Task-related modulation of crossed spinal inhibition between human lower limbs

Berthe Hanna-Boutros, Sina Sangari, Aliye Karasu, Louis-Solal Giboin, and Véronique Marchand-Pauvert

UPMC University of Paris 6, Paris, France

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Hanna-Boutros B, Sangari S, Karasu A, Giboin LS, Marchand-Pauvert V. Task-related modulation of crossed spinal inhibition between human lower limbs. J Neurophysiol 111: 1865–1876, 2014. First published February 5, 2014; doi:10.1152/jn.00838.2013.—Crossed reflex action mediated by muscle spindle afferent inputs has recently been revealed in humans. This raised the question of whether a complex spinal network involving commissural interneurons receiving inputs from proprioceptors and supraspinal structures, as described in cats, persists in humans and contributes to the interlimb coordination during movement. First, we investigated the neurophysiological mechanisms underlying crossed reflex action between ankle plantar flexors and its corticospinal control from primary motor cortex. Second, we studied its modulation during motor tasks. We observed crossed inhibition in contralateral soleus motoneurons occurring with about 3 ms central latency, which is consistent with spinal transmission through oligosynaptic pathway. The early phase of inhibition was evoked with lower stimulus intensity than the late phase, suggesting mediation by group I and group II afferents, respectively. The postsynaptic origin of crossed inhibition is confirmed by the finding that both H-reflex and motor-evoked potential were reduced upon conditioning stimulation. Transcranial magnetic stimulation over ipsilateral and contralateral primary motor cortex reduced crossed inhibition, especially its late group II part. Last, late group II crossed inhibition was particularly depressed during motor tasks, especially when soleus was activated during the walking stance phase. Our results suggest that both group I and group II commissural interneurons participate in crossed reflex actions between ankle plantar flexors. Neural transmission at this level is depressed by descending inputs activated by transcranial magnetic stimulation over the primary motor cortex or during movement. The specific modulation of group II crossed inhibition suggests control from monoaminergic midbrain structures and its role for interlimb coordination during locomotion.

H-reflex; TMS; commissural interneurons; corticospinal; locomotion

SEVERAL SPINAL PATHWAYS INVOLVING commissural interneurons mediate crossed reflex actions from proprioceptors. In cat lumbar cord, these pathways are closely interconnected and incorporated into complex networks coordinating muscle activity on both sides (Jankowska 2008). Two subpopulations of midlumbar commissural interneurons have been clearly distinguished based on their monosynaptic afferent inputs. One receives group I, reticulospinal and vestibulospinal afferents, while the other one is directly excited by group II afferents. Both groups are differentially controlled by other peripheral and descending inputs of various origins through oligosynaptic pathways, involving dorsal horn and long propriospinal interneurons, and the main effect in contralateral motoneurons is inhibition mediated by group II afferents (Jankowska et al. 2005; Jankowska and Edgley 2006). Commisural interneurons are rhythmically activated during locomotion (Matsuyama et al. 2004) and considered as part of the locomotor central pattern generator (Kiehn and Butt 2003).

In humans, the first evidence for a locomotor phase-related crossed reflex action was obtained by stimulating cutaneous afferents (Duyens et al. 1991). However, the reflex latency ranged from 70 to 100 ms, suggesting mediation through a transcortical loop (Nielsen et al. 1997; Christensen et al. 1999). More recently, new evidence for spinal crossed reflex action has been found by stimulating the posterior tibial nerve (PTN), producing inhibition in contralateral soleus (cSol) motoneurons with shorter latency (37–41 ms) mediated by proprioceptive afferents, probably of group I and group II origin (Stubbs and Mrachacz-Kersting 2009; Stubbs et al. 2011a). This short latency crossed inhibition is modulated according to the walking phase (Stubbs et al. 2011b), and its modifications after stroke suggest bilateral descending influence, involving contralateral motor cortex (Stubbs et al. 2012), much as in cat (Edgley et al. 2004; Jankowska and Edgley 2006; Stćcina et al. 2008).

These results indicate that human spinal cord might have common characteristics with feline spinal cord, and our hypothesis is that the neural transmission at the level of commissural interneurons might be particularly modulated during human walking, for interlimb coordination, compared with other motor tasks. However, the origin of the spinal pathway mediating the PTN-induced crossed inhibition to cSol motoneurons in humans remains unclear and needs further investigations. Indeed, 1) protocols based on modulation of averaged and rectified electromyogram (EMG) or Hoffmann (H) reflex (Stubbs and Mrachacz-Kersting 2009; Stubbs et al. 2011a) do not allow distinction between pre- vs. postsynaptic inhibition. 2) The central latency of crossed inhibition in humans was estimated at 7 ms (Stubbs and Mrachacz-Kersting 2009), whereas 3- to 5-ms central latency has been found in cat (Arya et al. 1991). 3) Conventionally, the threshold intensity is evaluated according to motor threshold (MT) to determine the origin of the peripheral inputs (Pierrot-Deseilligny and Burke 2012), and there is no clear evidence for group I crossed inhibition (Stubbs and Mrachacz-Kersting 2009). Therefore, our first objective was to further characterize the origin of the crossed inhibition produced by PTN stimulation in cSol motoneurons. Second, we investigated whether corticospinal inputs to commissural interneurons from ipsi- and contralateral
motor cortex influence neural transmission at this level. Last, we investigated whether the crossed inhibitions, likely mediated by group I and group II afferents, are differentially modulated according to motor tasks in humans.

