Short-interval intracortical inhibition with incomplete spinal cord injury

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Abstract

Objective: Short-interval intracortical inhibition (SICI) in leg and hand muscles was characterized in individuals with incomplete spinal cord injury (SCI) to understand how such inhibition limits corticospinal drive after spinal insult.

Methods: We compared SICI during a voluntary contraction in 16 SCI and 14 control subjects, the latter group tested over a larger range of conditioning and test stimulus (CS and TS) intensities to best match the SCI data.

Results: The average peak SICI in the tibialis anterior muscle was typically 3–4 times lower in the SCI subjects compared to controls. When matched for absolute TS intensity, in terms of maximum stimulator output, both U-shaped SICI recruitment curves were produced by similar CS intensities. SICI in the first dorsal interosseous muscle of the hand tended to be larger than in the ankle flexor.

Conclusions: Incomplete SCI reduces SICI compared to controls, but the absolute CS intensities that produce the U-shaped SICI recruitment curves are unchanged.

Significance: These findings suggest that although the relative excitability profile of cortical SICI networks is unchanged after SCI, the effective inhibition of corticospinal tract output by these neurons is reduced.

1. Introduction

Gamma-aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the brain and is crucial for the maintenance of cortical muscle representations in humans (reviewed in Chen et al., 2002) and animals (Jacobs and Donoghue, 1991; Capaday and Rasmusson, 2003). GABA-mediated inhibitory circuits producing short-interval intracortical inhibition (SICI) (Kujirai et al., 1993; Ziemann et al., 1996) have also been implicated in shaping the excitatory drive from the motor cortex during movement. For example, the decrease in excitability of SICI networks just before a muscle contraction may facilitate cortical excitability, and hence corticospinal drive, to the target muscle (Reynolds and Ashby, 1999). On the other hand, increases in SICI to a target muscle after a no-go signal (Sohn et al., 2002) or to adjacent, uninvolved muscles during rhythmic movements (Stinear and Byblow, 2003), are
thought to suppress unwanted muscle activity. Further, afferent inputs from the hand and leg reduce SICI (Rosenkranz and Rothwell, 2003; Devanne et al., 2009; Roy and Gorassini, 2008) and may contribute to movement-related modulation of cortical inhibitory networks (Reis et al., 2008). Thus, the reduced corticospinal drive and excessive co-activation of muscles during skilled movements after incomplete spinal cord injury (SCI) (Alexeeva et al., 1997) may, in part, be mediated by a disruption in the control over GABAergic SICI networks in response to the partial disruption of ascending sensory inputs to the sensorimotor cortex (Hayes et al., 1992; Roy et al., 2010).

Cortical GABAergic inhibition may be reduced after incomplete SCI (Davey et al., 1998; Smith et al., 2000a,b; Shimizu et al., 2000; Saturno et al., 2008), in line with other movement disorders, such as dystonia, amyotrophic lateral sclerosis and multiple sclerosis (Ridding and Rothwell, 1995; Vucic et al., 2009; Conte et al., 2009). In patients with SCI, such inhibition has been primarily studied using the application of sub-threshold transcranial magnetic stimulation (TMS), a technique that temporarily inhibits the ongoing electromyogram (EMG) (Davey et al., 1994; Roy, 2009), likely through the activation of cortical inhibitory neurons with oligo- or possibly disynaptic, connections onto fast-conducting corticospinal tract neurons that drive voluntary contractions (Butler et al., 2007). Within several weeks of SCI, the onset of EMG suppression is ~25 ms longer than the latency of the motor-evoked potential (MEP), while the latency difference is only ~13 ms in uninjured controls (Davey et al., 1998; Smith et al., 2000a). However, a greater involvement of slow-conducting corticospinal tract axons to voluntarily activated EMG may also explain the greater delay in EMG suppression (see also Davey et al., 1998). In addition to the technique of TMS-induced EMG suppression, two single-subject reports have shown that the reduction of a test MEP by a prior sub-threshold conditioning stimulation (i.e., SICI) in hand muscles is diminished after incomplete SCI (Shimizu et al., 2000; Saturno et al., 2008). While various inter-pulse intervals (i.e., 1–5 ms) were tested, SICI was examined only with a single conditioning and test stimulus (CS and TS) intensity.

