Afferent Regulation of Leg Motor Cortex Excitability After Incomplete Spinal Cord Injury

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Submitted 8 October 2009; accepted in final form 18 February 2010

Roy FD, Yang JF, Gorassini MA. Afferent regulation of leg motor cortex excitability after incomplete spinal cord injury. J Neurophysiol 103: 2222–2233, 2010. First published February 24, 2010; doi:10.1152/jn.00903.2009. An incomplete spinal cord injury (SCI) impairs neural conduction along spared ascending sensory pathways to disrupt the control of residual motor movements. To characterize how SCI affects the activation of the motor cortex by spared ascending sensory pathways, we examined how stimulation of leg afferents facilitates the excitability of the motor cortex in subjects with incomplete SCI. Homo- and heteronymous afferents to the tibialis anterior (TA) representation in the motor cortex were electrically stimulated, and the responses were compared with uninjured controls. In addition, we examined if cortical excitability could be transiently increased by repetitively pairing stimulation of spared ascending sensory pathways with transcranial magnetic stimulation (TMS), an intervention termed paired associative stimulation (PAS). In uninjured subjects, activating the tibial nerve at the ankle 45–50 ms before a TMS pulse in a conditioning-test paradigm facilitated the motor-evoked potential (MEP) in the heteronymous TA muscle by twofold on average. In contrast, prior tibial nerve stimulation did not facilitate the TA MEP in individuals with incomplete SCI (n = 8 SCI subjects), even in subjects with less severe injuries. However, we provide evidence that ascending sensory inputs from the homonymous common peroneal nerve (CPN) can, unlike the heteronymous pathways, facilitate the motor cortex to modulate the TA MEP (n = 16 SCI subjects) but only in subjects with less severe injuries. Finally, by repetitively coupling CPN stimulation with coincident TA motor cortex activation during PAS, we show that 7 of 13 SCI subjects produced appreciable (>20%) facilitation of the MEP following the intervention. The increase in corticospinal tract excitability by PAS was transient (<20 min) and tended to be more prevalent in SCI subjects with stronger functional ascending sensory pathways.

INTRODUCTION

Cortical representations of motor and sensory areas are plastic and are continuously modified by experience (Buenomano and Merzenich 1998). In general, sensorimotor areas are allocated based on the proportional use or disuse of a pathway in line with the mechanism of use-dependent plasticity studied in the human motor cortex (Butefisch et al. 2000). A steady stream of sensory input maintains the integrity of cortical networks, while in contrast, alterations in afferent activation promote sensorimotor reorganization (Brasil-Neto et al. 1992; Ziemann et al. 1998; for review see Chen et al. 2002). After an incomplete spinal cord injury (SCI), damage to the ascending and/or descending pathways induces widespread modifications to the sensorimotor system (Curt et al. 2002; Levy et al. 1990; Topka et al. 1991; although see Brouwer and Hopkins-Rosseel 1997). Imaging studies have shown that the activity in the primary motor cortex is reduced shortly after SCI and progressively reappears over the course of motor recovery (Jurkiewicz et al. 2007). With functional recovery of walking, SCI subjects show strengthening of corticospinal projections as seen by an increase in the motor-evoked potential (MEP) elicited using transcranial magnetic stimulation (TMS) (Thomas and Gorassini 2005; Wirth et al. 2008).

Cortical networks supplying leg muscles are markedly facilitated in uninjured individuals by prior stimulation of ascending afferent pathways from homonymous and/or neighboring heteronymous pathways (Deletis et al. 1992; Nielsen et al. 1997; Petersen et al. 1998; Roy and Gorassini 2008). In both upper and lower limbs, sensory-induced MEP facilitation is associated with decreases in the activation of inhibitory intracortical pathways (Rosenkranz and Rothwell 2003) and in the facilitation of excitatory intracortical pathways (Aimonetti and Nielsen 2001; Devanne et al. 2009; Rosenkranz et al. 2003; Roy and Gorassini 2008). Following a complete or nearly complete spinal cord lesion, afferent projections in the dorsal column are impaired. The disruption to the ascending pathways can abolish the sensory-induced MEP facilitation produced in the ankle flexor muscle (Hayes et al. 1992). Although it is likely that the potentiation of cortical circuits by peripheral nerve stimulation is graded according to the severity of the injury, the interaction of homo- and heteronymous afferent inputs on corticospinal circuits has yet to be investigated in SCI subjects having some spared ascending pathways.

Repetitive electrical stimulation of the common peroneal nerve (CPN) in uninjured individuals produces facilitation of the MEP in ankle flexors that can last for ≥1 h (Khaslavaia et al. 2002; Knash et al. 2003). In addition, a recent study has shown that CPN stimulation delivered over the course of several months, via a neuroprosthesis for foot drop, improves walking function and increases corticospinal tract function in subjects having CNS disorders, including multiple sclerosis, stroke, and incomplete SCI (Everaert et al. 2010; Stein et al. 2010). By pairing sensory afferent excitation with a cortical stimulus, it has been possible to accelerate the rate of MEP potentiation in an intervention called paired associative stimulation (PAS) (Stefan et al. 2000). In the lower leg, pairing afferent inputs from the CPN with a TMS pulse increases MEPs in ankle flexors for ≥30 min when the afferent input is timed to arrive at the motor cortex with or shortly after the cortical stimulus (Mrachacz-Kersting et al. 2007; Roy et al. 2007). In the upper limb, this MEP facilitation involves the activation of the N-methyl-D-aspartate (NMDA) receptor (Ste-
fan et al. 2002), reminiscent of long-term potentiation (LTP) in animal experiments (for review, see Dan and Poo 2006) and may give insight into the LTP-like properties of the human motor cortex (Rosenkranz et al. 2007). After stroke, PAS can potentiate MEPs in wrist muscles (Castel-Lacanal et al. 2007, 2009), and there is preliminary evidence that repeated exposure to PAS (daily sessions for 4 wk) increases corticospinal connections to leg muscles (Uy et al. 2003). To our knowledge, changes in corticospinal tract excitability by PAS have not been reported in individuals with an incomplete spinal cord lesion.

