Long-latency, inhibitory spinal pathway to ankle flexors activated by homonymous group 1 afferents

Ephrem T. Zewdie,1,6 Francois D. Roy,3,4,6 Yoshino Okuma,4 Jaynie F. Yang,2,4,5 and Monica A. Gorassini1,4,6

1Department of Biomedical Engineering, University of Alberta, Edmonton, Canada; 2Department of Physical Therapy, University of Alberta, Edmonton, Canada; 3Department of Surgery, University of Alberta, Edmonton, Canada; 4Centre for Neuroscience, University of Alberta, Edmonton, Canada; 5Faculty of Rehabilitation Medicine, University of Alberta, Edmonton, Canada; and 6Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada

Submitted 18 September 2013; accepted in final form 25 March 2014

Zewdie ET, Roy FD, Okuma Y, Yang JF, Gorassini MA. Long-latency, inhibitory spinal pathway to ankle flexors activated by homonymous group 1 afferents. J Neurophysiol 111: 2544–2553, 2014. First published March 26, 2014; doi:10.1152/jn.00673.2013.—Inhibitory feedback from sensory pathways is important for controlling movement. Here, we characterize, for the first time, a long-latency, inhibitory spinal pathway to ankle flexors that is activated by low-threshold homonymous afferents. To examine this inhibitory pathway in uninjured, healthy participants, we suppressed motor-evoked potentials (MEPs), produced in the tibialis anterior (TA), by a prior stimulation to the homonymous common peroneal nerve (CPN). The TA MEP was suppressed by a triple-pulse stimulation to the CPN, applied 40, 50, and 60 ms earlier and at intensities of 0.5–0.7 times motor threshold (average suppression of test MEP was 33%). Whereas the triple-pulse stimulation was below M-wave and H-reflex threshold, it produced a long-latency inhibition of background muscle activity, approximately 65–115 ms after the CPN stimulation, a time period that overlapped with the test MEP. However, not all of the MEP suppression could be accounted for by this decrease in background muscle activity. Evoked responses from direct activation of the corticospinal tract, at the level of the brain stem or thoracic spinal cord, were also suppressed by low-threshold CPN stimulation. Our findings suggest that low-threshold muscle and cutaneous afferents from the CPN activate a long-latency, homonymous spinal inhibitory pathway to TA motoneurons. We propose that inhibitory feedback from spinal networks, activated by low-threshold homonymous afferents, helps regulate the activation of flexor motoneurons by the corticospinal tract.

spinal inhibition; tibialis anterior; common peroneal nerve

Inhibitory networks in the spinal cord are important for controlling the force, timing, and ultimately, precision of movement [reviewed in Pierrot-Deseilligny and Burke (2005)]. Feedforward control of spinal inhibitory interneurons comes from descending motor commands, whereas feedback control comes from the sensory afferents activated during movement. The interaction between descending and peripheral inputs onto spinal inhibitory networks can be studied by examining the suppression of spinal reflexes by transcortical magnetic stimulation (TMS) to the primary motor cortex or alternatively, the suppression of cortically evoked responses by afferent nerve stimulation. For example, the short-latency, short-lasting suppression of the soleus H-reflex by TMS is thought to be mediated by premotoneuronal (i.e., directly synapsing onto motoneurons), Ia-reciprocal, inhibitory interneurons that are activated by the corticospinal tract innervating the antagonist, tibialis anterior (TA) (Kudina et al. 1993; Nielsen et al. 1993). Inhibitory interneurons that are located more upstream from the motoneuron have also been examined. For example, group I afferents from the common peroneal nerve (CPN) supplying the TA muscle converge, along with corticospinal inputs, onto inhibitory interneurons that synapse onto lumbar propriospinal neurons—the latter with projections onto the quadriceps motoneuron pool (Marchand-Pauvert et al. 1999). This was evidenced by the strong suppression of quadriceps motor-evoked potentials (MEPs) by a prior low-intensity stimulation of the CPN at interstimulus intervals (ISIs) of 4–11 ms, latencies consistent with the activation of propriospinal pathways. Suppression of MEPS by heteronymous afferents can also occur at much longer intervals between TMS and peripheral nerve stimulation. For example, suppression of the TA MEP by stimulation of the tibial nerve at the ankle (Roy and Gorassini 2008) or suppression of the soleus MEP by CPN stimulation (Geertsen et al. 2011) occurs at ISIs near 35–45 ms. This long-latency suppression is thought to be mediated by spinal inhibitory interneurons synapsing onto lumbar propriospinal neurons that receive convergent inputs from heteronymous group I sensory and feedforward corticospinal inputs (Geertsen et al. 2011).

The long-latency inhibition of motoneurons from these upstream, inhibitory interneurons has only been shown for heteronymous pathways (Geertsen et al. 2011; Iglesias et al. 2008; Roy and Gorassini 2008). Here, we refer to “homonymous” as afferents originating from the tested muscle and “heteronymous” as afferents originating from surrounding or antagonist muscles [cf. Pierrot-Deseilligny and Burke (2005)]. In this paper, we show, for the first time, properties of a long-latency, spinal inhibitory pathway to the TA motoneuron pool that is activated by low-threshold muscle and cutaneous afferents from the homonymous CPN, which may be involved in regulating or damping the activation of ankle flexor muscles by the corticospinal tract. The CPN was activated using a train of three pulses, delivered at various intervals before the TMS pulse and at very low-stimulation intensities (≤0.7 × motor threshold × MT). This triple pulse of stimulation was below the threshold of recruiting either the M-wave or H-reflex, so we were able to examine inhibition of the TA MEP without effects...
from motoneuron refractoriness or recurrent inhibition. The purpose of these experiments was to: 1) characterize the effect of stimulating the CPN afferents over a range of intensities on the TA MEP and voluntarily activated electromyogram (EMG), 2) determine the site of origin (e.g., cortical, subcorti-
cal, and/or spinal) of the TA MEP suppression, and 3) de-
termine which afferents contribute to the TA MEP suppression (muscle and/or cutaneous).