Studying SICI over a range of intensities has become increasingly important to control for the recruitment of neurons by the TS pulse that are differentially susceptible to SICI modulation (Ilić et al., 2002; Roshan et al., 2003; Garry and Thomson 2009; Wagle-Shukla et al., 2009) and to rule out contributions from short-interval intracortical facilitation activated by the CS (Ortu et al., 2008; Peurala et al., 2008; see also Roy, 2009). Moreover, because the intensities chosen to administer SICI are frequently based on the motor threshold, damage to the descending motor fibres in SCI will invariably raise these TMS intensities resulting in overactivation of the intact motor cortex. Therefore, it is important to evaluate the recruitment of SICI networks using CS intensities that are above and below the motor threshold of uninjured subjects when characterizing SICI after incomplete SCI.

On the basis that the motor cortex undergoes functional reorganization after incomplete SCI (Levy et al., 1990; Topka et al., 1991; Curt et al., 2002; although see Brouwer and Hopkins-Rosseel, 1997), it is conceivable that a reduction in SICI after SCI can be explained by a decrease in the excitability of cortical inhibitory networks. However, because incomplete SCI is also associated with excitability changes in spinal inhibitory networks caudal to the lesion (Pierrot-Deseilligny and Burke, 2005; Norton et al., 2008) and myelin loss near the zone of injury (Becerria et al., 1995), it is plausible that sub-cortical mechanisms can also contribute to the alteration of SICI after injury. Thus, in the present study we examine whether spinal and/or cortical factors may underlie alterations in SICI after SCI.

In this study, we expanded the experiments by Shimizu et al. (2000) and Saturno et al. (2008) by measuring SICI at incrementing CS intensities to produce a full recruitment curve in both leg and hand muscles of subjects with incomplete SCI. A TS intensity producing a test MEP of half maximum was used in the SCI subjects. We used a larger range of CS and TS intensities in the uninjured controls to match the data obtained in the SCI subjects. We show that the amplitude of SICI is reduced after incomplete SCI but the absolute CS intensities that produce SICI are unchanged.

2. Methods

2.1. 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. The majority of the incomplete SCI subjects were recruited to participate in locomotor or hand rehabilitation, and all experiments were done on a day when the subjects were not training. Our sample comprised 16 subjects (3 female) with incomplete SCI, aged 23–69 (47.7 ± 13.2, mean ± SD; Table 1) and 14 healthy control subjects (6 females) aged 18–68 (29.8 ± 13.6). The control subjects were, on average, younger than the SCI subjects; however, the magnitude of SICI tested during a background contraction, as performed in this study, is not affected by age (McInigle et al., 2010). All injured participants were classified as either C or D according to the American Spinal Injury Association Impairment Scale (AIS), with C being motor and sensory incomplete; (with more than half of key muscles below the neurological level having a muscle grade less than 3;3 or more for AIS D; Maynard et al., 1997). Data from different limbs of an SCI subject were considered to be independent because of the asymmetry of the lesion location and because the maximum MEP (MEP_max) evoked by TMS in left and right limbs could differ by a factor of three. Bilateral stimulation was also performed in most uninjured controls to be consistent with the SCI data.

2.2. Recordings and stimulation

Surface EMG was collected using pairs of Ag–AgCl electrodes (Kendall, Chicopee, MA) from the tibialis anterior (TA) and first dorsal interosseous (FDI) muscles. The EMG signals were amplified (500 or 1 k gain) and filtered with a 10- to 1000-Hz band-pass (Octopus, Bortec Technologies, Calgary, AB). Signals were digitized using Axoscope hardware and software at a rate of 5 kHz (Digidata 1200 Series, Axon Instruments, Union City, CA). Rectified and heavily smoothed EMG (100-ms time constant) from the target muscle was displayed on an oscilloscope to help the individuals maintain a tonic level of EMG. At the start of the experiment, subjects performed three maximum voluntary contractions (MVC) in the target muscle (2–3 s in duration) with verbal encouragement from the experimenter. A subject’s MVC was assessed by first smoothing the rectified EMG (using a 500-ms sliding window) and then selecting the maximum values from the three EMG bursts (see Table 1).