In the following, contralateral and ipsilateral for the peripheral and cortical stimulations are used compared with the left spinal cord.

**MATERIALS AND METHODS**

**Ethical Approval**

The experiments were carried out on 15 healthy subjects (10 women, 27.9 ± 1.6 yr old, range 22–40 yr), all of whom had given informed, written consent to the experimental procedures, which had been approved by the ethics committee of the Pitié-Salpêtrière Hospital (CPP Ile de France VI). The study conformed to the standards set by the latest revision of the Declaration of Helsinki.

**Recordings**

EMG activity of right and left soleus was recorded with bipolar surface electrodes placed medially on the posterior aspect of the legs, 2–3 cm below the gastrocnemius muscles (ZeroWire EMG, Aurion Srl, Milan, Italy). EMG activity was filtered (EMG bandwidth 10–1,000 Hz) and amplified (×1,000) before being digitally stored (2-kHz sampling rate) on a personal computer for off-line analysis (Power 1401 and Signal Software, CED, Cambridge, UK). In five subjects, the EMG activity of tibialis anterior, biceps femoris and vastus lateralis was also recorded during the various motor tasks investigated in experiment 3.

**Stimulations**

**Electrical stimuli.** One-millisecond rectangular electrical pulses were delivered to the right and left PTN, by constant current stimulators (DS7TH, Digitimer, Hertfordshire, UK) through surface electrodes: a 7-cm² brass hemispheric electrode placed in the popliteal fossa (cathode) and a 21-cm² brass plaque above the patella. The optimal stimulation sites were determined clinically by palpating the Achilles tendon upon stimulation, to check that stimuli did not encroach the common peroneal nerve and thus activated ankle dorsiflexors and evertors. The maximal motor response (Mmax) was evaluated in the right soleus to normalize the test [H-reflex and motor-evoked potential (MEP)] and M responses, to counteract the intersubject variability. Then, the intensity of the right PTN stimulation (test stimuli) was adjusted to evoke a sizeable test H-reflex in the right soleus between 20 and 30% Mmax, in the ascending phase of its recruitment curve (M response was less than the H-reflex; see Figs. 4, A and B, and 6, B and C). On the left side, the intensity of conditioning PTN stimuli was adjusted according to the threshold intensity for direct motor response in the soleus EMG (MT). To determine the MT, the stimulus intensity was gradually decreased, and the intensity retained as MT was that at which stimuli did not produce any M response in the left soleus EMG. The left PTN stimuli were then adjusted according to MT, as performed conventionally to determine the origin of the afferent inputs responsible for the effects observed (Pierrot-Deseilligny and Burke 2012). We tested the effect of left PTN stimuli on the right, cSol H-reflex.

**Magnetic stimuli.** In a first series of experiments, transcranial magnetic stimulation (TMS) was delivered through a double cone coil (Magstim Rapid, Whitland, UK), to investigate the modulation of the right soleus MEP by conditioning left PTN stimuli (experiment 1). The cone coil was held over the longitudinal fissure, at a position where the MEP in the right soleus EMG was the largest and the most reproducible in five consecutive trials and producing plantar flexion [optimal stimulation site; mean TMS intensity 64 ± 2% maximal stimulator output (MSO), range 56–74%]. In a second series of experiments, TMS was delivered through a figure-of-eight coil to investigate the effects of ipsi- and contralateral corticospinal inputs onto the left spinal cord (i.e., possibly onto commissural interneurons mediating crossed inhibition: experiment 2). We did not use the cone coil as in the first series of experiments, because we had to produce a more focal electrical field in the brain. The figure-of-eight coil was placed over the primary motor cortex, 2–3 cm lateral and 1.5–2.5 cm anterior from Cz, to limit the diffusion of the electrical field in the opposite hemisphere. The left (ipsilateral) and the right (contralateral) primary motor cortexes were successively stimulated (Fig. 1). The positions of the coil were determined during tonic contraction of the cSol (~15% EMG activity recorded during maximal tonic voluntary plantar flexion while sitting, i.e., %maximal voluntary contraction or MVC), and MEPs could only be evoked at about 93–97% MSO. When the subjects relaxed and intensity was 95% MSO, TMS was subliminal for a MEP, but we hypothesized that it produced descending volleys (Lackmy-Vallee et al. 2012). TMS intensity was thus fixed at this intensity in all subjects to study its effects on crossed inhibition. It has been noted that no MEP was evoked in the left soleus when using the cone coil (experiment 1; see Fig. 4E) and in the soleus ipsilateral to TMS when using the figure-of-eight coil (experiment 2; Fig. 1).