METHODS

All experiments were carried out with the approval of the Human Research Ethics Board at the University of Alberta and with informed consent of the participants. Our sample was comprised of 14 neuro-
logically intact control participants (nine men), ranging in age from 19 to 58 yr (30.5 ± 10.4, mean ± SD).

EMG Recordings and Peripheral Nerve/Skin Stimulation

A pair of Ag-AgCl electrodes (3.8 cm × 2.2 cm; Kendall; Covi-
dien, Mansfield, MA) was used to record surface EMG from the TA and soleus muscles with a ground electrode placed on the patella. The EMG signals were amplified by 1,000, band-pass filtered from 10 to
1,000 Hz (Octopus; Bortec Biomedical, Calgary, Alberta, Canada), and then digitized at a rate of 5,000 Hz using AxoScope 10 hardware and software (Digidata 1400 Series; Axon Instruments, Union City, CA). The full wave-rectified and smoothed EMG (100 ms time constant; NeuroLog NL703 EMG integrator; Digitimer, Hertford-
shire, UK) from the TA muscle was displayed on an oscilloscope screen. To measure the EMG from a maximum voluntary contraction (MVC), participants were seated in a chair with their ankle and knee joints positioned at 90°. The foot was strapped to a metal plate for isometric dorsiflexion, and the knee was held stable by strapping. Participants were given verbal encouragement and a visual display of their rectified and smoothed TA EMG to help them contract as hard as possible. Contraction trials were repeated until two consistent peak
EMG levels, varying <10%, were held for at least 1 s. Unless otherwise stated, all responses were collected during a tonic dorsi-
flexion of 10% MVC.

Peripheral nerves and skin were stimulated electrically using a constant current stimulator (DS7A; Digitimer) in a bipolar arrange-
ment with Ag-AgCl electrodes (2.2 cm × 2.2 cm; Kendall).

Experiment 1: Conditioning of TA MEPs by Incrementing Intensities of CPN Stimulation

The effect of conditioning the TA MEP by a prior stimulation of the homonymous CPN was examined in 12 participants. MEP re-
ponses were evoked in the right TA muscle, which was the dominant leg in all participants. TMS, inducing posterior-anterior currents in the motor cortex, was delivered to the contralateral motor cortex using a custom-made, figure-of-eight stimulation coil (part number 15857; 90-mm diameter) that was connected to a Magstim 200 stimulator (Magstim, Carmarthen, UK). First, the hotspot for the TA muscle was determined, while evoking MEPs that were ~300 μV in size, as the participant maintained a background contraction of 10% MVC. Typically, the hotspot for the TA muscle was 1–2 cm lateral and 0–2 cm posterior or anterior to vertex. A TA MEP amplitude of ~500 μV was used as the unconditioned test response, which is near one-half of the maximum TA MEP in uninjured, healthy individuals (Roy and Gorassini 2008). The TA MEP was then conditioned by stimulating the CPN at ISIs of 40, 50, and 60 ms (triple-pulse stimulation, 1-ms pulse width) before the TMS. The CPN was stimulated in increasing order at intensities of 0.1, 0.2, 0.3, 0.5, 0.7, 0.9, and 1.1 × MT for a total of seven intensities of stimulation. MT was determined with single-pulse stimulation and determined as the current intensity, producing a discernable and reproducible, direct motor response (M-
wave) at test. Bipolar stimulation was used to stimulate the CPN, with the cathode placed below the neck of the fibula after the optimal site to produce small, isolated contractions of the TA muscle, with minimal to no peroneal muscle activation, was identified. The anode was placed 2 cm proximal to the cathode near the outer edge of the popliteal fossa. At each of the seven intensities of CPN stimulation, two unconditioned TA MEPs (14 in total) and six conditioned TA MEPs (42 in total) were evoked in random order. In nine of the 12 participants, single-pulse CPN stimulation was also tested at an ISI of 40 ms at the same intensities used for the triple-pulse stimulation. All conditioned MEPs were measured peak to peak and expressed as a percentage of the unconditioned test MEP. The amount of MEP suppression at a given intensity was averaged across all participants.

