Abnormal absences of effects of contralateral group I muscle afferents on presynaptic inhibition of Ia terminals in humans and cats

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Submitted 9 September 2011; accepted in final form 1 June 2012

Crossed actions between the right and left limbs via spinal pathways following afferent stimulation have been well documented since the work of Sherrington (1910). The somatosen-sory influences from both ipsilateral and contralateral origin have a significant relevance to the modulation of reflex responses and the shaping of locomotor patterns (Collins et al. 1993; Dietz et al. 2003; Duysens et al. 1991). The study of crossed effects from a variety of sources is useful to understand the functional aspects of motor control involved, for example, in interlimb coordination during locomotion (Haridas and Zehr 2003; Mezzarane et al. 2011).

Cat experiments allow the investigation of specific contralateral actions from different classes of afferents (Baxendale and Rosenberg 1976, 1977; Eccles et al. 1964; Rosenberg 1970). More recently, experiments performed in both humans and cats have provided evidence of prominent crossed actions from group II muscle (Corna et al. 1996; Edgley et al. 2003) and cutaneous (Aggelopoulos and Edgley 1995; Edgley and Port 1995; Zehr et al. 2001) afferents.

Interestingly, data from animal preparations showed that contralateral group I afferents present a weak direct influence upon the ipsilateral motor nucleus (Harrison and Zytnicki 1984). Subsequent studies indicate that these afferents exert their contralateral influences mainly via commissural interneurons located in laminae VI–VII that synapse on both motoneurons and premotor interneurons (Jankowska et al. 2009), implying the existence of both direct and indirect (via interneurons) crossed actions. Although the absence of effects from contralateral group I afferent stimulation on dorsal root potential in the cat was reported previously (Devanandan et al. 1965), it should be stressed that none of the previous work in cats employed direct measurements of these crossed effects on presynaptic inhibition (PSI).

Indirect segmental effects from contralateral group I muscle spindle afferents in both upper and lower limbs of human subjects have been observed through conditioning of the H-reflex. For instance, electric stimulation of contralateral group I afferents (Ia fibers from extensor carpi radialis (ECR)) changed the reciprocal inhibition from ECR to the flexor carpi radialis muscle (Delwaide and Pepin 1991). In lower limb experiments, a presynaptic mechanism has been proposed to explain the observed soleus (SO) H-reflex inhibition in response to activation of contralateral group I muscle afferent by either passive leg pedaling movements (Cheng et al. 1998) or mechanical Achilles tendon stimulation (Koceja and Kamen 1992). It is important to point out that these experiments did not employ any direct evaluation of PSI onto Ia terminals.

Therefore, indirect evidence in the human suggests the possibility that contralateral group I afferents exert influence on the mechanisms subserving monosynaptic reflex (MSR) gain control. PSI of Ia terminals could be a potential target for such crossed effects, as it modulates reflex actions in different motor contexts (Rudomin and Schmidt 1999).
ABSENCE OF CONTRALATERAL EFFECTS ON PRESYNAPTIC INHIBITION

meet the requirements of a variety of motor tasks, the PSI onto Ia terminals can be altered by ipsi- or contralateral peripheral input and descending pathways, in both humans (Hultborn et al. 1987a; Mezzarane and Kohn 2002; Roby-Brami and Bussel 1990) and cats (Gossard and Rossignol 1990; Quevedo et al. 1995).

While several studies have reported weak contralateral influence from group I activation to the ankle extensors in the cat (Harrison and Zytnicki 1984; Holmqvist 1961; Perl 1958), significant crossed effects in human lower limbs have generally been ascribed to group I activation (Cheng et al. 1998; Koceja and Kamen 1992; Stubbs and Mrachacz-Kersting 2009) among other possible influences (e.g., cutaneous and muscle group II) (Iles 1996; Stubbs et al. 2011). However, the crossed effects from contralateral group I activation onto PSI of Ia MSRs remain to be addressed. Hence, in view of the recent human data, it appears worthwhile to reopen the question put forward by Devanandan et al. (1965) and conduct complementary experiments in both cats and humans.

