralateral SOL Ia afferents results in a long-latency depression of the ipsilateral SOL H-reflex (Stubbs and Mrachacz-Kersting, 2009), resembling largely actions of commissural interneurons.

5. Conclusion

This study showed that noninvasive transspinal stimulation of the thoracolumbar region in healthy humans while lying supine induces a short-latency, long-lasting SOL H-reflex depression. Similarly, muscle spindle group Ia afferents conditioning stimulation produces a pronounced depression in TEPs recorded from the ipsilateral ankle flexor/extensor muscles. The inhibitory effects were evident at times consistent with neural interactions occurring at different levels of the CNS. Based on our current and published findings (Knikou, 2013a, 2013b, 2014, 2017; Dixon et al., 2016), transspinal stimulation can be utilized in upper motor neuron lesions to normalize spinal reflex hyper-excitability and alter excitation thresholds of peripheral nerve axons.

Author contributions

MK developed the experimental protocol, performed experiments, analyzed data, and wrote the manuscript. LMM performed experiments and proofread the manuscript. MK and LMM approved the final version of the manuscript before submission.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Financial disclosure statement

Nothing to report.

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