We measured the onset latency when the conditioned MEP became smaller than the unconditioned test MEP. This was done to determine if the inhibition occurred directly onto the TA motoneurons (i.e., conditioned MEP was suppressed immediately at its onset) or if the inhibition occurred a few nanoseconds upstream from the TA motoneu-
rons [i.e., suppression of MEP was delayed by a few milliseconds, per Chais et al. (1997)]. To reveal the differences better in the conditioned and unconditioned MEP from the background EMG, the cumulative sum (CUSUM) of the rectified MEP was first calculated (Brinkworth and Turker 2003) using the following equation

\[
\text{CUSUM} (t) = \sum_{i=1}^{t} (\text{EMG}(t) - \text{mean(bkdeEMG)})
\]

where EMG (t) is the EMG at the time (t) in milliseconds, and mean(bkdeEMG) is the average of the rectified EMG measured, 100–200 ms before TMS or CPN stimulation. The TMS pulse was delivered at t = 0. Because the data were sampled at 5,000 Hz, t was increased in 0.2 ms increments. A CUSUM was calculated for the test MEP (average of 14 trials) and the average of the conditioned MEP at 0.5 and 0.7 × MT (average of 12 trials), by applying the above equation to the EMG at 10–70 ms after the TMS to avoid the TMS stimulation artifact within the first 10 ms. The 0.5 and 0.7 × MT trials were both used, because they had the most consistent MEP suppres-
tion. To compare the latency of MEP suppression when antidromic activation of the motoneuron and recurrent inhibition were strongly activated (Meunier et al. 1994), in five subjects, we also stimulated the CPN at 1.5 × MT (average of six trials). Here, we predicted that the latency of MEP suppression would be earlier, given that the motoneu-
ron would be inhibited by shorter pathways. The difference between the CUSUMs of the test and conditioned MEPs (Δ CUSUM) was also calculated. The onset of the MEP was best determined visually from the unrectified EMG. The latency when the conditioned MEP began to deviate from the test MEP was measured at the start of the steep slope in the Δ CUSUM line (see Fig. 2) and also compared with when the unrectified MEP and CUSUM traces began to deviate from one another.

Experiment 2: Time Course of TA EMG and MEP Suppression by CPN Stimulation

The effect of a triple-pulse CPN stimulation on voluntary EMG activity, generated in the TA muscle, was examined in eight partici-
pants, all of whom participated in experiment 1. The CPN was stimulated with three pulses (10 ms ISI) every 3 s for 40 trials, while the participant maintained a steady dorsiflexion of 10% MVC adjusted so that there was a clear suppression of the voluntary TA EMG that was not preceded by a prior excitatory H-reflex or M-wave. On average, the stimulation intensities used were between 0.2 and 0.7 × MT (0.44 ± 0.16 × MT). To determine the duration of the EMG suppression, the onset and offset of the suppres-
sion were measured as the time points where the rectified and smoothed EMG (second-order Butterworth filter at 200 Hz low pass) fell below (for onset) and then crossed above (for offset) a level that was three times the SD of the EMG, measured 200–10 ms before the
CPN stimulation. To measure the average background EMG suppression across the eight participants, the rectified and smoothed EMG data were binned every 5 ms, and the average value for each bin was then averaged across the eight participants. For each participant, either the 0.5 × MT or 0.7 × MT trial was used, whichever intensity resulted in the largest EMG suppression.

The time course of the suppression of the TA MEP was also examined in more detail by systematically varying the ISI of the triple-pulse CPN stimulation in these same participants. Triple pulses with ISIs, starting at 10, 20, and 30 ms, were increased gradually to triple pulses with ISIs of 45, 55, and 65 ms in steps of 5 ms (e.g., 10/20/30 ms, 15/25/35 ms... to 45/55/65 ms), for a total of eight different ISIs. Again, stimulation intensities were used that did not produce an early, short latency-excitatory reflex or M-wave, with an average stimulation intensity of 0.54 ± 0.1 × MT. At each ISI, two unconditioned MEPs (16 in total) and eight conditioned MEPs (64 in total) were evoked in random order.

Experiment 3: Conditioning of TA MEPs Evoked by Cervicomedullary and Spinal Cord Stimulation

To examine if the site of MEP suppression by the prior CPN stimulation occurred at a spinal site, we tested if MEP suppression still occurred when the axons of the corticospinal tract were activated directly at the level of the pyramidal decussation or at the level of the thoracic spinal cord. Cervicomedullary MEPs (CMEPs) were elicited in three participants (two of whom also participated in experiments 1 and 2), with a double-cone coil placed behind the inion in two participants [per Taylor and Gandevia (2004)] and with electrical transmastoid stimulation in the third participant, because TMS at the inion did not produce CMEPs in this participant. Because of the shorter conduction time from the site of brain stem stimulation to the lumbar spinal cord (∼5 ms), the CMEP was conditioned with a prior triple-pulse CPN stimulation at ISIs of 45, 55, and 65 ms, again using intensities that were below excitatory reflex threshold in these participants. Ten unconditioned and 10 conditioned CMEPs were evoked every 8 s. To activate the axons of the corticospinal tract at the level of the thoracic spinal cord in two of these three participants on a separate experiment day, a cathode comprising a gel electrode (3.8 cm × 2.2 cm; Kendall) was placed between the T3 and T4 vertebrae, and an anode was placed 10 cm above the cathode at approximately C7 (per Martin et al. (2008)). The spinal cord was then electrically stimulated using a high-voltage, multipulse current stimulator (D185-Mark IIa; Digitimer) with currents of 1.3 A and 1.6 A in the two participants. The spinal MEP (SMEP) was then conditioned by a prior triple-pulse CPN stimulation at ISIs of 50, 60 and 70 ms, at intensities below excitatory reflex threshold (∼0.9 × MT). Again, longer ISIs were used to account for the shorter conduction time from spinal stimulation to the lumbar spinal cord (∼10 ms). Five unconditioned and five conditioned SMEPs were evoked every 8 s. In the same respective experiment, suppression of the CMEP or SMEP was compared with suppression of the MEP evoked by TMS over the motor cortex. The latencies of the unconditioned test MEP, CMEP, and SMEP were determined visually from the average of 10 unrectified traces.