The combination of human and cat experiments can provide a broader view of the neuronal processes within the spinal cord involved in motor control (see, e.g., Hultborn et al. 1987a). This approach was utilized in the present work. To examine whether a presynaptic mechanism mediates crossed influences from group I afferents in humans, the PSI onto SO Ia terminals was condition with a vibratory stimulus applied to the contralateral triceps surae tendon. In the cat, two different protocols were used: 1) stimulation of either ipsilateral or contralateral afferents from posterior biceps and semitendinous (PBS) as a conditioning stimulus for the MSR elicited in the ipsilateral gastrocnemius/soleus muscle (GS) and 2) vibratory stimulus applied to the contralateral tendon of the GS muscle as conditioning for ipsilateral PSI. This last protocol was the same as used in humans. These experimental protocols were expected to reveal a possible presynaptic reflex modulation in response to contralateral group I activation in both species.

METHODS

Human Experiments

Subjects. Eleven subjects (8 men and 3 women) aged 30.27 ± 3.6 yr (mean ± SD) volunteered for the experiments. The protocol was approved by the local ethics committee according to the Declaration of Helsinki. None of the subjects had any history of neurological disorders. Subjects were seated in an armchair with ankle, knee, and hip angles at −90°.

Data acquisition and stimulation. Surface electrodes (Ag/AgCl with 0.8-cm diameter) were placed bilaterally on the belly of the SO and tibialis anterior (TA) muscles of the right (ipsilateral) leg, with an interelectrode distance of 2 cm. The skin was prepared for electrode placement with an abrasive solution. To obtain the H-reflex of the SO muscle, an electrical rectangular pulse (1-ms duration) was delivered to the ipsilateral posterior talib nerve (PTN) at the popliteal fossa. The reference amplitude of the H-reflex ranged between 10% and 30% of the maximal direct SO muscle response (Mmax) (Crone et al. 1990).

The presence of a constant M-wave in the recordings indicated constant stimulus efficacy to the PTN. However, 3 of 11 subjects did not present an M-wave accompanying the H-reflex within the range of 10–30% Mmax. Therefore, the stimulus efficacy test for these subjects was achieved by applying between trials an electrical stimulus to the PTN that evoked an M-wave amplitude of 10% Mmax in the SO muscle. The M-wave amplitude did not change across the trials (for the same stimulus intensity); therefore stimulus efficacy was assumed to be constant.

The H-reflex was conditioned by an electrical stimulus (1-ms duration) applied to the ipsilateral common peroneal nerve (CPN), using a bipolar electrode placed (2 cm apart) at the neck of the fibula to assess the level of PSI. A conditioning-test (C-T) interval of 100 ms (Iles 1996) was selected, and the conditioning stimulus intensity was 1.0 × motor threshold (MT) of the TA (stimuli at 0.9 × MT were also employed in a separate series, see below). Activation of Ia afferents from the TA muscle was assessed before the beginning of the experiment for those subjects who did not show a detectable H-reflex in the TA muscle in a relaxed state, the stimulus effectiveness at the CPN was confirmed by the presence of an H-reflex during contraction.

To examine whether the induced PSI was not the result of cutaneous afferent stimulation, the conditioning stimulus electrode was moved 2–3 cm distally from the original position on the CPN in two subjects. As previously found (Mezzarane and Kohn 2007), no reduction in the conditioned H-reflex amplitude was observed.

Procedures. The PSI pathway (from the CPN to SO Ia afferents) was conditioned by a brief sinusoidal vibration (3 cycles at 180 Hz) applied to the Achilles tendon of the contralateral (left) leg with a vibratory device (mini-shaker type 4810, Bruel & Kjær). The intensity of the tendon vibration corresponded to the maximal output of the mini-shaker amplifier and was maintained constant across the trial (see below). This stimulus preferentially activates group I afferents (Baxendale and Rosenberg 1976) and has been shown to successfully induce PSI (Hultborn et al. 1987a).

Figure 1 depicts a simplified diagram showing the location and time interval of the stimuli (S1 to S3). This protocol was applied in eight subjects. The H-reflex conditioned by electrical CPN stimulation was called “PSI_control” (Fig. 1; gray traces on Fig. 2A). The H-reflex response elicited by stimulus S1 in Fig. 1 and Fig. 2A conditioned by both a vibratory stimulus to the contralateral Achilles tendon (stimulus S3 in Fig. 1 and Fig. 2A) and an electrical stimulus to the ipsilateral CPN (stimulus S2 in Fig. 1 and 2A) was called “PSI_conditioned” (black traces in Fig. 2A). The negative interstimulus intervals (ISIs) in Fig. 1 indicate that the contralateral vibration (S3) was applied before the electrical stimulus to the CPN (S2). Positive ISIs indicate that the contralateral vibration was applied after the electrical stimulus to the CPN. When the ISI is equal to zero both stimuli were applied simultaneously (see also Fig. 2A). The “*” symbol in Fig. 1 represents the unknown commissural neuronal pathway that mediates excitatory/inhibitory effects on the last-order inhibitory interneuron that establishes synaptic contact on Ia terminals of the ipsilateral side.