In this study, we investigated whether homonymous and/or heteronymous afferent inputs can provide immediate modulation of motor cortex excitability in SCI subjects by conditioning MEP responses with a prior peripheral nerve stimulus. We also examined whether an intervention of PAS, involving repeated pairs of homonymous peripheral nerve and central stimulations, can induce transient increases in cortical excitability in subjects with incomplete SCI. Here we provide evidence that in our population of SCI subjects, afferent inputs from the homonymous, but not the heteronymous, nerve can reach the motor cortex to immediately potentiate the tibialis anterior (TA) MEP, and PAS can transiently (<20 min) increase the resting MEP response in some SCI subjects. Parts of the data from the uninjured controls have been presented in a modified format in Roy and Gorassini (2008).

METHODS

Subjects

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 subjects. Most of the subjects with an incomplete SCI were recruited to participate in locomotor rehabilitation. Experiments involving immediate conditioning of the MEP by sensory inputs were done on a rest day between training sessions, and all PAS experiments were done when the subjects were no longer training. Experiments done in the same subject were conducted ≥5 days apart. Our sample comprised 22 subjects (6 female) with an incomplete SCI (either American Spinal Injury Association (ASIA) Impairment Scale C or D; see Table 1) aged 20–69 (45.3 ± 13.2; mean ± SD; Table 1) and 16 uninjured control subjects (7 females) aged 18–68 (31.8 ± 13.2).

EMG recordings and maximum voluntary contractions

Surface electromyography (EMG) was collected from the TA, soleus, and abductor hallucis muscles using pairs of Ag-AgCl electrodes (Kendall; Chicopec, MA). The signals were amplified (500–2,000 gain), filtered (10–1,000 Hz band-pass; Octopus, Bortec Technologies; Calgary, AB, Canada) and digitized at a rate of 5 kHz using Axoscope hardware and software (Digidata 1200 Series, Axon Instruments, Union City, CA). At the start of each experiment, the maximum voluntary contraction (MVC) was determined by having the subjects generate three maximum isometric contractions (2–3 s in duration) with verbal encouragement from the experimenter. To calculate MVC, the rectified EMG was first smoothed using a 500-ms sliding average. MVC was then quantified as the average maximum activity produced by each of the three EMG bursts.

Transcranial magnetic and peripheral nerve stimulation

TMS was delivered using a Magstim 200 stimulator (Magstim; Dyfed, UK). TMS was applied to the contralateral motor cortex supplying the TA muscle using a double cone coil or a custom-made figure-of-eight barting coil (P/N 15857: 90-mm wing diameter). The coil was chosen based on the severity of the lesion and its ability to elicit MEPs below the injury, with the double cone coil being used in

TABLE 1. Details of subjects with incomplete SCI

<table>
<thead>
<tr>
<th>Code/Sex</th>
<th>Age, yr</th>
<th>Years Post Injury</th>
<th>Cause of Injury</th>
<th>Injury Level</th>
<th>ASIA Score</th>
<th>Side Tested (L, R)</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1M*</td>
<td>56.9</td>
<td>34.2</td>
<td>Trauma</td>
<td>C₉,6</td>
<td>D</td>
<td>L (128.2)</td>
<td>Oxybutynin</td>
</tr>
<tr>
<td>2M*</td>
<td>44.7</td>
<td>4.9</td>
<td>Trauma</td>
<td>C₉,5</td>
<td>C</td>
<td>R (91.1)</td>
<td>Baclofen, gabapentin, oxybutynin</td>
</tr>
<tr>
<td>3M</td>
<td>27.3</td>
<td>1.7</td>
<td>Trauma</td>
<td>C₉,10</td>
<td>C</td>
<td>R (51.5), L (8.2)</td>
<td>Baclofen</td>
</tr>
<tr>
<td>4M*</td>
<td>47.1</td>
<td>23.0</td>
<td>Trauma</td>
<td>C₉,2</td>
<td>C</td>
<td>R (34.6), L (20.6)</td>
<td>None</td>
</tr>
<tr>
<td>5M</td>
<td>45.0</td>
<td>2.4</td>
<td>Trauma</td>
<td>L₂, L₃</td>
<td>D</td>
<td>R (NA), L (NA)</td>
<td>Gabapentin, hydromorphone</td>
</tr>
<tr>
<td>6F</td>
<td>44.2</td>
<td>1.0</td>
<td>Surgical Bleed</td>
<td>Tₑ₂-Tₑ₄</td>
<td>C</td>
<td>R (40.3), L (129.6)</td>
<td>Baclofen</td>
</tr>
<tr>
<td>7F*</td>
<td>48.1</td>
<td>1.3</td>
<td>Trauma</td>
<td>C₉,4</td>
<td>C</td>
<td>R (74.3), L (72.6)</td>
<td>Baclofen, gabapentin, oxybutynin, oxycodone</td>
</tr>
<tr>
<td>8M*</td>
<td>41.7</td>
<td>1.1</td>
<td>Trauma</td>
<td>C₉,4</td>
<td>C</td>
<td>R (74.3), L (72.6)</td>
<td>Baclofen, gabapentin, oxybutynin, oxycodone</td>
</tr>
<tr>
<td>9M*</td>
<td>20.9</td>
<td>1.1</td>
<td>Trauma</td>
<td>C₇</td>
<td>C</td>
<td>R (12.3)</td>
<td>Baclofen, oxybutynin</td>
</tr>
<tr>
<td>10F</td>
<td>69.4</td>
<td>2.5</td>
<td>Surgery</td>
<td>Tₑ₂-L₃</td>
<td>D</td>
<td>R (88.4), L (84.5)</td>
<td>Baclofen, oxycodone</td>
</tr>
<tr>
<td>11M*</td>
<td>63.3</td>
<td>20.0</td>
<td>Trauma</td>
<td>C₉,6</td>
<td>D</td>
<td>R (187.6), L (196.6)</td>
<td>None</td>
</tr>
<tr>
<td>12M*</td>
<td>33.6</td>
<td>1.1</td>
<td>Trauma</td>
<td>C₉,6</td>
<td>C</td>
<td>R (155.3), L (259.9)</td>
<td>Baclofen, pregabalin</td>
</tr>
<tr>
<td>13M</td>
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<td>2.4</td>
<td>Trauma</td>
<td>C₉</td>
<td>D</td>
<td>L (129.0)</td>
<td>Baclofen, pregabalin</td>
</tr>
<tr>
<td>14M</td>
<td>44.1</td>
<td>17.6</td>
<td>Trauma</td>
<td>Tₑ₁₂</td>
<td>D</td>
<td>R (170.6), L (149.6)</td>
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</tr>
<tr>
<td>15M*</td>
<td>25.0</td>
<td>1.0</td>
<td>Trauma</td>
<td>Tₑ₄-L₁₂</td>
<td>C</td>
<td>R (75.5), L (144.5)</td>
<td>Gabapentin</td>
</tr>
<tr>
<td>16F</td>
<td>42.7</td>
<td>1.9</td>
<td>Trauma</td>
<td>Cₑ₆, Tₑ₈</td>
<td>C</td>
<td>R (86.6)</td>
<td>None</td>
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<tr>
<td>17M*</td>
<td>56.8</td>
<td>1.2</td>
<td>Trauma</td>
<td>Cₑ₇, Tₑ₈</td>
<td>C</td>
<td>R (2.2)</td>
<td>Baclofen, dantrium, oxybutynin</td>
</tr>
<tr>
<td>18M*</td>
<td>52.3</td>
<td>2.6</td>
<td>Trauma</td>
<td>Tₑ₈</td>
<td>C</td>
<td>R (180.2)</td>
<td>None</td>
</tr>
<tr>
<td>19M*</td>
<td>41.6</td>
<td>1.6</td>
<td>Trauma</td>
<td>Cₑ₇, Cₑ₈</td>
<td>D</td>
<td>R (268.5)</td>
<td>Baclofen, pregabalin</td>
</tr>
<tr>
<td>20F*</td>
<td>58.5</td>
<td>4.9</td>
<td>Idiopathic transverse myelitis</td>
<td>Tₑ₁₂</td>
<td>D</td>
<td>R (142.4)</td>
<td>Baclofen, gabapentin</td>
</tr>
<tr>
<td>21M</td>
<td>23.4</td>
<td>1.0</td>
<td>Trauma</td>
<td>Tₑ₉</td>
<td>C</td>
<td>R (153.5), L (50.1)</td>
<td>None</td>
</tr>
<tr>
<td>22F</td>
<td>50.1</td>
<td>1.8</td>
<td>Tumor removal</td>
<td>Tₑ₉</td>
<td>D</td>
<td>R (119.2), L (137.5)</td>
<td>Baclofen, oxybutynin</td>
</tr>
</tbody>
</table>