Experiment 4: Conditioning TA MEPs by Skin Stimulation

Suppression of the TA MEP could occur at very low intensities of CPN stimulation, sometimes as low as 0.2–0.3 × MT. To determine if activation of afferents innervating the skin just underneath the stimulating electrode contributed to the MEP suppression or if sensory axons activated within the CPN were responsible, the anode was moved 4 cm away from its position over the CPN, closer to the midline of the shin but within the sensory dermatome of the CPN (see “OFF Nerve” location in Fig. 5). Six of the 12 participants from experiment 1 participated in these experiments on a different day.

Similar to experiment 1, stimulation intensities of 0.1, 0.2, 0.3, 0.5, 0.7, 0.9, and 1.1 × MT were used with triple pulses at ISIs of 40, 50, and 60 ms with two unconditioned (14 in total) and six conditioned (42 in total) MEPs per stimulation intensity. In the same experiment, suppression of the TA MEP was also confirmed by placing the cathode over the CPN (“ON Nerve” location).

The skin between the first and second toes, which is innervated by the deep peroneal nerve (DPN), the cutaneous branch of the CPN, was also stimulated to determine further if cutaneous afferents contributed to the MEP suppression. In six of the 12 participants from experiment 1, the cathode was placed on the lateral side of the first toe, and the anode was placed at the base of the first and second toe (see Fig. 5 for location of stimulating electrodes, 2.2 cm × 2.2 cm). The skin was stimulated with a triple-pulse (0.2-ms-wide) train at ISIs of 45, 55, and 65 ms to compensate for the longer time that it takes the afferent signal to reach the spinal cord when traveling from the foot compared with the knee. In this way, the afferent signal reached the spinal cord at the same time, relative to the arrival of the descending activation of the spinal cord from TMS, as in the CPN experiment. Stimulation intensities at 0.1, 0.3, 0.5, 1.0, 1.5, 2.0, and 2.5 × sensory perception threshold (PT) were used to determine the maximum (i.e., peak) suppression of the TA MEP, i.e., the maximum suppression at any of the stimulation intensities applied. On another experiment day, suppression of the H-reflex at a DPN stimulation intensity that produced peak TA MEP suppression was compared in four participants (three from a previous experiment). To evoke an H-reflex, the CPN was stimulated (1-ms pulse width; 45/55/65 ms ISI) at an intensity that produced one-half of the maximum H-reflex.

Statistical Analysis

To compare the effect of CPN stimulation on the TA MEP at the various stimulation intensities (experiment 1) or ISIs (experiment 2), a one-way ANOVA for repeated measures was used with post hoc Student’s t-tests (Bonferroni corrected) to determine the stimulation intensities or ISIs where the conditioned MEP was smaller than the unconditioned test MEP. All statistical analyses were performed using SPSS 20 software. Single- and triple-pulse CPN stimulation (experiment 1) and ON and OFF nerve stimulation (experiment 4) were compared using two-way, repeated-measures ANOVA, treating the stimulation type (single or triple pulse; ON and OFF nerve) and stimulation intensity (0.1, 0.2 × MT, etc.) as within-subject factors. A paired Student’s t-test was used to compare the effect of CPN stimulation on the MEP and SMEP (experiment 3) and the effect of DPN and CPN stimulation on the unconditioned test TA MEP and H-reflex (experiment 4), because the data were normally distributed. Data are presented in figures as mean ± SE and in the text as mean ± SD, unless stated otherwise. Significance was set at P < 0.05.

RESULTS

Experiment 1: Conditioning of TA MEP by Incrementing Intensities of CPN Stimulation

The conditioning of the test TA MEP by a prior triple-pulse stimulation to the homonymous CPN at ISIs of 40/50/60 ms decreased the amplitude of the MEP starting at a stimulation intensity of 0.2 × MT and peaked at an intensity of 0.7 × MT, as shown for a single participant in Fig. 1A. Here, an M-wave or H-reflex from triple-pulse CPN stimulation was not seen until 1.1 × MT. In the 12 participants examined, there was a significant main effect on the conditioned MEP, expressed as a percentage of the test MEP, at the various CPN stimulation intensities used [ANOVA: F(7,11) = 9.56, P < 0.001; Fig. 1B]. Post hoc analysis revealed that the conditioned MEP was...
A visible M-wave or H-reflex appeared only at stimulation intensities between 0.9 and 1.1 × MT. As an effect of this, the mean amplitude of the rectified EMG, measured during the time period where an expected M-wave or H-reflex should be, remained within ±5% of the prestimulus mean EMG for stimulation intensities, ≤0.7 × MT, but not at the 0.9 or 1.1 × MT intensities. Thus we only considered MEP suppression at stimulation intensities of 0.7 × MT or less to rule out effects from recurrent inhibition or motoneuron refractoriness. When averaged across the 0.5 and 0.7 × MT stimulation intensities (Fig. 1B), the TA MEP was reduced by 32.7 ± 14.7%. In contrast to the triple-pulse stimulation, single-pulse stimulation of the CPN, at an ISI of 40 ms (Fig. 1C), produced little to no effect on the TA MEP at low intensities of stimulation (≤0.7 × MT), as tested in nine of the 12 participants on a different experiment day. There was a significant interaction with the number of pulses used (single vs. triple) and stimulation intensity [two-way, repeated-measures ANOVA: F(7, 8) = 2.665, P = 0.036], with post hoc analysis revealing larger TA MEP suppression at 0.5 and 0.7 × MT with the triple-pulse stimulation. Similar results were found when using single-pulse stimulation at ISIs of 50 and 60 ms (data not shown).