TMS was delivered to the contralateral motor cortex using two MagStim 200 stimulators connected to a BiStim module (Magstim, Dyfed, UK). TMS was applied to the leg motor cortex using a custom-made batwing figure-of-eight coil (P/N 15857: 90-mm wing diameter) or a double cone coil. The hand motor cortex was stimulated using a flat figure-of-eight coil (90-mm wing diameter) with the handle orientated perpendicular to the direction of the central sulcus (30–45° to the midsagittal line). The optimal spot to the target muscle was identified using a suprathreshold TMS intensity and was marked on the scalp using a felt-tipped pen. TMS coils were orientated to induce postero-anterior currents in the brain. Because resting MEPs are small or absent in many individuals affected with SCI (Roy et al., 2010; Brouwer and Hopkins-Rosseel, 1997), all responses were evoked during a voluntary contraction.
MEP$_{\text{max}}$ was assessed by means of single pulse TMS given at increasing intensities (5–10% increments), reaching 100% MSO curve (i.e., producing test MEPs near ½ MEP$_{\text{max}}$). The TS intensity was set to the sensitive portion of the recruitment curve (i.e., the maximum tolerable intensity). The MEP$_{\text{max}}$ value was determined from the intensity that evoked the four largest peak-to-peak responses. To assess the relative strength of the spared corticospinal tract in SCI subjects vs. controls, MEP$_{\text{max}}$ values were taken from trials where the level of background EMG (in terms of µV) was matched between the two groups.

2.3. Experiment 1: SICI in the leg

SICI was tested in the contracted TA muscle using the protocol of Kujirai et al. (1993) with an inter-pulse interval of 3 ms. Thirteen SCI subjects (18 legs) and 5 uninjured controls (10 legs) were tested. Subjects maintained a tonic contraction corresponding to approximately 15–20% of their MVC, thereby controlling for voluntary effort. The TS intensity was set to the sensitive portion of the recruitment curve (i.e., the maximum tolerable intensity). The MEP$_{\text{max}}$ value was determined from the intensity that evoked the four largest peak-to-peak responses. To assess the relative strength of the spared corticospinal tract in SCI subjects vs. controls, MEP$_{\text{max}}$ values were taken from trials where the level of background EMG (in terms of µV) was matched between the two groups.

2.5. Experiment 3: Effect of matched TS intensity on SICI

Because the recruitment of SICI can differ when using a strong TS intensity (Ilić et al., 2002), we examined whether the actual TS intensities (in terms of MSO) were responsible for the high CS intensities needed to produce SICI in the SCI subjects (see Fig. 1E). SICI was evaluated in 5 uninjured subjects (9 legs) using a stronger TS intensity that was matched to the SCI data. CS intensities were varied from 40% to 130% of AMT, and the absolute level of background EMG was also matched to the SCI data.

2.6. Experiment 4: SICI in the hand

SICI in the hand motor cortex was examined in 4 SCI subjects (8 hands). SICI profiles were collected as described in experiment 1. Size-matched MEPs were collected in 4 uninjured control subjects (8 hands) for comparison.