The age of the subject, the number of years after the subject sustained a spinal cord injury (SCI) measured at the time of the experiment, and the cause of the injury are shown. The subject with transverse myelitis displayed scarring only in the spinal cord as assessed by magnetic resonance images. Side tested refers to the right (R) or left (L) tibialis anterior (TA) muscle recorded from and the associated maximum voluntary contraction value for that muscle. Medications: baclofen and dantrium are antispastic agents; gabapentin, hydromorphone, oxycodone, and pregabalin help alleviate pain; oxybutynin and tolterodine are used in the treatment of overactive bladder symptoms. Subjects who participated in the paired associative stimulation experiments are marked (*) in the table.
the more severely injured subjects. In addition, knowing that TMS can induce local pain, headache, discomfort, etc. (see Rossi et al. 2009), we mitigated these effects whenever possible by using the more focal figure-eight batwing coil rather than the diffuse double cone coil. TMS was administered using postero-anterior currents in the brain. The optimal spot to the TA muscle was identified using a low suprathreshold TMS intensity and was marked on the scalp with a felt-tipped pen (1-2 cm lateral of vertex with the batwing coil and often 1 cm more posterior with the double cone). Unless otherwise stated, all responses were collected during a tonic dorsiflexion. For SCI subjects with an appreciable MVC (>30 μV), a tonic contraction corresponding to 15–20% of the MVC was maintained (22.8 ± 1.9 μV on average). SCI subjects with weak volitional control (MVC <30 μV; 4 subjects) maintained a dorsiflexion contraction that was most repeatable (generally >50% of MVC; 8.3 ± 2.7 μV on average). Rectified and heavily smoothed EMG (100-ms time constant) from the TA muscle was displayed with a fast-time sweep on an oscilloscope to help the subjects maintain a steady contraction when needed. Peripheral nerves were electrically stimulated using a constant-current stimulator (DS7A, Digitimer). The tibial nerve (TN) at the ankle and the CPN were stimulated with the cathode below the medial malleole (ISI) between the single pulse nerve stimulus and the TMS pulse was varied from 30 to 80 ms (10-ms increments). Peripheral nerve stimuli were always delivered before the TMS pulse.

The effect of homonymous inputs from the CPN on the TA MEP was evaluated in 16 SCI subjects (data from 16 legs) at ISIs from 30 to 80 ms. The CPN was activated at 1.2 × MT (1-ms pulse) because higher stimulation intensities can produce excessive spinal inhibition. For comparison, four uninjured controls (data from 4 legs) were tested at ISIs ranging from 30 to 100 ms. A minimum of 6 conditioned responses were collected at each interval, intermixed with a minimum 12 single-pulse test responses. Stimuli were randomly delivered every 5–6 s.

**PAS**

Because afferent inputs from the CPN in incomplete SCI subjects can reach the motor cortex to potentiate the MEP (Fig. 2C) and repeated CPN stimulation paired with TMS can facilitate TA MEPs in uninjured controls (Mrachacz-Kersting et al. 2007; Roy et al. 2007; Stein et al. 2009; Stinear and Hornby 2005), we examined whether PAS can modify corticospinal excitability to the TA muscle after incomplete SCI. Thirteen SCI subjects were recruited, and two interventions (PAS or PAS-sham described in the following text) were tested with 12 of these subjects also participating in the CPN afferent conditioning experiments. The order of interventions (PAS or PAS-sham) was randomly delivered across subjects with 7 of the 13 subjects first receiving the PAS intervention. Only one leg was studied per subject. Baseline MEPs were collected at rest because PAS-induced aftereffects are more prominent in the relaxed compared with a contracted muscle (Ridding and Taylor 2001; Roy et al. 2007; Stefan et al. 2000; Stein et al. 2009). Resting MEPs were collected using either a double cone coil set to 80% MSO (maximum tolerable) in subjects where appreciable resting MEPs >50 μV could not be produced or using the batwing coil at a TMS intensity that produced a peak-to-peak MEP of 0.1–0.2 mV (i.e., a sizeable response in many SCI subjects). In one subject, who could only elicit a flicker of voluntary activation (subject 17M), resting MEPs were collected when the muscle was quiet, but immediately after trying to flex the ankle. Baseline MEPs were also recorded during a background contraction (active MEP) with a TMS intensity near 1/2MEPmax in the 12 subjects who could maintain a tonic contraction. To minimize subject discomfort, active MEPs were assessed using the batwing coil rather than the double cone coil whenever possible. A minimum of 10 responses were collected for each condition (see following text).