The onset latency, when the conditioned MEP became smaller than the test MEP, was measured, as demonstrated for a single participant in Fig. 2. To visualize the differences better between the unconditioned and conditioned MEP, the CUSUM of the rectified MEPs were calculated (Fig. 2) and subtracted from one another to give a Δ CUSUM (Fig. 2). The onset of the MEP was first determined visually from the unrectified EMG, and the latency, when the test and conditioned MEPs began to deviate from one another, was determined as the start of the steep slope in the Δ CUSUM line. The latency between the onset of the TA MEP and the start of the steep slope in the Δ CUSUM was 3.7 ms for this participant when the conditioning CPN stimulation was below MT (average of 0.5 and 0.7 × MT; Fig. 2A). When the stimulation intensity in this same participant was increased to suprathreshold levels (1.5 × MT), to evoke direct inhibition of the TA motoneurons by recurrent inhibition and from motoneuron refractoriness, the conditioned MEP was suppressed at its onset with a 0-ms delay (Fig. 2B). In the 12 participants from experiment 1, the average latency from the start of the MEP to when the conditioned MEP began to deviate from the test MEP was 4.2 ± 3.1 ms for the 0.5 and 0.7 × MT stimulation intensities. This is in contrast to the conditioned MEP at 1.5 × MT (suprathreshold), where a large M-wave and H-reflex were present and where the conditioned MEP was suppressed earlier at 0.2 ± 0.3 ms after the onset of the MEP (Mann-Whitney rank sum test, P = 0.008, n = 5).

Experiment 2: Time Course of TA EMG and MEP Suppression by CPN Stimulation

Triple pulses of low-intensity stimulation to the CPN also suppressed voluntary EMG activity in the TA muscle, as shown for three participants in Fig. 3A. Suppression of TA EMG activity below the mean background (Fig. 3A; average of 40 trials) occurred without a prior excitatory reflex response at stimulation intensities ≤0.7 × MT. The average onset of the EMG suppression occurred at 65.9 ± 7.1 ms after the first pulse of CPN stimulation and ended at 112.9 ± 11.6 ms (see experiment 2 in methods for defining the period of EMG suppression).
suppression). In Fig. 3B, the duration of the TA EMG suppression is displayed for each of the eight participants tested with time 0 ms, starting at the first pulse of CPN stimulation. Also shown on the same time axis is a single trial where a TA MEP was conditioned by a prior triple-pulse CPN stimulation at the standard ISIs of 40/50/60 ms. Note that at these ISIs, the TA MEP occurred during the same time period as when the voluntary EMG was suppressed. That is, the average onset and offset time of the TA MEP following the first pulse of CPN stimulation was 88 ± 110 ms, and occurred within the average window of time that the voluntary TA EMG was suppressed (between 65 and 115 ms), the latter shown by solid circles in Fig. 3B. Note that the average amount of EMG suppression over this entire window was 15.4 ± 11.7% of the prestimulation EMG at the 0.5 and 0.7 × MT intensities.

In these same participants (plus one), we also examined in more detail the time course of TA MEP suppression by using different ISIs of triple-pulse CPN stimulation, starting at ISIs of 10/20/30 ms before TMS and increasing to ISIs of 45/55/65 ms in steps of 5 ms (see Fig. 3C for ISIs used). Overall, there was a significant main effect of ISIs on the TA MEP [ANOVA: F(7,8) = 3.623, P = 0.003]. Similar to experiment 1, the TA MEP was suppressed significantly (by 23.6 ± 16.1%, P = 0.003) when using a triple-pulse conditioning stimulation at the 40/50/60-ms ISI. In addition, the TA MEP was suppressed significantly (by 28.0 ± 23.8%, P = 0.009) at an earlier triple-pulse ISI of 25/35/45 ms.

To examine if the suppression of background EMG activity by low-intensity CPN stimulation was the main contributor to the suppression of the TA MEP, we examined if the average profile of the EMG suppression for all participants matched the averaged profile of MEP suppression across the various ISIs measured in Fig. 3C. Unlike the MEP suppression profile, which increased, decreased, and then increased again across the different ISIs (Fig. 3C), the average background EMG suppression displayed a single U-shaped profile (Fig. 3D), peaking at 85 ms after the first pulse of CPN stimulation. The location of the 16-ms-duration MEP for three example ISIs is also indicated in Fig. 3D and demonstrates that although the
conditioned MEP at the 35/45/55-ms ISI occurred near the peak of background EMG suppression, it was not suppressed compared with the test MEP (as marked by the arrow in Fig. 3C). The same holds true for the 30/40/50-ms ISI, where the conditioned MEP was not suppressed but occurred during similar levels of background EMG suppression as the MEPS evoked at the 25/35/45- and 40/50/60-ms ISIs, which were suppressed (Fig. 3C). Thus there was not a 1:1 relationship between the amount of MEP and background EMG suppression produced by the low-intensity CPN stimulation. Likewise, MEPS evoked at rest, i.e., with no background EMG, could still be suppressed by CPN stimulation and were more prominent at the lower stimulation intensities. When tested in four of the participants from this experiment, the average MEP suppression at rest was 27.5 ± 12.6% (P = 0.02) across the 0.3 and 0.7 × MT stimulation intensities (test MEP size: 364.7 ± 109.5 μV).