**Table 1**

<table>
<thead>
<tr>
<th>Subject No./ Sex</th>
<th>Age (y)</th>
<th>Years post injury</th>
<th>Cause of Injury</th>
<th>Injury level</th>
<th>AIS$^a$ Score</th>
<th>Medication$^b$</th>
<th>Muscle(s) tested and MVC$^c$ (µV)</th>
</tr>
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<tbody>
<tr>
<td>1 M</td>
<td>56.9</td>
<td>34.2</td>
<td>Trauma</td>
<td>C5–6</td>
<td>D</td>
<td>oxybutynin</td>
<td>ITA(131.9)</td>
</tr>
<tr>
<td>2 F</td>
<td>48.1</td>
<td>1.3</td>
<td>Trauma</td>
<td>C6</td>
<td>C</td>
<td>baclofen, gabapentin, tolterodine</td>
<td>ITA(146.9)</td>
</tr>
<tr>
<td>3 M</td>
<td>41.7</td>
<td>1.1</td>
<td>Trauma</td>
<td>C3–4</td>
<td>C</td>
<td>baclofen, gabapentin, oxybutynin</td>
<td>ITA(53.9), ITA(76.4)</td>
</tr>
<tr>
<td>4 F</td>
<td>69.4</td>
<td>2.5</td>
<td>Surgery</td>
<td>T4–15</td>
<td>D</td>
<td>oxybutynin, oxycodeine</td>
<td>ITA(88.4)</td>
</tr>
<tr>
<td>5 M</td>
<td>63.3</td>
<td>20.0</td>
<td>Trauma</td>
<td>C4–5</td>
<td>D</td>
<td>none</td>
<td>ITA(187.6), ITA(196.6)</td>
</tr>
<tr>
<td>6 M</td>
<td>33.6</td>
<td>1.1</td>
<td>Trauma</td>
<td>C4–5</td>
<td>D</td>
<td>baclofen, pregabalin</td>
<td>ITA(132.4), ITA(284.1)</td>
</tr>
<tr>
<td>7 M</td>
<td>60.7</td>
<td>2.4</td>
<td>Trauma</td>
<td>C5</td>
<td>D</td>
<td>baclofen, pregabalin</td>
<td>ITA(131.9), ITA(122.5)</td>
</tr>
<tr>
<td>8 M</td>
<td>44.1</td>
<td>17.6</td>
<td>Trauma</td>
<td>T1–2</td>
<td>D</td>
<td>none</td>
<td>ITA(150.6), ITA(131.4)</td>
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<td>T4, 11, 12</td>
<td>C</td>
<td>gabapentin</td>
<td>ITA(137.8)</td>
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<td>10 F</td>
<td>42.7</td>
<td>1.9</td>
<td>Trauma</td>
<td>C6, 7</td>
<td>C</td>
<td>none</td>
<td>ITA(82.2)</td>
</tr>
<tr>
<td>11 M</td>
<td>52.3</td>
<td>2.6</td>
<td>Trauma</td>
<td>T5–6</td>
<td>C</td>
<td>none</td>
<td>ITA(191.1)</td>
</tr>
<tr>
<td>12 M</td>
<td>41.6</td>
<td>1.6</td>
<td>Trauma</td>
<td>C3, C4</td>
<td>D</td>
<td>baclofen, pregabalin</td>
<td>ITA(252.5), rFDI(197.3), IFDI(331.0)</td>
</tr>
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<td>13 M</td>
<td>23.4</td>
<td>1.0</td>
<td>Trauma</td>
<td>T6–9</td>
<td>C</td>
<td>none</td>
<td>ITA(105.2)</td>
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<td>58.5</td>
<td>5.1</td>
<td>Trauma</td>
<td>C3, C6</td>
<td>C</td>
<td>baclofen, gabapentin, morphine sulphate</td>
<td>rFDI(181.3), IFDI(52.6)</td>
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<td>1.2</td>
<td>Trauma</td>
<td>C6/7, T8</td>
<td>C</td>
<td>baclofen, dantirim, oxybutynin</td>
<td>rFDI(68.2), IFDI(80.0)</td>
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<td>44.7</td>
<td>4.9</td>
<td>Trauma</td>
<td>C3–5</td>
<td>C</td>
<td>baclofen, gabapentin, oxybutynin</td>
<td>rFDI(33.0), IFDI(54.0)</td>
</tr>
</tbody>
</table>

$^a$ AIS = American Spinal Injury Association Impairment Scale.