**Afferent conditioning of the TA MEP**

Using a conditioning-test paradigm, we have previously shown that heteronymous nerve inputs from the TN at the ankle can facilitate TA MEPs in uninjured control subjects (open symbols in Fig. 2B) (normalized values shown in Roy and Gorassini 2008). Here we examined whether these same peripheral inputs can condition TA MEPs in eight SCI subjects (data from 8 legs). The TMS intensity used to evoke the test (unconditioned) MEP in the SCI subjects was adjusted to the sensitive portion of the recruitment curve near 1/2MEPmax, determined during the experiment to increase the likelihood of facilitating or inhibiting the MEP from the conditioning afferent stimulus. The unconditioned MEPs in the 10 uninjured controls (data from 10 legs) were well below 1/2MEPmax but typically twice that of the SCI subjects (controls: 0.62 ± 0.05 mV; SCI: 0.30 ± 0.05 mV). The TN at the ankle was stimulated at 1.5–2 × motor threshold (MT; 0.2-ms pulse) as per Roy and Gorassini (2008); and the interstimulus interval (ISI) between the single pulse nerve stimulus and the TMS pulse was varied from 30 to 80 ms (10-ms increments). Peripheral nerve stimuli were always delivered before the TMS pulse.
stimulated before the TMS pulse. During PAS-sham, the afferent excitation (3 pulses at 100 Hz) was delivered at randomly-distributed ISIs starting from 80 to 200 ms because afferent input arriving many milliseconds before the cortical stimulation does not affect the MEP (see 60-ms ISI in Mrachacz-Kersting et al. 2007; Roy et al. 2007). Because voluntary drive enhances the induction of PAS (Kujirai et al. 2006; Mrachacz-Kersting et al. 2007), the stimuli were triggered when the voluntarily activated TA EMG crossed a predefined threshold (15–20% of MVC). The subjects were cued to dorsiflex their ankle using a computer program (every 5 s). The TMS intensity during PAS was the same as that used to evoke 1/2MEPmax during a background contraction. In some of the weaker subjects (n = 2), the TMS intensity was capped at 65% MSO when using the double cone coil to keep stimulation intensities within a similar range across SCI subjects and because repeated stimulation at higher intensities can elicit discomfort (see preceding text). Neuro-modulatory interventions composed of stimulation blocks can facilitate MEPs (Kido Thompson and Stein 2004; Knash et al. 2003). Therefore the 120 sets of stimuli were delivered in two blocks of 60 (~15 min in duration) to allow for a rest period and reduce the effects of fatigue in the SCI subjects. Resting and active MEPs were measured 10 and 20 min after the intervention.

Data analysis

MEPs from the TA muscle were measured peak-to-peak for each single sweep and then averaged across a given trial. From the recruitment curve data, MEPmax was determined as described previously and compared between SCI and control subjects using an unpaired Student’s t-test. The MEPmax value was plotted against the background EMG that was measured in the 100-ms window before the stimulus. It was also plotted against the corresponding MVC value, and the relationships between these different measurements were evaluated using the Pearson product–moment correlation (r). Changes in the MEP following peripheral nerve stimulation were determined within a subject group using a one-way repeated-measures ANOVA treating the ISI as the within subject factor followed by post hoc paired t-test. For the CPN data, the peak MEP facilitation/disinhibition was compared with the peak inhibition at the 30-ms ISI rather than to the test MEP given that spinal inhibition is present following homonymous nerve stimulation (see Poon et al. 2008). In the PAS/PAS-sham experiments, the resting MEPs that were normalized to baseline were log-transformed to produce a normal distribution of data points. The active MEPs were not log-transformed because the data were normally distributed. Resting and active MEPs recorded at baseline, and 10 and 20 min after PAS/PAS-sham were analyzed using two-way repeated measures ANOVA, treating the intervention (PAS or PAS-sham) and time as within subjects factors. Significance was set at P < 0.05. Data are presented as means ± SE unless otherwise indicated.

RESULTS

MEP recruitment curves in the TA muscle

To compare the amount of corticospinal tract integrity within our SCI population, recruitment curves were obtained by plotting the mean peak-to-peak MEP against the corresponding TMS intensity. As shown for an example subject with a C3–4 lesion (subject 8M in Table 1), the size of the MEP response (Fig. 1A) measured during a background contraction of 15–20% MVC generally followed a sigmoid curve and plateaued at high stimulation intensities (i.e., MEPmax; Fig. 1B) (see also Thomas and Gorassini 2005). The mean MEPmax in the SCI subjects (0.65 ± 0.08 mV) was considerably reduced compared with the uninjured controls (2.6 ± 0.3 mV; P < 0.0001, Fig. 1C). However, there was some overlap in MEPmax values between both groups that may have been partly due to an overlap in the level of background EMG used (see Fig. 1D). MEPmax was positively correlated with background EMG (in µV) for the whole group in both SCI subjects (r = 0.73; P < 0.0001) and uninjured controls (r = 0.75; P = 0.0021; Fig. 1D), although the relationship was not systematically tested for each subject. Interestingly, the MEPmax in the SCI subjects was also positively correlated to the maximum voluntary EMG that could be produced by the parietal muscle (Fig. 1E; r = 0.72; P < 0.0001), indicating that individuals with the largest MEPmax values could also elicit the strongest voluntary contractions.