**Experimental Procedures**

**Experiment 1: Origin of the PTN-induced inhibition in cSol (nine subjects).** The subjects were sitting in a comfortable reclining armchair, with head support. The legs were supported such that the joint angles were 110° for the hips, 150° for the knees (semiflexed) and 100° for the ankles (10° plantar flexion). The intensity of left PTN stimulation was adjusted at 2 × MT, and the intervals between left PTN (conditioning stimuli) and right, contralateral PTN (test stimuli) varied from 0 to 40 ms, to investigate the time course of the effects produced by left PTN stimuli in cSol at rest (see diagram of the protocol in Fig. 3). Then the short (3–7 ms) and the long (15 ms) interstimulus interval (ISI) at which the right cSol was more depressed were chosen (see Fig. 3C), and the intensity of the left PTN stimulation was changed between 0.4 and 2.5 × MT (0.1 × MT stepwise) to investigate the intensity curve of the left PTN-induced inhibition of cSol at rest (see Fig. 3, D and E). Lastly, seven of nine subjects (2 did not accept TMS) were asked to sustain a tonic plantar flexion (~15% MVC) to compare the effect of the left PTN stimulation (2–2.5 × MT) on the right soleus H-reflex and MEP (produced by the cone coil). The ISIs between the left and right PTN stimulations were 3–7 ms and 12–15 ms, and the ISIs between the left PTN stimulation and TMS were 1–10 ms and 11–17 ms (see diagram of the protocol in Fig. 4). Difference in ISIs for H-reflex and MEP can be explained by the difference in conduction time in group Ia afferents and corticospinal volleys, and the multiple corticospinal volley (Pauvert et al. 1998). Despite our effort to adjust the intensities of PTN stimulation and TMS so as to evoke test responses of similar size (Morita et al. 1999), on average, the amplitude of the test H-reflex size was larger than that of test MEP (17.6 ± 2.7% vs. 6.4% Mmax, paired t-test, P < 0.05).

**Experiment 2: Corticospinal control of PTN-induced inhibition in cSol (seven subjects).** Subjects were sitting at rest. Left PTN stimuli (2–2.5 × MT) were delivered 5 (short ISI) and 15 ms (long ISI) before right PTN stimuli, producing test H-reflex in the right cSol. TMS (figure-of-eight coil) was applied 1 over the left hemisphere, i.e., ipsilateral to the conditioning left PTN stimuli (iTMS), and 2 over the right hemisphere, i.e., contralateral to the conditioning left PTN stimuli (cTMS). The effects of TMS 1 on right soleus H-reflex, 2 on short ISI, and 3 on long ISI crossed inhibition, both produced by left PTN stimuli in the right cSol motoneurons, were tested in the same recording session. ISI between TMS and the right PTN stimulation (evoking the test H-reflex) was set at 6, −4, −2 and 0 ms [TMS was
delivered after \((-6, -4, -2\) ms) or at the same time \((0\) ms) than the right PTN stimulation] to determine in each individual the optimal ISI for the convergence of peripheral and corticospinal inputs at spinal level (see diagram of the protocol in Fig. 5). The optimal ISI was determined statistically by comparing the algebraic sum of the effects on separate stimuli and the effects on combined stimuli (see RESULTS, Corticospinal Control of Crossed Inhibition).

Experiment 3: Modulation of PTN-induced inhibition in cSol during motor tasks \((11\) subjects). Based on the results of experiment 1, ISI between left and right PTN stimuli was set at 5 (except in 2 subjects, short ISI was 3 and 7 ms) and 15 ms \((long\) ISI). The intensity of the right PTN stimulus was adjusted to produce test H-reflexes of similar size across conditions, and thus to test the same proportion of the motoneuron pool in each motor task \((Crone et al. 1990)\). The effect of the left PTN stimulation \((2–2.5 \times MT)\) on the right cSol H-reflex was examined \(1\) at rest and during tonic plantar flexion \((\sim15\%\) MVC) in sitting position \((quiet\) and tonic sitting, respectively); \(2\) while standing at rest or slightly on the tip of the toe \((quiet\) and tonic standing, respectively); and \(3\) during the walking stance phase of the right leg (see EMG activities in Fig. 2). During treadmill locomotion \((Biodex Medical Systems, Shirley, NY)\), a pressure transducer was placed on the right heel to detect the time of heel strike and to trigger left PTN stimulations according to EMG activity in cSol during stance. The subjects first walked on the treadmill for 5–10 min before recordings, to accustom themselves to treadmill walking, and to determine their preferred speed \((mean 3.6 \pm 0.1\) km/h, range \(3–4)\). Left PTN stimuli were delivered in the ascending \((cSol+)\) and descending part of cSol EMG burst \((cSol−); see Fig. 6A), at a similar level of EMG activity \((76.4 \pm 10.8\) vs. \(88.2 \pm 12.3\) mV; paired \(t\)-test, \(P = 0.13)\). On average, stimuli were triggered at \(34.6 \pm 2.1\) and \(49.1 \pm 2.3\%\) of the total duration of the step cycle, i.e., \(48.1 \pm 4.2\) and \(81.0 \pm 2.7\%\) of the duration of cSol EMG burst.