**Experiment 3: Conditioning of TA MEPs Evoked by Cervicomedullary or Spinal Cord Stimulation**

To determine if the suppression of the TA MEP by a prior CPN stimulation was mediated at subcortical sites, we compared the conditioning effect of CPN stimulation on evoked potentials produced by stimulating the corticospinal tract directly, either at the cervicomedullary junction (CMEP) or at the level of the thoracic spinal cord (SMEP). As shown for a single participant in Fig. 4A, a prior triple-pulse CPN stimulation (see figure legend for ISIs used) reduced the peak-to-peak amplitude of the cortical MEP, brain stem CMEP, and spinal SMEP.

The amount of MEP and CMEP suppression was similar in the three participants tested (Fig. 4B; P = 0.77). In the two participants tested with spinal cord stimulation (who were also tested with the brain stem stimulation on a different day), the amount of SMEP suppression was also similar to the amount of MEP suppression (Fig. 4B). This indicates that the MEP suppression from TMS was likely mediated at subcortical and/or spinal sites. Similar to the MEP, the conditioned CMEP and SMEP began to deviate from the test (unconditioned) responses at 3.0 ± 0.6 and 3.3 ± 0.8 ms, respectively, as demonstrated for the single participant in Fig. 4A.

The evoked responses recorded in the TA muscle were similar when using TMS over the motor cortex and when using electrical stimulation over the thoracic spinal cord (compare MEP with SMEP in Fig. 4C), suggesting that the corticospinal tract was activated in a similar manner, either at rest or during a background contraction. On average, the onset latency of the SMEP was 21.2 ± 0.1 ms, the CMEP 24.2 ± 1.2 ms, and the MEP 30.1 ± 1.2 ms.

**Experiment 4: Conditioning TA MEPs by Skin Stimulation**

Because suppression of TA MEPs by CPN stimulation could occur at very low intensities (<0.7 × MT), we examined if activating cutaneous receptors, directly underneath the cathode, contributed to the MEP suppression or if activation of low-threshold afferents within the CPN was necessary. When the cathode was moved medially away from the CPN (OFF Nerve; Fig. 5A), the TA MEP was not suppressed at low-stimulation intensities compared with when the cathode was
located over the CPN (ON Nerve). In the six participants
tested, the conditioning of the TA MEP with OFF Nerve
stimulation did not suppress the TA MEP at low-stimulation
intensities (Fig. 5A). There was a significant interaction be-
tween “stimulation site” (ON and OFF Nerve) and “stimulation
intensity” \[ F(5, 6) = 3.356, P = 0.03 \], with larger MEP
suppression occurring with ON Nerve stimulation at the 0.5
and 0.7 \( \times \) MT intensities, indicating that low-threshold affer-
ents activated within the CPN contributed to the TA MEP
suppression.

Based on the results above, low-threshold muscle or cuta-
naneous afferents within the CPN likely contributed to the TA
MEP suppression. To determine if low-threshold cutaneous
afferents played a role, we selectively activated the cutaneous
branch of the CPN, the DPN, which innervates the skin
between the first and second toe of the foot (Fig. 5B). In the six
participants tested (on a separate experiment day from the ON
and OFF nerve experiment), the PT, when stimulating the skin
of the toe with triple-pulse stimulation, was 0.68 \( \pm \) 0.1 mA.
The peak suppression of the TA MEP, when testing a range of
stimulation intensities above and below PT, was 27.0 \( \pm \) 5.9%
of the test MEP across all six participants (Fig. 5B; \( P < 0.001 \))
and occurred at an average stimulation intensity of 0.72 \( \pm \) 0.5 \( \times \)
PT. In comparison, the peak suppression of the TA MEP from
triple-pulse CPN stimulation (across all intensities tested),
recorded on the same day, was 40.66 \( \pm \) 14.6% of the test MEP
(Fig. 5B; \( P < 0.01 \)) and occurred at an average stimulation
intensity of 0.50 \( \pm \) 0.25 \( \times \) MT.

In contrast to the TA MEP, there was no suppression of the
TA H-reflex by DPN stimulation. In four participants tested,
DPN stimulation (0.8 \( \pm \) 0.5 \( \times \) PT) produced a peak MEP
suppression of 28.2 \( \pm \) 7.4% (Fig. 5B; \( P < 0.01 \)), whereas the
H-reflex was facilitated, but not significantly, by 10.1 \( \pm \) 24.5%
(\( P = 0.47 \)).