Afferent conditioning of the TA MEPs

As shown in Fig. 2A for a single SCI subject (top), a prior conditioning stimulation of the TN at the ankle had no noticeable effect on the TA MEPs at the various ISIs. The conditioned MEPs were similarly unchanged in the SCI group [ANOVA: F(6,42) = 0.90, P = 0.51; Fig. 2B, ○] even though five of the eight SCI subjects had relatively well preserved corticospinal tract function (MEPmax >0.5 mV where 0.5 mV was near the group mean) with four of these subjects being functional ambulators. This lack of effect from prior heteronymous nerve stimulation is in direct contrast to the uninjured controls, as shown for the example control subject in Fig. 2A (middle). As a group (n = 10), the controls demonstrated a twofold increase in the MEP in the contracted TA muscle [ANOVA: F(12,108) = 3.57, P < 0.001; Fig. 2B, ○]. The un-normalized MEPs were significantly increased when the peripheral nerve was stimulated 45–50 ms before the TMS pulse, latencies that are consistent with a transcortical loop, with suppression of the MEP occurring at the 35-ms ISI (all P < 0.05) (see also Roy and Gorassini 2008).

For homonymous CPN stimulation, MEP responses in the TA muscle were frequently diminished, likely because the TA motoneuron pool was still refractory from the prior antidromic activation of the motoneurons (see large M-wave in lower traces of Fig. 2A, SCI subject: CPN; see DISCUSSION (see also Poon et al. 2008). Conditioned MEPs in the SCI subjects were generally suppressed compared with the test MEP (Fig. 2C), especially at the 30-ms ISI before afferent inputs have time to reach the motor cortex. Note that the amount of MEP inhibition at the 30-ms ISI in the SCI subjects was weaker compared with the uninjured controls (Fig. 2D). During the period when the MEP was inhibited, there was nonetheless evidence that homonymous CPN inputs delivered 50 ms before the TMS pulse could provide some facilitation of the contralateral TA motor cortex in SCI subjects with less severe damage to the corticospinal tract. In the 11 stronger SCI subjects with MEPmax values >0.5 mV (Fig. 2C, ○), the MEP amplitude was modulated by the CPN [ANOVA: F(6,60) = 2.64, P = 0.025]. In particular, the MEP response at the 50-ms ISI (0.45 ± 0.07 mV) was significantly larger than the MEP recorded at the 30-ms ISI (0.34 ± 0.05 mV; P = 0.010). In this instance, we used the MEP at the 30-ms ISI as a baseline comparison rather than the test MEP to better reveal the amount of afferent-induced MEP facilitation that needed to break through the superimposed spinal inhibition from the homonymous nerve stimulation. A similar transient facilitation/disinhibition of MEP responses from homonymous CPN stimulation occurred 10 ms earlier in the uninjured control subjects at the 40-ms ISI (Fig.
suggesting a delay in transmission of afferent inputs to the TA motor cortex in the SCI subjects. In contrast, MEP facilitation/disinhibition following CPN stimulation was absent/weak in the five SCI subjects with more damage to the corticospinal tract, i.e., subjects having MEPmax values /H11021 \(0.5\) mV (Fig. 2C, U).

In five SCI subjects having a MEPmax \(>0.5\) mV, we compared conditioning the MEP with both TN and CPN stimulation. In these subjects, even though the size of the test MEPs were well matched (0.31 mV TN experiment vs. 0.38 mV CPN experiment), the CPN stimulation resulted in a 26% increase in the MEP at the 50- to 60-ms ISI (at the transcortical latency) compared with the MEP averaged over the 30- to 40-ms ISI (before the transcortical latency). However, heteronymous TN stimulation depressed the MEP by 1% (data not shown), providing evidence that afferent-induced excitation of the TA motor cortex by homonymous pathways was less affected by SCI than it was for heteronymous pathways.

PAS
An intervention of PAS was tested in 13 SCI subjects (5 ASIA D, 8 ASIA C; Table 1, *) to investigate whether afferent stimulation below the lesion paired with TMS (120 pairings...
over 15 min) could potentiate corticospinal excitability to the ankle flexor muscle. To measure changes in corticospinal excitability, MEPs were evoked in the resting muscle using a double cone coil (in 9 subjects; 76.4 ± 1.8% MSO) or a batwing coil (in 4 subjects; 93.8 ± 4.7% MSO). There was a significant intervention effect on the log-transformed MEPs collected at rest [Fig. 3A; ANOVA: \( F(1,12) = 5.191, P = 0.042 \)]. The log-transformed resting MEPs were significantly facilitated 10 min after PAS (\( P = 0.038 \)), but the facilitation tended toward baseline values at 20 min (\( P = 0.089 \)). None of the resting values were affected by PAS sham (all \( P > 0.35 \)). Resting MEPs averaged 10–20 min after PAS were facilitated by \( 20\% \) in 7 of 13 subjects (termed responders: Fig. 3C, —), whereas only 2 subjects showed similar MEP increases following the sham treatment (Fig. 3D, —). Although MEP responses recorded during a background contraction (active MEPs) tended to be facilitated rather than inhibited, neither PAS or PAS-SHAM had a significant effect on the active MEP (Fig. 3B), which were \( 0.43 ± 0.8 \) and \( 0.46 ± 0.8 \) mV at baseline, respectively.

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Effect of peripheral nerve stimulation on MEPs. **A:** raw sweeps showing the effect of a preceding tibial nerve (TN) stimulus [delivered at interspike intervals (ISIs) of 40–70 ms] on the tibialis anterior (TA) MEP in an SCI subject (top 5 traces) and an uninjured control (middle 5 traces) and from a preceding common peroneal nerve (CPN) stimulus (bottom 3 traces). **B:** MEPs in 8 SCI subjects (●) and 10 uninjured controls (○) conditioned by TN stimulation. A normalized version of data from the controls in B is published in Roy and Gorassini (2008). **C:** the effect of CPN stimulation on MEPs in 11 SCI subjects having an MEP\(_{\text{max}}\) > 0.5 mV (●) and 5 subjects with an MEP\(_{\text{max}}\) < 0.5 mV (○). **D:** the effect of CPN stimulation on MEPs in 4 uninjured controls. —, the test MEP in each group. *, significant differences as compared with the test MEP in B and as compared with the MEP at the 30-ms ISI in C (*\( P < 0.05 \)).
Predictors for PAS facilitation