The efficacy of the vibratory stimulus (applied to the contralateral leg) to activate the corresponding muscle spindle Ia afferents was assessed by comparing the H-reflex amplitudes of the contralateral leg with and without the vibration applied 600 ms before the stimulus to the PTN in the same leg. Twenty responses were obtained every 10 s before the beginning of the experiment. The first 5 responses from the train of 20 were termed “Control 1” (without any conditioning) and were followed by 5 conditioned responses (“Vibration 1”). To achieve full recovery from vibration, this procedure was repeated one more time (after full recovery) to obtain “Control 2” and “Vibration 2” (see Fig. 2B), totaling 20 responses. A reduced H-reflex amplitude (e.g., due to homosynaptic depression; Cisi and Kohn 2007; Kohn et al. 1997) suggests that the Ia afferents have been effectively recruited by the brief vibratory stimulus. However, a small contribution from group Ib afferents cannot be excluded (Burke et al. 1983).

The ISI between the conditioning vibratory stimulus to the contralateral tendon and the stimulus applied to the ipsilateral CPN (S3–S2) was chosen pseudorandomly from −60 to 60 ms in steps of 10 ms (Fig. 2A). The intervals between the contralateral vibratory stimulus and the test stimulus applied to the PTN (S3–S1) ranged...
from \(/H11002\) fixed at 100 ms. The interstimulus interval (ISI) between S3 and S2 stimuli interval between conditioning (S2) and test (S1) stimuli (C-T interval) was applied and the time course of the experiments. The symbol "?" represents the Fig. 1. Simplified schematic showing the locations where the stimuli were

motoneuron; Ia, muscle Ia afferent; PSI, presynaptic inhibition. vibratory tendon stimulation; In, last-order inhibitory interneuron; Mn, SO stimulus to the ipsilateral common peroneal nerve (CPN); S3: contralateral experiments were done to explore the possibility that the lack of MT to the CPN for two ISIs (\(/H11002\) 40 ms and \(/H11002\) 60 ms in steps of 10 ms (see Fig. 2). S1: stimulus to the posterior tibial nerve (PTN) to obtain soleus (SO) H-reflex; S2: conditioning stimulus to the ipsilateral common peroneal nerve (CPN); S3: contralateral vibratory tendon stimulation; In, last-order inhibitory interneuron; Mn, SO motoneuron; Ia, muscle Ia afferent; PSI, presynaptic inhibition.

from \(-160\) ms to \(-40\) ms (100 ms was subtracted from the ISIs that correspond to the C-T interval between CPN and PTN stimulation).

At the beginning of each trial, five H-reflex responses were elicited every 10 s to bring the central nervous system to a steady state. This procedure was adopted to prevent a possible bias due to a transient change in the spinal cord circuitry. PSI_control and PSI_conditioned responses were obtained for each of the 13 ISIs (gray and black traces, respectively, in Fig. 2A) in a pseudorandom alternated fashion. The H-reflex conditioned by CPN stimulation alone (PSI_control) was then compared with the H-reflex conditioned by both CPN stimulation and mechanical stimulus (i.e., PSI_conditioned). In addition, at the end of each trial (after the delivery of the control and conditioned stimuli in all 13 ISIs), five H-reflex responses without any conditioning stimulus (electrical or mechanical) were obtained (this was called “Control”) to check for PSI efficacy.

An interval of 10 s was used between consecutive H-reflex responses to minimize the effects of homosynaptic depression (Kohn et al. 1997). Each trial was repeated 10 times, resulting in 260 H-reflex responses (10 PSI_control and 10 PSI_conditioned by vibration for each of the 13 ISIs). Each experiment lasted \(\sim 2\) h. Subjects were allowed to relax between each 6-min trial (to sprawl, move the head, and stretch the arms, back, etc.) as long as needed.