Analysis

For each experimental paradigm, one recording session consisted of 20 test stimuli delivered alone \((evoking H\text{-reflex or MEP in the EMG activity of the right soleus})\) and 20 combined to conditioning stimuli \((left PTN, iTMS, cTMS)\), randomly alternated \((0.33\) Hz). Peak-to-peak amplitude of H-reflex was measured to compare the size of conditioned H-reflexes to the mean size of the test H-reflex. Because MEPs were produced by multiple descending corticospinal volleys, the right soleus EMG activity was rectified to analyze the MEP area, to take into account the summation of all descending volleys at spinal level and their interaction with conditioning peripheral inputs. We have compared the area of conditioned MEPs to the mean area of the test MEP, estimated within the same window of analysis limited to the MEP latency and its duration. Test responses \((H\text{-reflex and MEP})\) and \(M\) responses were normalized to \(M\)max in the right soleus \((evaluated in each experimental condition or motor task)\), for interindividual comparisons and to ensure that the effects of conditioning stimuli were studied on the same proportion of the...
motoneuron pool (Crone et al. 1990; Lackmy and Marchand-Pauvert 2010). Mean level of rectified EMG was measured to compare the level of background activity during motor task (Table 1). Mean values are indicated ± 1 standard error of the mean (SEM).

**Statistics**

Two-tailed paired t-tests were performed to compare the control and conditioned responses in each individual. Because normality and homogeneity of variances were not respected in the group data, nonparametric Wilcoxon signed-rank test was performed to compare the threshold intensity of short and long ISI crossed inhibition. The mean levels of short and long ISI crossed inhibition were tested with single-sample t-tests. The effects of subthreshold iTMS and cTMS on short and long ISI crossed inhibition were tested using two-tailed paired t-tests. Pearson correlation analyses were undertaken to determine whether the size of the test response (H-reflex or MEP) and the background EMG activity had influenced the level of short and long ISI crossed inhibition. Then, 1) analysis of covariance (ANCOVA) was performed to compare the level of short and long ISI inhibition between H-reflex and MEP, and between motor tasks at long ISI; and 2) analysis of variance (ANOVA) was undertaken to compare the background EMG, the M responses, and the short ISI inhibition between tasks. If ANCOVA and ANOVA provided significant F values, Fisher’s least significant difference (Bonferroni and Sidak corrections) tests were performed for comparison of two means.

Because of data distribution, the test size of the right soleus H-reflex between motor tasks was compared using the Friedman test. Statistical analysis was achieved using StatEL software (www.adscience.eu), and the significance level was set at P value < 0.05.

**RESULTS**

**Characteristics of PTN-induced Inhibition of cSol H-reflex**

Figure 3B shows the time course of cSol H-reflex inhibition produced by left PTN conditioning stimuli in one subject. H-reflex depression occurred at ISI 2 ms, was significant at 3 ms (paired t-test, P < 0.05) and disappeared at ISI 40 ms. The soleus H-reflex latency was visually determined, based on the raw signal and the cumulative sum, between 34 and 34.5 ms on the right side (Fig. 3A) and between 35 and 35.5 ms on the left side. Because stimulating and recording electrodes were positioned approximately at an equal distance to the spinal cord, the simultaneous arrival of the fastest group Ia volley (produced by stimulating PTN nerves) at motoneuron level would occur at 0.5–1 ms ISI. The inhibition manifested at 3 ms, i.e., with an extra time of 2–2.5 ms. Similar calculation was performed in each subject, and the mean latency of H-reflex on the right side was 30.9 ± 0.9 ms vs. 31.1 ± 0.9 ms on the left side, i.e., 0.2 ± 0.3 ms difference (range 1 to 2 ms). On average, the left PTN-

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**Table 1. Mean background cSol motoneuron activity during motor tasks**

<table>
<thead>
<tr>
<th></th>
<th>Quiet Sitting</th>
<th>Tonic Sitting</th>
<th>Quiet Standing</th>
<th>Tonic Standing</th>
<th>Stance cSol+</th>
<th>Stance cSol−</th>
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<tbody>
<tr>
<td>EMG</td>
<td>3.7 ± 0.2 μV</td>
<td>24.5 ± 5.0 μV</td>
<td>15.8 ± 2.7 μV</td>
<td>35.3 ± 3.3 μV</td>
<td>76.4 ± 10.8 mV</td>
<td>88.2 ± 12.3 mV</td>
</tr>
<tr>
<td>M response</td>
<td>2.5 ± 0.7</td>
<td>2.2 ± 0.5</td>
<td>2.8 ± 0.6</td>
<td>2.6 ± 0.7</td>
<td>3.1 ± 0.6</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>H-reflex</td>
<td>25.6 ± 2.6</td>
<td>25.1 ± 2.1</td>
<td>20.1 ± 1.5</td>
<td>23.6 ± 1.8</td>
<td>33.5 ± 5.7</td>
<td>27.6 ± 3.6</td>
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Values are means ± SE. EMG, mean right contralateral soleus (cSol) background EMG according to motor task; M response, mean M response in the right cSol expressed as %maximal motor response (Mmax) evaluated during each motor task; H-reflex, mean test H-reflex in the right cSol expressed as %Mmax evaluated during each motor task; cSol+ and cSol−, ascending and descending part of cSol EMG burst, respectively.

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induced inhibition of cSol H-reflex occurred at 3.1 ± 0.6 ms ISI (range 1 to 7 ms ISI), i.e., with a mean extra time of 2.9 ± 0.7 ms (range 0 to 7 ms). Accordingly, in the group of nine subjects, significant inhibition was produced between ISIs 3 and 30 ms (0.05 < P < 0.01), with two phases occurring at short (2–7 ms) and longer ISI (>10 ms; Fig. 3C). In the following, we have investigated the characteristics of the two inhibitory phases. First, we tested different ISIs to determine the optimal ISI for short and long ISI inhibitions (i.e., when inhibition was significant) in each subject; difference in optimal ISI across subjects and experimental sessions can be explained by interindividual variability (subject height for instance) and electrode location.