**DISCUSSION**

**Summary of Findings**

In uninjured, healthy participants, TA MEPs, produced from
TMS to the motor cortex, were consistently suppressed by a
prior subthreshold triple-pulse but not single-pulse stimulation
to the CPN, applied 40, 50, and 60 ms earlier. In fact, in five
participants, the conditioning of the TA MEP by a prior CPN
stimulation was tested on 3 separate experiment days, and the
absolute amount of MEP suppression differed each day by only
7.3% on average at the 0.5 and 0.7 \( \times \) MT stimulation inten-
sities. Muscle activity, generated by a weak, voluntary con-
traction, was also suppressed by low-intensity, triple-pulse
CPN stimulation and occurred in the absence of a prior excit-
atory reflex response. Evoked responses from direct activation
of the corticospinal tract at the level of the brain stem and

![Fig. 4. Suppression of TA MEP, cervicomed-
ullary MEP (CMEP), and spinal MEP
(SMEP) by CPN stimulation. A: average of 5
unconditioned test (gray trace) and 5 CPN-
conditioned (Cond; black trace) TA MEPs,
CMEPs, and SMEPs, recorded from a single
participant at 40/50/60 ms, 45/55/65 ms, and
50/60/70 ms ISIs, respectively. B: the peak-
to-peak amplitude of the conditioned MEP,
CMEP, and SMEP is expressed as a percent-
age of the corresponding unconditioned test
response. The average unconditioned test
MEP and CMEP were 429.24 \( \pm \) 106.5 \( \mu \)V
and 819.88 \( \pm \) 1,071.0 \( \mu \)V, respectively (\( n = 3 \)), and the unconditioned test MEP and
SMEP (\( n = 2 \); recorded on a separate day)
were 532.35 \( \pm \) 108.1 \( \mu \)V and 919.00 \( \pm \) 284.3
\( \mu \)V, respectively (conditioned MEP on this
day was suppressed by 30.5%; not shown).
C: average of 5 unconditioned test MEPs and
SMEPs from a single participant, recorded at
rest (left) and during dorsiflexion (right). All
traces are aligned to the start of the TMS or
spinal electrical stimulation pulse. Error bars
represent mean \( \pm \) SE. Calibration lines are for
both A and C.
spinal cord were also suppressed by appropriately timed low-threshold CPN stimulation. Moreover, stimulation of the skin, innervated by the DPN, which contains the cutaneous branch of the CPN, also suppressed the TA MEP. We discuss below that the suppression of TA MEPs and voluntary EMG likely resulted from the activation of spinal inhibitory networks by low-threshold homonymous muscle and cutaneous afferents and provides a negative-feedback system to help regulate the activation of flexor motoneurons by the corticospinal tract.

**Origin of TA MEP and Voluntary EMG Suppression by Triple-Pulse CPN Stimulation**

**Spinal location.** The suppression of the TA MEP and voluntarily activated EMG was likely mediated by low-threshold CPN afferents, activating inhibitory networks in the spinal cord. This is supported by the finding that responses evoked by direct stimulation to the corticospinal tract, both at the level of the brain stem (at pyramidal decussation) (Gandevia et al. 1999; McNeil et al. 2009; Ugawa et al. 1991) and the spinal cord (C7–T4) (Martin et al. 2008), were also suppressed by appropriately timed CPN stimulation. The activation of the corticospinal tract by direct stimulation to the spinal cord likely activated similar fast-conducting corticospinal tract axons, as were activated with TMS over the motor cortex, given the very similar shapes of the evoked potentials (SMEPs) recorded in the TA muscle (e.g., Fig. 4C). A selective contribution by the corticospinal tract to the SMEP may result from its strong monosynaptic connection to the TA motoneuron pool compared with other descending tracts in the spinal cord (Lemon 2008). Thus, the preserved suppression of the similarly shaped SMEPs by low-threshold CPN stimulation strongly suggests that the TA MEPs were suppressed via processes occurring in the lumbar spinal cord. This is in agreement with other studies showing that afferent inputs from the leg, unlike that from the upper limb, do not inhibit cortical circuits but mainly activate inhibitory circuitry in the spinal cord (Geertsen et al. 2010, 2011; Roy and Gorassini 2008).

**Spinal interneurons.** The fact that the TA MEP/voluntary EMG could be suppressed by CPN stimulation at intensities that were below M-wave or H-reflex threshold indicates that processes, such as recurrent inhibition or motoneuron refractoriness, were not involved (Meunier et al. 1994; Tucker et al. 2005). Rather, activation of spinal inhibitory interneurons, interposed between the CPN afferents and the TA corticospinal tract, likely mediated this inhibition. One likely candidate is inhibitory interneurons that synapse onto propriospinal-like interneurons innervating the TA motoneuron pool, given the long (40–60 ms) conditioning intervals needed to suppress the TA MEPs by low-threshold muscle and cutaneous (i.e., DPN) afferents. A similar propriospinal site of action has been suggested recently for inhibitory, heteronymous afferent pathways from the CPN to the soleus motoneuron pool (Geertsen et al. 2011) and from tibial nerve afferents to the TA motoneuron pool (Roy and Gorassini 2008), both of which also require long (approximately 35–45 ms) conditioning-test stimulation intervals. These propriospinal-like interneurons may form part of an indirect corticospinal pathway to the motoneuron pool (Geertsen et al. 2011). The conditioning-test intervals (35–45 ms) that suppress the soleus and TA MEP are longer than that required for the activation of inhibitory, CPN-activated in-
terneurons that synapse directly onto quadriceps propriospinal neurons—the latter a trisynaptic pathway that requires conditioning-test intervals of only 4–11 ms (Marchand-Pauvert et al. 1999). Thus the homonymous afferent pathway studied here may involve more than three synapses and is likely different than the di- and trisynaptic, nonreciprocal Ib and Ia inhibitory pathways studied previously (Cavallari and Katz 1989; Forget et al. 1989a, b; Malagnen and Pierrot-Deseilligny 1988; Pierrot-Deseilligny et al. 1981). The inhibition of the TA MEP at ISIs of 25/35/45 ms, which had overlapping ISIs with 40/50/60 ms ISIs, could have recruited similar pathways or other inhibitory pathways with fewer synapses.