The protocol described above was repeated at a later date in five subjects with conditioning stimulus intensities of 1.0 \(\times\) MT and 0.9 \(\times\) MT to the CPN for two ISIs (\(-40\) ms and \(-30\) ms). These extra experiments were done to explore the possibility that the lack of crossed effects would be due to an already saturated presynaptic inhibitory pathway. In these experiments a different vibration device (Labworks model LW-126-13) was chosen because it was easier to quantify the movement of its tip with an inbuilt accelerometer (see below).

To verify the variability of the mechanical stimulus, an experiment focusing on the mini-shaker tip displacement was performed. The stimulus intensity (corresponding to the maximal output of the mini-shaker amplifier) was measured by the displacement of the mini-shaker tip when in contact with the tendon (~0.6 mm). In this experiment, the displacement of the mini-shaker tip was measured by a kinematic analysis system (Optotrak Certus, Northern Digital) that detected the movement of an active optical marker attached to the tip at a sample frequency of 800 Hz. To assess the consistency of the mini-shaker tip displacement, the movement during 28 vibratory stimuli applied to the tendon with an interval of 1 s was recorded. The same procedure could not be adopted to evaluate the displacement during a complete trial because of the overheating of the active markers. In these experiments, an accelerometer (ADXL193; Analog Devices) was attached to the main cylinder located inside the armature of the shaker (LW-126-13) whose tip remained in contact with the contralateral triceps surae tendon (similar procedure was employed by Fornari and Kohn 2008). The consistency of mechanical stimulation could be assessed throughout the trial, as acceleration directly corresponds to displacement.

Signal processing and data analysis. The EMG signals were amplified and filtered (10 Hz to 1 kHz) by a MEB 4200 system (Nihon-Kohden). The signals were fed into the PC-based acquisition and processing system WorkBench (DataWave Technologies) that sampled each signal at 2,500 Hz. Two independent stimulators (of the MEB 4200 system) delivered the electrical stimuli, triggered by the PC-based signal acquisition system. This system also triggered the vibratory stimulus at the appropriate timings. The resulting data files in ASCII were processed by programs written in MATLAB (MathWorks).

To ensure that the induced PSI was effective, the last five responses of a given trial (the “Control” response without any conditioning, either electrical or mechanical, described in Procedures) were compared to the PSI_control (reflex responses conditioned by only CPN stimulus). An average of all 13 values (corresponding to the 13 ISIs) of PSI_control was computed for each trial and compared to the average of 5 Control responses obtained in the respective trial. The average of all PSI_control and Control reflexes, evaluated for all 10 repetitions (dashed and solid traces, respectively, in Fig. 3A), was estimated for each subject to calculate the overall PSI effect shown in Fig. 3B.

Cat Experiments

Preparation. Experiments were performed on 17 adult cats (weight range 2.2–4.0 kg) initially anesthetized with pentobarbital (35 mg/kg ip). Blood pressure was monitored through the carotid artery. The left radial vein was also cannulated to administer additional doses (10 mg/kg) of pentobarbital to maintain deep anesthesia. Guidelines contained in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (85-23, revised 1985) were strictly followed.

The lumbo-sacral and low thoracic spinal segments were exposed, and the dura mater was removed. After the surgical procedures, the animal was restrained in a stereotaxic apparatus with spinal and pelvic clamps. L5–L7 ipsilateral and contralateral ventral roots were dissected and sectioned. Pools were formed with the skin around the exposed tissues, filled with mineral oil (after placement of the electrodes), and maintained at a constant temperature (37°C). Blood pressure was continuously monitored and maintained at 100–120 mmHg.

J Neurophysiol • doi:10.1152/jn.00831.2011 • www.jn.org
Stimulation. In a group of 12 animals contralateral and ipsilateral GS afferents were stimulated with single pulses of 1.2–1.4 times the threshold level of afferent volleys recorded on the cord dorsum (Fig. 4A). Conditioning stimuli (3 electrical pulses at 100 Hz; Enriquez-Denton et al. 2004) were applied to ipsi- or contralateral PBSt afferents (Fig. 4A). Figure 4, B and C, right, show the ipsilateral GS-MSR conditioned by stimulation to both the ipsilateral and contralateral PBSt nerves, respectively. C-T intervals from 0 to 120 ms were analyzed. The frequency of stimulation was adjusted to 0.5 Hz. A Master-8 system (and TTL pulses) was used to produce simultaneous pulses of stimulation. In a group of five animals a brief conditioning vibration (3 cycles at 180 Hz with a Chubbuck mechanical stimulator transducer) was applied to the contralateral GS tendon in order to activate the corresponding muscle Ia afferents (see Fig. 5). Conditioning ipsilateral PBSt stimulation consisted of a train of 3 pulses at 100 Hz (Enriquez-Denton et al. 2004). The ISIs between contralateral tendon vibration and ipsilateral PBSt stimulus varied from −60 to +60 ms. Negative ISIs indicate that the vibratory stimulus was delivered before the conditioning electrical stimulus to the PBSt nerve (to induce PSI on the ipsilateral side). These negative ISIs were considered for statistical analysis. This procedure was analogous to that implemented for the human experiments. The C-T interval between the electrical stimulus to the ipsilateral PBSt and GS nerves was fixed at 25 ms.