Figure 3D shows the intensity curve of the left PTN-induced inhibition of cSol H-reflex in another subject. At short ISI (5 ms), the inhibition occurred with stimulus intensity adjusted between 0.6 and 0.7 × MT. At longer ISI (15 ms), the inhibition arose with higher stimulus intensity, above 1 × MT. On average (Fig. 3E), crossed inhibition was produced with higher stimulus intensity at long ISI (15 ms; 1.0 ± 0.1 × MT) than at short ISI (3–7 ms; 0.7 ± 0.1 × MT; Wilcoxon signed-rank test, P < 0.05).

Figure 4, A–D, shows the inhibition of the right soleus H-reflex and MEP, produced by left PTN conditioning stimuli in one subject. Note that TMS delivered through the double cone coil did not produce any MEP in the left soleus in this
subject and in the six other subjects so investigated (iSol; Fig. 4E). Given the difference between the test size of soleus H-reflex and MEP (on average, $17.6 \pm 2.7$ vs. $6.4\% M_{\text{max}}$; paired $t$-test, $P < 0.05$), we first tested the correlation between the crossed inhibition and the size of the test response. At both short and long ISI, the crossed inhibition decreased when the size of the test response increased (Pearson correlation analysis, $P < 0.001$ for both ISI; $R^2$ was 0.7 and 0.8 for short and long ISI, respectively). By taking into account the difference in the size of the test responses, ANCOVA revealed that crossed inhibition at short ISI was similar between H-reflex and MEP ($P = 0.25$). On the other hand, at long ISI, the inhibition of soleus MEP was significantly smaller than that of H-reflex ($P < 0.01$; Fig. 4F). The main result to retain is that left PTN conditioning stimuli could inhibit cSol motoneurons while testing the size of H-reflex (single-sample $t$-test, $P < 0.01$ at both ISI) or MEP ($P < 0.05$ at both ISIs).

**Corticospinal Control of Crossed Inhibition**

The algebraic sum of the effects of separate subthreshold TMS and left PTN stimuli on the right cSol H-reflex was 80–85% in the group of subjects, at short (left bars) and long ISI (right bars) when test response was an H-reflex (shaded bars) or a MEP (open bars). Vertical bars are $\pm 1$ SE. $**P < 0.01$.

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**Fig. 4.** Effects of left PTN stimulation on cSol H-reflex and MEP. **Top:** diagram of the stimulation procedure for experiment 1 with TMS. $A–D$: mean test (gray line) and conditioned responses (dark line) at short (5 ms; $A$ and $C$) and long (15 ms; $B$ and $D$) ISI between left PTN and test stimuli applied to PTN evoking H-reflex ($A$ and $B$) or TMS producing MEP ($C$ and $D$) in the right soleus (cSol) EMG, in one subject. The intensity of the left PTN conditioning stimuli was $2–2.5 \times M_{\text{T}}$. $E$: mean left soleus (iSol) EMG activity after TMS evoking the MEP in the right soleus (cSol), in the same subject as in $A$ and $D$. $F$: mean conditioned response (% mean test response) in the group of subjects, at short (left bars) and long ISI (right bars) when test response was an H-reflex (shaded bars) or a MEP (open bars). Vertical bars are $\pm 1$ SE. $**P < 0.01$. 

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compared with the effects on combined stimuli. Paired t-tests in each individual revealed only significant TMS-induced depression of crossed inhibition (6/7 subjects). This depression was observed at one or two out of the four ISIs tested, depending on the subject. Data were thus grouped according to the ISI (−6, −4, −2, or 0 ms) at which this depression occurred or was maximal in each individual. On average, both short and long ISI crossed inhibitions (Figs. 5, A and B, respectively) were significantly depressed by TMS when applied ipsilaterally (iTMS) or contralaterally (cTMS) to the left spinal cord (paired t-test, P < 0.05, **P < 0.01). The level of depression (7–10% on average) was compared for both hemispheres, and we found no significant difference (P = 0.7 and 0.6 for short and long ISI inhibition, respectively; Fig. 5C). We noticed that the depression occurred 1 ms earlier for cTMS (−3.7 ± 0.7 ms) than for iTMS (−2.6 ± 0.6 ms).

Modulation of Crossed Inhibition According to Motor Task

Left PTN conditioning stimuli were delivered during the stance phase of walking, at similar level of background EMG (Table 1), when the right soleus was activated (cSol+) or deactivated (cSol−; Fig. 6A). At the same time, the left soleus was silent (bottom trace in Fig. 6A). During the other tasks investigated, the right soleus background EMG was significantly less (ANOVA, P < 0.001; post hoc analyses, P < 0.001; see top traces in Fig. 2). We, therefore, tested whether the background EMG had influenced the level of crossed inhibition at short and long ISI, regardless of the motor task. Pearson correlation analyses revealed no relationship between the level of background EMG and that of crossed inhibition (P = 0.3 and 0.1 for short and long ISI inhibition, respectively).