As shown in Fig. 3, some subjects with initial resting MEPs that were small in amplitude (<50 µV) displayed MEP potentiation with PAS, whereas others with higher resting MEPs (>100 µV) did not, suggesting that the size of the resting MEP was not a strong predictor of the facilitatory effect of the intervention. Likewise, using the double cone (n = 5) versus batwing (n = 8) coil during PAS had no effect on the MEP potentiation (P = 0.63) nor did using an ISI interval of 40–60 ms (n = 6) versus 50–70 ms (n = 7; P = 0.91). Finally, the degree of damage to the descending corticospinal tract, as assessed using the MEP_{max} values measured before the PAS intervention, was not correlated to the magnitude of the PAS effect (Fig. 3E; r = 0.42; P = 0.15) nor was there a difference in MEP_{max} values between subjects who responded to PAS (responders: >20% facilitation; 0.76 mV MEP_{max}) and those that did not (nonresponders: <20% facilitation; 0.65 mV MEP_{max}; P = 0.70). In contrast, it appeared that the integrity of the spared ascending pathways to the motor cortex played a larger role in determining if a subject responded to PAS or not. To test this, we compared the effect of CPN stimulation on the MEP by using the same conditioning-test paradigm shown in

FIG. 3. The effect of paired associative stimulation (PAS) on the MEP in SCI subjects. A: graphs show the effect of PAS (■) and PAS-sham (○) on the log-transformed resting MEP. A value of 0 represents no change to the MEP size as compared with baseline. Active MEPs recorded during a voluntary contraction are shown in B for both PAS and PAS-sham. Individual subject data showing the resting MEPs before and after PAS (C) and PAS-sham (D); —, subjects showing >20% MEP facilitation. ×, instances when resting MEPs were absent in >20% of the trials. The background noise had a peak-to-peak amplitude of ~10 µV (□). E: scatter plot shows the strength of the PAS-induced effect (post-resting MEP as % of baseline) plotted in relation to MEP_{max} with no significant correlation between the 2 (r = 0.42; P = 0.15). F: short-term conditioning of the MEP by CPN stimulation (12 subjects tested). The profiles are grouped according to the subjects that showed a >20% PAS-induced facilitation (responders: 7 subjects) and those that did not (nonresponders: 5 subjects). *, significant differences as compared with the baseline MEP in A; and between MEP amplitudes at 30–40 and 50–60 ms for the responders in F (*P < 0.05).
Offspring BW and expression level of Bmi1 were assessed in the offspring born to the WT and the Bmi1 knock-out (KO) mice. No significant differences were observed in offspring BW (WT: 24.3 ± 0.48 g, KO: 24.2 ± 0.57 g) or in the expression level of Bmi1 (WT: 1.0 ± 0.1, KO: 0.0 ± 0.0).

Figure 2. Bmi1 expression level and offspring BW in WT and Bmi1 KO mice. The expression level of Bmi1 was assessed in the offspring born to the WT and the Bmi1 knock-out (KO) mice. No significant differences were observed in offspring BW (WT: 24.3 ± 0.48 g, KO: 24.2 ± 0.57 g) or in the expression level of Bmi1 (WT: 1.0 ± 0.1, KO: 0.0 ± 0.0).

DISCUSSION

In this study, we show that after incomplete SCI, the facilitation of MEP responses from heteronymous afferent stimulation is considerably diminished in comparison to uninjured controls. However, in a group of SCI subjects with comparatively less severe injuries, we argue that afferent inputs from homonymous nerve stimulation can provide excitatory drive to the TA motor cortex, but the effect is delayed in comparison to the uninjured controls. By pairing afferent and TMS inputs, we show that an intervention of PAS can transiently increase corticospinal connections of SCI subjects with functional, ascending sensory pathways.

Recruitment curves and background EMG

MEP responses elicited using a stimulation intensity that was maximally tolerable (i.e., using a double cone stimulation at 80% MSO) were negligible or absent in the resting TA muscle of several SCI subjects (see resting MEPs in Fig. 3, C and D), as compared with the markedly larger resting MEPs of uninjured individuals (see Roy and Gorassini 2008). This indicates that the resting MEP response may be a sensitive measure of damage to the descending corticospinal system (Brouwer and Hopkins-Rosseel 1997; Calancie et al. 2001; Davey et al. 1999). In contrast, when MEPmax responses were measured during a low background contraction (<60 μV, Fig. 1D), comparably sized responses were occasionally evoked in SCI subjects and uninjured controls. For example, SCI subjects with comparatively higher background contractions (with respect to the SCI group) had similar MEPmax amplitudes to those of uninjured subjects using comparatively low background contractions (with respect to the uninjured group). However, during high levels of background contraction (>100 μV), SCI subjects will invariably have much lower MEP responses than the uninjured controls (see Davey et al. 1999; van Hedel et al. 2007; personal observations) given the limited extent to which a background contraction can facilitate the remaining corticospinal pathways. Nonetheless, these findings highlight that when comparing the maximum connectivity of the corticospinal tract (MEPmax) within the same group, it is important to match low contraction levels in terms of a set amount of absolute EMG (in μV). Using a set percentage of MVC may introduce variability because different subjects will have different absolute levels of MVC and hence, different absolute levels of background EMG (in μV). When comparing corticospinal function between SCI and uninjured subjects, it may be better to compare responses at higher levels of background EMG to reveal differences between the two groups, such as at 40% of MVC (van Hedel et al. 2007) where excitability of the spinal cord is likely saturated in both groups (Davey et al. 1999; Day et al. 1987, Di Lazzaro et al. 1998).

Despite the potential complication from the background EMG, the MEPmax value obtained during a 15–20% MVC contraction was a relatively accurate predictor of peak EMG activity produced during an MVC (Fig. 1E) (see also Everaert et al. 2010). This relationship may have been strengthened if subjects were tested during stronger volitional contractions.