Electrophysiological recordings. Bilateral MSRs were recorded simultaneously from proximal L6 ventral roots. When a stimulus was applied to the ipsilateral GS nerve no reflexes were evoked in the contralateral ventral root. Bilaterally evoked afferent volleys and spontaneous cord dorsum potentials were recorded at L6 by using two silver ball electrodes placed on the cord dorsum. Another electrode was inserted in the back muscles as a reference. Low-noise and high-gain differential amplifiers (Grass model P511) were used to amplify the potentials.

Statistical Analyses
A two-tailed paired t-test was used to detect the PSI effect in humans by comparing H-reflex of SO with (PSI_control) and without (Control) conditioning by ipsilateral CPN stimulation. In the cat, the
PSI effect was detected by comparing the MSR of GS with and without conditioning by either ipsilateral or contralateral PBSt stimulation. The same test was used to detect possible differential effects of contralateral tendon vibration on reflex responses conditioned by ipsilateral PSI across the 13 ISIs in humans and 6 ISIs in cats. The statistical package SPSS (v. 16.0) was used to perform the analyses. A significance level was set as being lower than 5%.

RESULTS

Human Experiments

Figure 2A shows typical records obtained from one subject during an experiment. The black traces are the EMG recorded from the SO muscle representing the PSI_conditioned responses (mean of 10 repetitions), i.e., the H-reflexes conditioned by both S2 (ipsilateral CPN electrical pulse applied 100 ms before the PTN) and S3 (contralateral tendon vibration at the 13 ISIs) stimuli. The gray traces in Fig. 2A, right, represent the averaged reflex responses with no contralateral vibration (PSI_control).

The amplitudes of these responses (PSI_conditioned and PSI_control), which look very similar to each other as in the example of Fig. 2A, were compared as previously described. Indeed, considering all subjects, there were no significant differences between PSI_control and PSI_conditioned amplitudes for each of the 13 ISIs (Table 1). These results indicate an absence of effect from contralateral vibration on the ipsilateral PSI, i.e., there is no effect from the contralateral Ia afferents on the PSI of the ipsilateral Ia SO terminals in relaxed humans. The difference between PSI conditions are of near-significance at ISIs of −30 ms and 20 ms (P = 0.056 and P = 0.062, respectively). However, the P values at neighboring ISI values were not marginal (0.98/0.52 and 0.46/0.88, respectively), indicating absence of a possible physiologically significant difference between conditions. Additionally, the repetition of the experiment for ISIs of −30 ms and −40 ms (Fig. 3C) confirmed the lack of effects at these latencies (see below).

Figure 6A shows EMG recordings from the SO muscle in one subject. The dotted, solid, and dashed lines represent the averaged Control, PSI_control, and PSI_conditioned reflexes, respectively. There is a clear effect of the CPN conditioning in this subject. Figure 6B demonstrates the same effect in all subjects. Both individual and overall data show an absence of differences (P > 0.05) between PSI_control and PSI_conditioned for the ISIs depicted (Fig. 6).

The comparison between PSI_control and the H-reflex amplitudes obtained at the end of each trial (Control) for all subjects showed that the overall effect from CPN stimulation was significant (P < 0.05) (Fig. 3, A and B). The conditioned H-reflex decreased by ~40% from its control value (Fig. 3B).

Conditioning stimuli. To examine the possibility that the induced level of PSI by CPN conditioning was saturated (i.e., reached a maximum value), the experiment was repeated in five human subjects with two different conditioning intensities to the CPN (1.0 × MT and 0.9 × MT) at 2 ISIs (−40 ms and −30 ms) (Fig. 3C). The results presented in Fig. 3C show a lack of contralateral effects at both conditioning intensities. Moreover, the 0.9 × MT conditioning stimulus effect was less than that observed with 1.0 × MT. These findings suggest that the absence of crossed effects is not a result of saturation in the inhibitory pathway.