Figure 6, B and C, shows the mean test and conditioned H-reflexes in cSol after left PTN stimuli during the various motor tasks in one subject. The intensity of the right PTN stimuli was adjusted to evoke test H-reflex in the right cSol of similar size across motor tasks (with small M response); it was varied in a small range, less than 1 mA. Accordingly, the M response evoked before the test H-reflex did not significantly change across conditions in the group (ANOVA, P = 0.85; Table 1). Nevertheless, the mean size of the right soleus H-reflex was not exactly the same in this subject, as in the group data, especially during quiet standing and walking (Table 1). However, Friedman test indicated that these differences were not statistically significant in the group (P = 0.14). Despite this, and because we found a significant influence of the size of the test response in experiment 1 (H-reflex vs. MEP, see above), we tested whether the size of the test H-reflex had influenced the level of crossed inhibition, regardless of the motor task. We did not find any relationship with the level of short ISI inhibition (Pearson correlation analysis, P = 0.06), but the long ISI inhibition significantly decreased for large right H-reflex test size (P < 0.05, R² = 0.06).

In the subject illustrated in Fig. 6, B and C, the conditioned H-reflex was smaller than the test H-reflex in all motor tasks, except at long ISI in early stance, when the right soleus was activated (Stance cSol+). We tested whether the level of crossed inhibition changed according to the motor task in the group. ANOVA revealed no significant modulation of short ISI inhibition (3–7 ms) during motor tasks (P = 0.5; Fig. 7A).

![Figure 5](http://jn.physiology.org/)

**Figure 5.** Contralateral and ipsilateral corticospinal control. Top: diagram of the stimulation procedure for experiment 2. A and B: mean level of crossed inhibition (%mean test H-reflex) in the group of subjects, resulting from the algebraic sum of separate effects (open bars) or produced on combined stimuli (shaded bars) at short (A) and long ISI (B). The data were grouped according to the ISI (between TMS and peripheral stimuli) at which TMS reinforced the crossed inhibition. This ISI varied from one subject to another between 0 and −6 ms. C: mean difference between the effects on combined stimuli and the algebraic sum of separate effects in the group of subjects at short (left bars) and long ISI (right bars) when TMS was applied over the ipsilateral (iTMS; open bars) or contralateral motor cortex (cTMS; shaded bars). Vertical bars are ± 1 SE. *P < 0.05, **P < 0.01.

Given its relationship with the H-reflex test size, the possible change in long ISI (15 ms) crossed inhibition during motor task was tested using ANCOVA. We found that the relationship between the H-reflex test size and the level of inhibition did not change between tasks (P = 0.14), but the level of inhibition was significantly modified (taking into account the size of the
test H-reflex; \( P < 0.01 \). To sum up, post hoc analyses revealed that the crossed inhibition was the largest during quiet sitting and the smallest during stance, when cSol was activated (cSol+), compared with other motor tasks (Fig. 7B; \( P \) values are indicated in Table 2).

**DISCUSSION**

This study has shown that conditioning PTN stimuli reduced the size of both cSol H-reflex and MEP, with a mean central latency of about 3 ms. The early phase of crossed inhibition was produced with lower stimulus intensity than the late phase. While both phases were reduced by ipsilateral and contralateral corticospinal inputs to the left spinal cord, only the late phase was depressed during motor tasks, especially when the right cSol was activated during the walking stance phase.

**Origin of Crossed Inhibition**

Crossed inhibition in humans has been investigated so far, by testing the effect of PTN stimuli on rectified and averaged EMG and H-reflex size whose modulations depend on the level of presynaptic inhibition of group Ia afferents. However, this hypothesis has never been proposed as a possible mechanism underlying crossed inhibition. Because PTN stimuli significantly reduced the MEP size, to the same extent than that of H-reflex at short ISI but to a lesser extent at long ISI (see below), our results further confirm the postsynaptic origin of crossed inhibition (Nielsen and Petersen 1994).

As observed in a previous study based on H-reflex modulation (Stubbs et al. 2011a), crossed inhibition was produced on average at about 3 ms ISI. Investigating short ISIs, with 1 ms step, allowed us to determine with more precision the central latency. Indeed, in a previous study using the modulation of rectified EMG (Stubbs and Mrachacz-Kersting 2009), whose resolution time is less than that of H-reflex technique, the central latency has been estimated between 7 and 11 ms, which was longer than that found in animals (3–5 ms; Arya et al. 1991). In the present study, we found a central latency of \( \sim 3 \) ms, which is compatible with animal data and further supports the spinal origin of crossed inhibition and its mediation through oligosynaptic pathways (Fig. 8).

PTN-induced depression of cSol EMG occurred with conditioning stimuli adjusted between M threshold and a M response of 25% Mmax in iSol, which was estimated to
PTN stimuli could reduce the MEP size at both short and long ISIs, corresponding to group I and group II crossed inhibition. The inhibition of cSol MEP was weak compared with H-reflex, especially when testing the group II crossed inhibition. This suggests that corticospinal inputs from primary motor cortex depressed the transmission of crossed inhibition of spinal motoneurons, especially that mediated by group II afferents.