Afferent facilitation of the MEP with heteronymous inputs

In uninjured controls, afferent stimulation of the TN at the ankle can facilitate TA MEPs at a latency that is a few milliseconds longer than the latency of the somatosensory-evoked potential (i.e., P40 or P37 occurring near ~40 ms) (Hauck et al. 2006; Nuwer 2008) and is consistent with an interaction along the transcortical loop (Roy and Gorassini 2008). There is additional evidence that part of the MEP facilitation (at this latency) is mediated at a cortical level on the basis that cortical MEPs, and not subcortically evoked responses, are potentiated by the afferent input from the leg (Nielsen et al. 1997; Petersen et al. 1998; Roy and Gorassini 2008). In the present study, the lack of MEP facilitation in the SCI subjects with damaged ascending sensory pathways suggests that supraspinal neurons rostral to the injury site are important for such MEP potentiation (Hayes et al. 1992). The lack of facilitation may have occurred because of a loss of afferent input reaching the cortex, or alternatively, because the descending volleys potentiated by the sensory inputs were attenuated by the injury. Admittedly, the group of SCI subjects tested with TN stimulation had small test MEP responses compared with the uninjured controls. However, small MEPs close to 0.3 mV can be strongly facilitated by TN stimulation in the relaxed muscle of uninjured subjects (Roy and Gorassini 2008), suggesting that the size of the MEP was not responsible for the diminished sensory-induced facilitation.

Afferent modulation of the MEP with homonymous inputs

For homonymous CPN stimulation, MEP responses in the TA muscle of uninjured individuals were generally depressed, likely because when the corticospinal volley activated by TMS arrived at the TA motoneuron pool, some motoneurons were still in a refractory state produced by a prior antidromic activation from the CPN stimulation (Poon et al. 2008). Suppression of the conditioned MEP was most prominent at the 30-ms ISI, a latency before afferent inputs can reach the motor cortex to facilitate the MEP. Despite such underlying spinal inhibition, there was a transient facilitation/disinhibition of the MEP when the CPN was stimulated 40 ms before the cortical stimulus compared with the size of the MEP at the 30-ms ISI. In uninjured controls, this latency is consistent with the arrival of the afferent input at the motor cortex, and the mechanism is in general accordance with the results obtained when stretching the TA muscle (Petersen et al. 1998; van Doornik et al. 2004; Zuur et al. 2009). Although some of the MEP facilitation at the 40-ms ISI compared with the 30-ms ISI may have arisen due to a time-dependent decrease in motoneuron refractoriness (Poon et al. 2008), this cannot account for all of the observed MEP disinhibition. For instance, if motoneuron refractoriness were...
the sole mechanism, one would expect the MEP to increase monotonically at increasing interstimulus intervals in contrast to the transient increase observed at the 40-ms latency.

In the SCI subjects, the MEPs tended to be less inhibited by the CPN stimulus, potentially due to a reduction of spinal inhibition that occurs post injury (reviewed in Knikou 2007; Norton et al. 2008; Pierrot-Deseilligny and Burke 2005). In addition, the MEP facilitation/disinhibition by CPN stimulation was delayed by 10 ms and is consistent with delays observed in sensory-evoked potentials after SCI (Li et al. 1990; Restuccia et al. 2000). Moreover, the MEP facilitation/disinhibition by CPN stimulation was related to the severity of the injury. For example, individuals with stronger corticospinal connections, as measured by MEP_{max}, exhibited larger afferent-induced MEP facilitation/disinhibition compared with those with weaker connections. Moreover, there was a tendency for the MEP to be more facilitated by homonymous CPN inputs compared with the heteronymous TN in the five subjects studied with both nerves and having test MEP responses closer to controls. This evidence suggests that afferent connections to the TA muscle after SCI may be more strongly regulated by homonymous CPN rather than heteronymous TN inputs (see also Deletis et al. 1992) even though evoked potentials over the somatosensory cortex may be more clearly defined for the TN rather than the CPN (Chabot et al. 1985; Nuwer 2008). It is plausible that the heteronymous afferent pathway in our subjects was more affected after SCI because it contains more cortical interneuronal relays compared with the homonymous pathway. This reduction of heteronymous afferent facilitation to the motor cortex may partly contribute to the disruption of motor coordination and strength observed after SCI.

PAS

An intervention of PAS potentiated the MEPs recorded at rest, and this increase persisted for ≥10 min after the intervention. As a large number of subjects were taking baclofen at the time of the experiment (see Table 1), it is possible that the drug may have hindered the induction of PAS given that baclofen, a GABA_B receptor agonist, decreases PAS-induced facilitation in uninjured subjects (McDonnell et al. 2007). However, there were no differences in the PAS-induced facilitation of the resting MEP between groups (P = 0.71; 5 subjects off baclofen vs. 8 subjects taking baclofen). Although the effect of PAS was variable, facilitating the resting MEPs by >20% in only 7 of the 13 subjects, MEPs were not significantly altered by the sham treatment, suggesting that appropriately timed afferent and cortical inputs contributed to modifying the strength of the corticospinal connections.

In the present study, we cannot determine if the MEP facilitation from PAS occurred at cortical or subcortical levels. However, given that PAS increases the amplitude of descending corticospinal volleys measured from epidural recordings (Di Lazzaro et al. 2009) but has no effect on the H-reflex recorded in the TA muscle (Marachacz-Kersting et al. 2007; Roy et al. 2007), it is likely that part of the MEP facilitation involves cortical elements. However, changes to spinal excitability, as shown in wrist flexors following PAS, cannot be entirely excluded (Meunier et al. 2007). In contrast to the resting MEP response, none of the MEPs measured during a voluntary contraction were significantly altered by PAS, similar to other experiments in the upper limb (Ridding and Taylor 2001; Stefan et al. 2000). In the ankle flexor, MEP facilitation due to PAS is weaker in the contracted versus the relaxed muscle (Roy et al. 2007; Stein et al. 2009), and it is possible that PAS mainly affects the excitability of cortical circuits to low-threshold corticospinal tract neurons, which are already activated during the voluntary contraction.