An H-reflex was evoked in the contralateral leg 600 ms after the vibration to establish whether the conditioning mechanical stimulus used in the present experiments was enough to activate Ia afferents. The traces in Fig. 2B, left, are the raw data from one representative subject showing the 20 reflex responses elicited within 10-s intervals. The aver-

\[ \text{A} \]
\[ \text{B} \]
\[ \text{C} \]
age peak-to-peak reflex responses conditioned by the mechanical stimulus are presented in Fig. 2B, right. The depression was significant for all subjects tested (P < 0.001). A full recovery of reflex amplitude after the 10-s interval (compare Vibration 1 with Control 2) is evident in the bar graph. The overall reflex depression in response to vibration was 47%.

The mechanical stimuli (tendon vibration with 3 cycles at 180 Hz) were very consistent along the experiment. The mean ± SD displacement of the tip of the mini-shaker in contact with the tendon in response to the application of 28 stimuli (evoked at 1-s intervals) was 0.56 ± 0.008 mm, yielding a very small coefficient of variation (CV = 1.4%). Experiments with the second shaker system (adjusted to give similar tip displacements) also yielded a very small CV (3.5%), with a measured acceleration of 25 g (where g is the acceleration of gravity). This value corresponded to a displacement of ~0.7 mm (Hultborn et al. 1987a).

**Cat Experiments**

Figure 4B shows that the conditioning stimulation (around 10 ms of C-T time interval) to the ipsilateral PBSt nerve was associated with a statistically significant reduction (P < 0.05, t-test) in amplitude of the GS-MSR. On the other hand, no change was observed in GS-MSR amplitude when the conditioning stimulus was applied to the contralateral PBSt nerve (Fig. 4C). Similar results were obtained when the sides were switched, i.e., when the test reflex was recorded from the contralateral GS (not shown).

The open circles in Fig. 7 illustrate measurements of GS-MSR amplitudes for one cat for all values of C-T time intervals described in METHODS, but similar results were obtained in the other 11 cats. The filled circles in Fig. 7 show the GS-MSR mean values from the 12 cats, conditioned by either ipsilateral (Fig. 7, top) or contralateral (Fig. 7, bottom) stimuli to the PBSt nerve at 20 ms of C-T time interval. There was a significant decrease (P < 0.05, 12 cats) in the mean GS-MSR amplitude when the conditioning stimulus was applied to the ipsilateral PBSt nerve (Fig. 7, top). Conversely, there was no detectable change (P > 0.05, 12 cats) in the GS-MSR amplitude when the conditioning stimulus was applied to the contralateral PBSt nerve (Fig. 7, bottom).

The experiments using conditioning vibratory stimulation of the contralateral tendon in the cat, as illustrated in Fig. 5, yielded results similar to those obtained in humans. The pooled data for five animals are illustrated in Fig. 8. The filled bars in Fig. 8 show the mean amplitude of GS-MSRs under PSI of the ipsilateral PBSt afferents (PSI_control). The open bars show the mean amplitude of GS-MSRs during both electrical stimulation of ipsilateral PBSt and contralateral tendon vibration (i.e., activation of contralateral group Ia afferents) (PSI_conditioned). Figure 8 also shows the effects of this contralateral conditioning stimulation to the tendon for different ISIs (from −60 to 0 ms). In summary, the conditioning vibratory stimulation (S3) of the contralateral group Ia afferents was not associated with significant (P > 0.5) changes in amplitude of the GS-MSR (S1) subjected to PSI by the ipsilateral PBSt afferents (S2).

**DISCUSSION**

CROSSED INFLUENCES FROM AFFERENT INPUTS OF THE CONTRALATERAL LIMB CAN ADJUST THE EXCITABILITY OF REFLEX PATHWAYS VIA PRE- AND POSTSYNAPTIC MECHANISMS

CROSSED EFFECTS OF GROUP I AFFERENTS FROM THE CONTRALATERAL SO NERVE ON IPSILATERAL GS-MSRS

Evidence for Group I Afferents in Mediating Crossed Effects

In general, crossed reflexes have been extensively studied in humans and animal preparations. The current view is that...
Fig. 5. A: diagram of the experimental arrangement to explore the effects of contralateral group I afferents on PSI of the GS-MSR. Conditioning stretching stimulation (S3) was applied on the contralateral GS tendon (3 vibratory cycles). Conditioning electrical stimulation (S2) was applied on the ipsilateral PBSt nerve (3 electrical pulses), and the test stimulus (S1) was applied on the ipsilateral GS nerve (1 electrical pulse). The symbol “?” represents the unknown commissural pathway(s) that could mediate crossed actions. B: the stimulation sequence was similar to the stimulation illustrated in Fig. 1, but with C-T interval (between S2 and S1) of 25 ms instead of 100 ms used in the human experiments.