Alternatively, inhibition of the TA MEP could involve a spinal inhibitory mechanism with a long time course of activation to reach functional inhibition. For example, presynaptic inhibition occurs within the time scale of the homonymous suppression of the TA MEP (e.g., 40–200 ms) (El-Tohamy and Sedgwick 1983; Hultborn et al. 1987) and is also enhanced by spatial facilitation, i.e., three pulses of stimulation produce larger inhibition than one pulse (Burke et al. 1994). Thus presynaptic inhibition onto terminal axons of propriospinal or other excitatory interneurons interposed between the CPN and TA corticospinal tract could have mediated the long-latency spinal inhibition. Moreover, inhibition likely did not occur directly onto the TA motoneurons, i.e., at a premotorneuronal site (Iglesias et al. 2008). Unlike stimulation intensities above MT (at 1.5 × MT), where there was likely direct, recurrent inhibition onto the TA motoneurons (Meunier et al. 1994), only the later portions of the TA MEP were suppressed by low-intensity stimulation of the CPN (from 4.2 ms onward), suggesting that inhibitory inputs, a few synapses upstream from the TA motoneurons, were involved [as per Chaix et al. (1997)]. However, the lack of early MEP inhibition could have resulted from only the later-recruited motor units having either higher thresholds and/or activated by later I-waves, inhibited by the CPN stimulation. Interestingly, the responses evoked by cervicomedullary or spinal cord stimulation, which produce more of a single-descending volley, were also suppressed at later latencies (3 ms) from the onset of the unconditioned CMEP or SMEP, providing supportive evidence for a site of action upstream from the motoneuron. In addition, stimulation of the DPN, the cutaneous branch of the CPN, only suppressed the TA MEP and not TA H-reflex, suggesting that the MEP inhibition was not premotorneuronal (Mazevet et al. 1996). This is in agreement with findings from Roy and Gorassini (2008) and Geerts et al. (2011), where heteronymous nerve stimulation (tibial nerve to TA and CPN to soleus, respectively) produced a similar long-latency (35–45 ms) suppression of the MEP but not the H-reflex. As the inhibitory profile of these two pathways is quite similar to the homonymous CPN-TA MEP pathway studied here, it is likely that they share similar upstream inhibitory interneurons.

**Effects from the background contraction.** Given that TA MEPs were evoked during a background contraction and that triple-pulse CPN stimulation could also suppress low levels of voluntary EMG, the CPN stimulation could have suppressed the TA MEP by inhibiting upstream interneurons in corticospinal pathways activated by the voluntary background contraction. As demonstrated in Fig. 3, C and D, not all of the MEP suppression can be accounted for by a decrease in background EMG, because many MEPs at the various ISIs tested, which were evoked during similar levels of background EMG suppression, were not suppressed by the CPN stimulation. Likewise, MEPs could still be suppressed when evoked at rest, when there was no background EMG activity. Taken together, suppression of background EMG by the subthreshold CPN stimulation was likely not the only contributor to MEP suppression. Activation of spinal inhibitory interneurons, distinct from the ones producing the decrease in background EMG, likely also contributed to the suppression of the MEP at the 25/35/45- and 40/50/60-ms ISIs.

**Afferents.** Suppression of the TA MEP from triple-pulse CPN stimulation occurred at very low-stimulation intensities (<0.7 × MT) in the control participants. We ruled out that activation of afferents, innervating the skin directly below the cathode, contributed to the MEP suppression, because only when the cathode was over the CPN did MEP suppression occur and thus was likely activating low-threshold muscle (Ia and Ib) afferents in the CPN. In addition, activation of low-threshold cutaneous afferents likely also contributed, given that stimulation of the skin innervated by the DPN (the cutaneous branch of the CPN) also suppressed the TA MEP but to a lesser extent. Thus as with many spinal inhibitory interneurons and propriospinal neurons, there seems to be a convergence of afferents conveying different sensory modalities onto the homonymous, long-latency, spinal inhibitory pathway studied here.

**Future Directions**

In this study, we have characterized the activation of spinal inhibitory circuitry by low-threshold homonymous afferents. Future studies are required to examine directly if this pathway regulates the activation of the TA motoneuron pool by the corticospinal tract to help control the force, timing, and ultimately, the precision by which ankle flexors are activated during movement and if the excitability of this pathway is affected by injury to the central nervous system.

**ACKNOWLEDGMENTS**

We thank the participants for their large time commitment and effort in this study, in addition to the excellent technical assistance of Jennifer Nevett-Duchcherer. We are grateful to Dr. Dick Stein for his helpful suggestions on the manuscript.

**GRANTS**

Support for this work was provided by the Canadian Institute of Health Research (CIHR) grant MOP-106549 (to M. A. Gorassini). Salary support was provided by Alberta Innovates: Health Solutions (to M. A. Gorassini).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


*J Neurophysiol* • doi:10.1152/jn.00673.2013 • www.jn.org
REFERENCES