Subthreshold TMS over the ipsi- and contralateral (to the left spinal cord) primary motor cortex both reduced the level of crossed inhibition. However, comparing algebraic sum of the effects of separate stimuli and the effects on combined stimuli can be difficult due to possible saturation. Indeed, motoneurons cannot be more inhibited than that allowed by the afferent pathways. If afferent inhibitory pathways had been overstimulated, PTN stimuli would have reduced the MEP size at least to the same extent as that of H-reflex. Indeed, the electrical field inducing MEP, which was produced through the double-cone coil over the longitudinal fissure, may have spread on both ipsi- and contralateral motor area. Therefore, the results of TMS experiments, based on the modification of MEP size (double cone coil) and on the depression of crossed inhibition after iTMS and cTMS (figure-of-eight coil), suggest that neural transmission at the level of commissural interneurons is controlled by pyramidal tract neurons likely from both sides in humans as in cats (Stecina et al. 2008). While the difference did not reach the statistical significance, we found again the group II crossed inhibition more depressed than the group I inhibition, especially when stimulating the contralateral motor cortex.

Feline commissural interneurons receive direct and indirect (involving segmental and long propriospinal neurons) reticulospinal control (Bannatyn et al. 2003; Matsuyama et al. 2004). The control from ipsi- and contralateral pyramidal tract neurons is indirect, involving spinal interneurons and/or the reticular formation (Jankowska 2008; Jankowska et al. 2005; Jankowska and Edgley 2006). In humans, it is difficult to determine which pathways mediate the corticospinal volleys produced by TMS. Moreover, peripheral inputs can interact with direct and indirect corticospinal waves (Pauvert et al. 1998). Therefore, it is difficult to determine the exact timing and the circuitry involved in the interaction between corticospinal and peripheral inputs. However, to make sure that interaction occurred at spinal level, we investigated only short ISIs between TMS and peripheral volleys. We found, on average, that the TMS-induced ipsilateral inputs required 1 ms more than the contralateral inputs to influence the crossed inhibition. One possibility would be that the corticospinal control was from the contralateral motor cortex only, and the 1-ms delay corresponded to the time required for the spread of the current to the opposite hemisphere. However, this might not be the case at least for two reasons. J) The current propagating in the contralateral

Corticospinal Control

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### Table 2. Results of post hoc analyses of long interstimulus interval inhibition during motor tasks

<table>
<thead>
<tr>
<th>Tonic Sitting</th>
<th>Quiet Standing</th>
<th>Tonic Standing</th>
<th>Stance cSol+</th>
<th>Stance cSol−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quiet sitting</td>
<td>0.14</td>
<td>&lt;0.05*</td>
<td>&lt;0.05*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Tonic sitting</td>
<td>0.66</td>
<td>&lt;0.05*</td>
<td>&lt;0.01*</td>
<td>0.66</td>
</tr>
<tr>
<td>Quiet standing</td>
<td>0.49</td>
<td>&lt;0.05*</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tonic standing</td>
<td>0.8</td>
<td>&lt;0.05*</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Stance cSol+</td>
<td>&lt;0.05*</td>
<td>&lt;0.05*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stance cSol−</td>
<td></td>
<td></td>
<td>&lt;0.05*</td>
<td></td>
</tr>
</tbody>
</table>

*P* values for post hoc analysis (Bonferroni and Sidak corrections). *Significant difference.
hemisphere being less than that produced by TMS over the contralateral motor cortex, the depression of crossed inhibition would have been less when stimulating the ipsilateral hemisphere than when stimulating the contralateral one, which was not the case. 2) When TMS was applied over the longitudinal fissure, a MEP was evoked only in the right soleus, which suggests that the left hemisphere was mainly activated, i.e., the ipsilateral hemisphere to the left spinal cord. And thus, because the depression of the MEP was smaller than that of the H-reflex, the ipsilateral descending inputs may have interacted with subcortical structures to counteract the crossed inhibition. Therefore, and even if the hypothesis of current spread cannot be totally ruled out, we assume that the crossed spinal inhibition is modulated by descending inputs from both hemispheres, and the 1-ms delay suggests distinct descending pathways. However, in cats, the reverse has been shown, and it is impossible to argue in humans using indirect measurements.

The main message to retain is that we could only reveal that corticospinal inputs depressed the crossed inhibition. In animals, neural transmission involving commissural interneurons can be both depressed and enhanced by corticospinal inputs (Stecina et al. 2008). Testing ISI longer could have helped to reveal reinforcement of crossed inhibition by the motor cortex. But, on one hand, this would have left enough time for the activation of a transcortical loop by the test volley. On the other hand, it is not enough for possible interaction between corticospinal and conditioning group I and group II inputs. The mechanism underlying the corticospinal depression of crossed action is unclear, either involving control of commissural interneuron excitability and/or activation of other interneurons supplanting the crossed inhibition (Stecina et al. 2008).

Modulation During Motor Tasks

The early phase of the crossed inhibition, likely mediated by group I afferents, did not change according to the motor tasks,
while the late phase, likely mediated by group II afferents, was depressed during voluntary movement, compared with quiet sitting, especially during the walking stance phase, when cSol was activated.