Table 1. Percentage of inhibition of H-responses conditioned by both stimuli (S2 and S3) and by CPN stimulation (S2)

<table>
<thead>
<tr>
<th>ISI, ms</th>
<th>-60</th>
<th>-50</th>
<th>-40</th>
<th>-30</th>
<th>-20</th>
<th>-10</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>% PSL_conditioned</td>
<td>42</td>
<td>35</td>
<td>32</td>
<td>29</td>
<td>31</td>
<td>33</td>
<td>30</td>
<td>32</td>
<td>35</td>
<td>35</td>
<td>32</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>% PSL_control</td>
<td>44</td>
<td>31</td>
<td>32</td>
<td>38</td>
<td>34</td>
<td>32</td>
<td>33</td>
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<td>31</td>
<td>35</td>
<td>35</td>
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<td>35</td>
</tr>
<tr>
<td>P values</td>
<td>0.876</td>
<td>0.210</td>
<td>0.982</td>
<td>0.056</td>
<td>0.519</td>
<td>0.654</td>
<td>0.458</td>
<td>0.460</td>
<td>0.062</td>
<td>0.885</td>
<td>0.621</td>
<td>0.856</td>
<td>0.398</td>
</tr>
</tbody>
</table>

Values are % of inhibition of the H-responses conditioned by both stimuli (S2 and S3), termed PSL_conditioned, and by the common peroneal nerve (CPN) stimulation (S2), termed PSL_control. P values are from the 2-tailed paired Student’s t-test. This test was used to detect differences between PSL_conditioned and PSL_control for all 13 interstimulus intervals (ISIs) between stimulus S3 (tendon vibration) and S2 (electrical pulse to the CPN).

See the article for further explanation of the data presented in the table.
also be contralaterally activated (e.g., cutaneous and group II) (Iles 1996; Stubbs et al. 2011); hence the crossed effects would not be only associated with contralateral Ia afferents. Even though Devanandan et al. (1965) anticipated an absence of postsynaptic influence from contralateral Ia afferents onto motoneurons, later studies documented changes in excitability of the Ia inhibitory interneuron in response to contralateral group I stimulation in cat hindlimbs (Harrison and Zytnicki 1984) and in the upper limbs of humans (Delwaide and Pepin 1991). With respect to the latter study, one can speculate that Ia inhibitory interneurons could mediate these crossed influences in human lower limbs as well. Nevertheless, any comparison of crossed effects on reciprocal inhibition (or PSI) between upper and lower limbs would be speculative because of the lack of available data.

Altogether, these findings suggest that the influence of contralateral group I afferents onto interneurons involved in PSI of Ia terminals is feeble or absent. Reciprocal inhibitory pathways (postsynaptic action) might be relevant in the mediation of these crossed effects.

It is important to mention that, despite the very low intensity of vibratory stimulus, a recruitment of cutaneous afferents cannot be fully discarded in the present experiments in humans. A significant crossed effect on PSI from contralateral cutaneous activation has been reported (Iles 1996). One may argue that the train of four electrical shocks (1.5 × perceptual threshold) used by Iles (1996) is probably much more effective in firing cutaneous afferents than the vibration applied to the skin over the Achilles tendon as used in the present research. Thus putative cutaneous crossed effects should be expected to be of a lesser magnitude here than in Iles’s (1996) experiments.

Even considering the possibility of the interference from low-threshold cutaneous afferents (Hultborn et al. 1987a; Perl 1957), it is very unlikely that a cutaneous effect would counteract any possible group I crossed effect for all the ISIs used in the present study. The complementary experiments in the cat pathways (postsynaptic action) might be relevant in the mediation of these crossed effects.
According to the motor task (Liu et al. 2010), no morphological interneurons (with or without crossed axonal projections) suggested to be a mechanism to select intermediate zoneeral side. However, while PSI on group I afferents has been effects from group I afferents and modulate PSI in the ipsilateral laminae VI–VII (Bannatyne et al. 2009; Jankowska et al. 2009). These bilaterally projecting interneurons are of special interest, as one may speculate that they form (which produced the same results as in humans) support this hypothesis since the conditioning electrical stimulation was applied directly on group I PBSt nerves (without the participation of cutaneous afferents) and the vibratory conditioning was applied exclusively to the isolated tendon (without the skin).