In the group of SCI subjects tested (5 ASIA D, 8 ASIA C with an average TA MVC of 126 ± 24 μV), the amount of facilitation produced by PAS was not significantly correlated to the strength of the descending corticospinal pathway as assessed by MEP_{max} (Fig. 3E). In line with a subject’s volitional muscle strength, MEP_{max} can provide insight about the integrity of the corticospinal tract (Fig. 1E) (see also Everaert et al. 2010) but may not be a strong predictor of whose motor system will respond to PAS. In contrast, PAS-induced MEP facilitation was more prevalent in the subjects whose motor cortex responded more strongly to ascending CPN afferent inputs. Thus the amount of afferent-induced cortical facilitation may provide insight as to whether a SCI subject will respond to PAS or not. These findings also demonstrate the importance of ascending sensory pathways for the induction of PAS. Potentially, a stronger relationship between the integrity of the descending corticospinal tract and PAS facilitation could have occurred if our subject group contained less severely injured subjects (i.e., more ASIA D’s), as such subjects would likely have less damage to ascending sensory pathways. This was shown for the stronger subjects in Fig. 2C that contained a higher proportion of ASIA D injuries (55% with an average TA MVC of 153 ± 17 μV) compared with the PAS group (38%; see preceding text) and whose MEPs could be excited/disinhibited by the afferent CPN inputs. Likewise, if parameters such as the intensity, latency and frequency of CPN stimulation were tuned to provide maximum MEP facilitation for each subject, then perhaps a more consistent PAS-induced facilitation may have occurred across all subjects.

PAS and motor learning

Increases in corticospinal drive contribute to recovery of the functional walking after human SCI in response to intensive motor training (Norton and Gorassini 2006; Thomas and Gorassini et al. 2005; reviewed in Yang and Gorassini 2006). Eight of the 13 SCI subjects tested with PAS were also participating in locomotor training (Gorassini et al. 2009) within a few months of the PAS experiments. It is interesting that there was a trend for the group of SCI subjects who responded to PAS to also show increases in MEP_{max} from training (70.4 ± 30.1% increase in MEP_{max}; n = 5 subjects) compared with subjects who did not demonstrate a PAS effect (6.3 ± 8.8% increase in MEP_{max}; n = 3 subjects). Although it is unclear whether such a trend will persist when using a larger sample size, LTP in animals (for review, see Monfils et al. 2005) and PAS-induced changes in humans are both linked to motor learning (Rosenkranz et al. 2007; Ziemann et al. 2004) and may be related to a subject’s ability to increase corticospinal tract excitability and motor function after SCI.

Technical considerations

Because the resting MEPs were increased by >20% in only 7 of the 13 subjects, it is possible that the PAS facilitation may

J Neurophysiol • VOL 103 • APRIL 2010 • www.jn.org
have been more consistent across subjects had we used a
standard TMS intensity (with respect to motor threshold), a
single type of coil, and a single afferent stimulus. However, we
propose that these technical factors are secondary to the in-
duction of PAS for several reasons.

First, the TMS intensities used in most human experiments
are based on recruitment thresholds such as 1.2 × motor
threshold (or 1/2 MEP_max), and may be nonoptimal for
the induction of PAS in SCI subjects. Restricting the TMS inten-
sity in the more severely injured subjects to 65% MSO when
using the double cone coil, rather than setting the intensity to
a fixed percentage of AMT, resulted in stimulation intensities
that were more similar across all SCI subjects and to that
previously used in unjured volunteers (see Mrachacz-Kerst-
ing et al. 2007; Roy et al. 2007; Stinear and Hornby 2005). On
the basis that PAS likely has a strong cortical component
(discussed in preceding text) and that motor cortex neurons are
generally intact after SCI, it makes sense to keep TMS inten-
sities close to those used in unjured controls.

Second, although the effect of the TMS coils was not
directly assessed in our study, MEP potentiation during PAS
has been produced using various TMS coils (Mrachacz-Kerst-
ing et al. 2007; Roy et al. 2007; Stefan et al. 2000, 2002;
Wolters et al. 2003), suggesting that the size and shape of the
coch was not responsible for the lack of effect in some subjects.
This is supported by the fact that there were no systematic
differences in PAS facilitation between subjects when the
double cone or batwing coil was used.

Third, we predict that the facilitatory effect of using three
pulses at 100 Hz (at intervals starting from 40 or 50 ms) may
outweigh the putative contribution of inhibitory-PAS to the leg
motor cortex, which has been previously reported at ISIs of 20,
24, and 40 ms (Jayaram and Stinear 2008; Mrachacz-Kersting
et al. 2007; Stinear and Hornby 2005). In the resting lower limb,
we have previously found that PAS can be facilitatory over a
large range of stimulation ISIs (Roy et al. 2007), whereas
PAS-induced cortical inhibition occurs over more stringent
stimulation intervals (see Mrachacz-Kersting et al. 2007). In
addition, facilitation of the MEP by CPN stimulation occurs
most strongly using 100 Hz stimulation (Mang et al. 2010)
leading to the idea that the effect of PAS may be favored when
using three pulses at 100 Hz as compared with one pulse (see
also Roy et al. 2007). In summary, we believe that our PAS
protocol provided sufficient activation of the motor cortex to
induce neuroplasticity but was not successful in some subjects
due to weak activation of the motor cortex by the spared
ascending sensory pathways.

Summary and clinical implications

The present findings provide evidence that PAS can facili-
tate MEP responses in some SCI subjects having functionally
spared ascending inputs to the motor cortex. As has been
shown for repetitive TMS and transcranial DC stimulation in
stroke, priming the nervous system with PAS prior to motor
rehabilitation might facilitate the effects of motor training (see
Hummel et al. 2008). However, the resting MEP in the SCI
subjects was only increased for ~10 min after the intervention;
this would not leave much time to perform extensive motor
training. Longer periods of cortical facilitation in a larger
percentage of SCI subjects may potentially be induced by using
a larger number of PAS conditioning stimuli, by fine tuning the
peripheral nerve stimulation parameters to overcome the im-
paired afferent excitation or by using other forms of cortical
facilitation such as repetitive TMS (Hamada et al. 2008; Huang
et al. 2005) or transcranial DC stimulation (Jeffery et al. 2007;
Nitsche and Paulus 2000) that do not rely on ascending sensory
pathways.

Acknowledgments

We thank the many participants of the study for generous commit-
tment of time, and we thank therapists K. Brunton, K. Pope, and G. Hendricks, who
recruited the subjects for the study. We are also very grateful to J. Nevet-
Duchcerer and E. Takele for their excellent technical assistance and to Dr.
Richard Stein for helpful insight.

Grants

Funding for this work was provided by a Canadian Institutes of Health
Research Grant to Drs. Yang and Gorassini. F. D. Roy was supported by
studentships from the Natural Sciences and Engineering Research Council
of Canada and the Alberta Heritage Foundation for Medical Research (AHFMR).
M. A. Gorassini is a Senior Scholar funded by AHFMR.

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