When investigating the various motor tasks, the intensity of the right PTN stimuli had to be changed to evoke test cSol H-reflexes of similar size (%Mmax) across conditions. On average, the test H-reflex size was between 20 and 35% Mmax. It has been shown that changes in this range do not influence the effect of conditioning stimuli (Crone et al. 1990). Moreover, it is difficult to determine how the changes in stimulation intensity had influenced the afferent volley in the right PTN, given the joint position changes and the resulting changes in volume conductor, but this possibility has to be raised. To our knowledge, the contralateral afferent inputs (from the right side) do not interact with commissural interneurons (located in the left spinal cord). On the other hand, the afferent inputs from the right soleus may have influenced the spinal excitability on the right side, which has, in turn, influenced the crossed inhibition, especially at long ISI based on our results. At long ISI, recurrent inhibition occurred, but it has been shown enhanced at this time in the gait cycle (Lamy et al. 2008). Spinal group II excitation could also manifest, and it has been found particularly enhanced during walking (Marchand-Pauvert and Nielsen 2002; Mazzaro et al. 2006). The net result at motoneuron level might have been equal and might have had low influence on crossed inhibition. Moreover, if these mechanisms had interfered with crossed inhibition, we should have observed changes during quiet standing as well, because the right PTN intensity had to be particularly increased during quiet standing to evoke a test H-reflex of 20–30% Mmax due to increased presynaptic inhibition (Mynark et al. 1997).

We, therefore, assume that the modifications of crossed inhibition are task dependent. We found the crossed inhibition depressed during voluntary movement, compared with quiet sitting, especially during walking. This result further supports that descending inputs, whether produced voluntarily or artificially using TMS, depressed the crossed inhibition. However, during movement, we observed a specific depression of group II inhibition, which corroborates the results of our TMS experiments, showing that the depression of group II crossed inhibition, by descending inputs, seemed to be more marked than that produced by group I. This might be related to the higher sensitivity of spinal group II inputs to neuromodulation by monoamines (midbrain structures relaying motor cortex outflow). Indeed, excitatory spinal group II inputs at cervical and lumbar levels have been particularly depressed by α2-noradrenergic agonists, while group I inputs were unchanged (Corna et al. 1995; Lourenço et al. 2006; Marque et al. 2004; Maupas et al. 2004; Rémy-Néris et al. 2003). Therefore, while group I and group II commissural interneurons can receive common descending inputs, it appears that the two subpopulations can be controlled differentially rather than being coactivated during voluntary movement, as suggested in cats (Jankowska et al. 2005).

The group II crossed inhibition was abolished during the walking stance phase, when cSol was activated. This result further supports the hypothesis that group II afferent inputs are particularly involved in the control of spinal excitability during stabilized walking in humans (Marchand-Pauvert and Nielsen 2002; Mazzaro et al. 2006). As in cats (Matsuyama et al. 2004), the neural activity at the level of commissural interneurons is modulated during locomotion in humans, and the fact that group II crossed action was particularly depressed during walking suggests possible monoaminergic control from midbrain structures, analogous to the mesencephalic locomotor region.

Possible Mechanisms and Functional Implications

Group II crossed inhibition was depressed during tonic voluntary contraction and when subjects were standing up. In the standing position, the balance was not particularly challenged, because the subjects were asked to stand on both legs with a support polygon enough to limit body sway. The depression was stronger, leading to its abolition, during the walking stance phase when cSol was activated and iSol was silent. Crossed inhibition was thus tested during the single-support phase, when the balance is particularly challenged. Given the powerful vestibulospinal control onto commissural interneurons (Jankowska 2008; Krutki et al. 2003), the further depression of crossed action during walking could be due to vestibulospinal inputs to assist the upright posture during locomotion. However, at comfortable speed, vestibulospinal control is less than during slow or fast speed (Hirasaki et al. 1999; Jahn et al. 2000). Given the powerful cortical control of ankle muscles during walking (Petersen et al. 1998, 2001), it might be possible that the activation of midbrain structures by primary motor cortex contributes to the depression of group II crossed action. Alternatively, the depression occurred when the opposite leg was in the swing phase and soleus was stretched. The resulting afferent inputs, and those from other muscle groups of both legs (see the muscle activity on the same leg during walking compared with the other motor tasks in Fig. 2), likely contributed to the modulation of spinal interneurons and changes in spinal excitability, possibly by activating excitatory pathways counteracting the crossed inhibition. We did not observe a reversal of crossed inhibition in our study, but such effect has recently been observed during walking (Gervasio et al. 2013). Whatever the mechanism of control, our results further support that spinal neural networks interact for interlimb coordination, especially during walking.

Conclusions

The present study has shown that crossed action to contralateral motoneurons in humans is mediated through oligosynaptic spinal pathway involving commissural interneurons, likely controlled by group I and group II muscles afferents. We did not reveal any excitation to contralateral motoneurons, which suggests that the main crossed spinal action in humans is inhibitory, as in cats (Arya et al. 1991). We also showed that crossed inhibition is depressed by bilateral descending inputs from motor cortex. These findings support the idea that spinal pathways contribute to interlimb coordination during walking in humans. In cat spinal cord, two subsets of commissural interneurons have been identified, which are activated by group I and group II afferents (Jankowska 2008). In humans, it is difficult to definitively determine the pathways mediating group I and group II crossed inhibition using indirect electrophysiological tools. However, the present study provides support for a similar organization in human spinal cord, given that crossed inhibition was differentially modulated according to motor tasks and when testing artificial corticospinal inputs.

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Future research is needed to confirm these connections and explore their possible applicability.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