**Possible Commissural Interneurons Conveying Crossed Effects**

Commissural interneurons that convey the signals from contralateral synaptic inputs are not homogeneous, and their characterization is complex (Edgley and Aggelopoulos 2006; Jankowska et al. 2005, 2009; Jankowska and Edgley 2010).

An important target for monosynaptic input from group I afferents are the commissural interneurons located within laminae VI–VII (Bannatyne et al. 2009; Jankowska et al. 2009). Among these intermediate zone interneurons, those that project to contralateral motor nuclei have only contralateral axons and those that project bilaterally send axons to areas located outside the motor nuclei, indicative of targets other than motoneurons (Jankowska et al. 2009). These bilaterally projecting interneurons are of special interest, as one may speculate that they form contact with interneurons interposed in presynaptic inhibitory pathways, i.e., they also project to ipsilateral lamina VI, where the occurrence of GABAergic presynaptic synapses on Ia terminals has been reported (Maxwell et al. 1990) and where few GABAergic interneurons (probably primary afferent depolarization mediated) have been found (Bannatyne et al. 2009).

Therefore, commissural interneurons from the contralateral intermediate zone could be strong candidates to convey crossed effects from group I afferents and modulate PSI in the ipsilateral side. However, while PSI on group I afferents has been suggested to be a mechanism to select intermediate zone interneurons (with or without crossed axonal projections) according to the motor task (Liu et al. 2010), no morphological study has provided evidence of synaptic connections between these commissural neurons and ipsilateral last-order presynaptic inhibitory interneurons. Previous studies were also unable to confirm the existence of crossed oligosynaptic (or polysynaptic) pathways leading to modulation of PSI of Ia terminals. Absence of such crossed pathways could explain the present results obtained in humans and cats.

It is important to emphasize, however, that the general pattern of neuronal connectivity within the human spinal cord might be considerably different from that described in animal preparations. Thus, given the relatively long time course of PSI in humans (~100 ms; Hultborn et al. 1987b) and in cats (see Fig. 7), the use of a long range of ISIs in the present study was necessary to unravel possible crossed actions on ipsilateral PSI mediated by crossed pathways.

**Future Directions**

A complementary characterization of group I contralateral influences in humans could come from the analysis of motor units with different synaptic input thresholds within the SO motoneuronal pool. In the present experiments only the earliest recruited motor units were investigated by evoking test reflexes at around 20% M_max. Hence, it is conceivable that contralateral effects could be differentially manifested for a wider range of motor unit types, i.e., there could be an uneven distribution of effects (perhaps task dependent) throughout the pool of motoneurons belonging to the SO motor nucleus (Mezzarane et al. 2011). This possibility remains to be explored by performing an analysis based on a wider spectrum of H-reflex amplitudes.

Additionally, possible crossed effects conveyed by group I afferents from heteronymous muscles onto Ia PSI regulation deserve further investigation.

**Conclusion**

The present results from anesthetized cats and humans in a resting state suggest that the contribution of contralateral group I afferents to reflex modulation via PSI of Ia terminals is minimal or absent. If we can generalize the results obtained here for the specific pathways investigated, one may conclude that 1) the weak crossed effects found in cats in previous studies are not mediated by a presynaptic inhibitory mechanism and 2) if there is a group I crossed influence in humans, it is not mediated by PSI. Therefore, the presynaptic mechanism subserving reflex gain control is not triggered or regulated by contralateral group I afferent activation. One alternative mechanism operational in both species could be a postsynaptic reflex modulation.

**ACKNOWLEDGMENTS**

The authors thank Sandro A. Miqueleti and Fernando H. Magalhães for their invaluable technical help.

**GRANTS**

This work was supported by a CNPq (Brazil) grant to A. F. Kohn and CONACyT Grant 62610, VIEP-BUAP:103, PIFI-FOMES-BUAP, and “Cátedra Marcos Mohinsky” grants to E. Manjarrez (Mexico). R. A. Mezzarane was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (proc. no. 2010/15522-4) (Brazil).

**DISCLOSURES**

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