$^b$ baclofen and dantirim are antispastic agents; gabapentin, morphine sulphate, oxycodeine and pregabalin are analgesics; oxybutynin and tolterodine are for symptoms of an overactive bladder.

$^c$ MVC = maximum voluntary contraction.
crossed a threshold that was 2 × SD above the mean. This method was also used to evaluate the MEP latency and duration. SICI was plotted at incrementing CS intensities to generate a SICI recruitment curve. A one-way analysis of variance (ANOVA) and post hoc t-tests (2-tailed) were used to evaluate significant MEP modulation across the CS intensities. To calculate the average SICI in each individual, we selected the three adjacent CS intensities that produced the strongest MEP suppression in the group data (e.g., between 70% and 90% of AMT). The group mean was calculated from these averages, and statistical comparisons were done using t-tests (2-tailed). The Pearson product-moment correlation (r) was used to correlate each individual’s average SICI to MEP<sub>max</sub> TS intensity, and background EMG.

Because of the large differences in AMT between SCI and control subjects, the group SICI recruitment curves were also re-plotted in terms of % MSO to determine if SICI was produced by similar abso-
duce a CS of 80% AMT (marked by grey circle in Fig. 1B; 12.2 ± 4.2% inhibition; \( P = 0.010 \)) and the stronger CS of 110% AMT tended to facilitate the MEP in line with the recruitment of short-interval intracortical facilitation (\( P = 0.053 \)). In contrast, test MEPs in the control subjects were suppressed over a larger range of CS intensities from 60% to 90% AMT and were facilitated at 120% AMT (see grey triangles in Fig. 1C; ANOVA: \( F(8,81) = 9.93, P < 0.00001 \)).

Fig. 1D shows the average recruitment profile of SICI in both subject groups plotted as a function of AMT, which was 62.5 ± 3.4% and 53.7 ± 4.2% MSO in the SCI and control subjects, respectively (arrows in Fig. 1E). Although the MEPs were modulated in a similar U-shaped pattern, the average SICI (measured from 70% to 90% AMT) was significantly smaller in the SCI subjects (7.6 ± 3.9%) compared to the uninjured controls (29.4 ± 5.4%; \( P = 0.0030 \)). Likewise, the same CS reduced the MEP area by 14.1 ± 6.6% in the SCI subjects and by 40.0 ± 6.8% in the uninjured controls (\( P = 0.018 \)). Because incomplete SCI increases AMT (Davey et al., 1998; Smith et al., 2000a), the SICI recruitment curves were re-plotted as function of the % MSO intensities used (see Section 2.7) to control for the absolute CS intensities applied to the cortex. When doing so, the U-shaped SICI recruitment curve for the SCI group was shifted to the right relative to the uninjured controls (Fig. 1E). The absolute CS intensities (in % MSO) producing SICI>50% (SICI measurements that were >50% of the group mean, see Section 2.7) were greater in the SCI subjects compared to controls (KS-test: \( P = 0.0098 \), data not shown). Neither MEPmax, the TS intensity or the level of background EMG was correlated to an individual's average SICI (all \( r \) values >0.49 and all \( P > 0.10 \)). Similarly, there was no difference in the average SICI between SCI subjects taking and not taking baclofen, a GABA-B receptor agonist that alleviates spasticity (8 limbs ON baclofen and 10 limbs OFF baclofen; \( t \)-test: \( P = 0.40 \)).

3. Results

3.1. Experiment 1: SICI in the leg

SICI in the TA muscle was examined using a test MEP that was at a similar location on the TMS recruitment curve in both groups, i.e., the test MEP occurred near \( \frac{1}{2} \) MEPmax and along the sensitive portion of the curve (Thomas and Gorassini, 2005). TMS during a voluntary contraction yielded test MEPs of 0.41 ± 0.04 (SEM) mV in the SCI subjects (using a TS intensity of 78.3 ± 3.4% MSO) and 1.24 ± 0.20 mV in the uninjured controls (TS intensity of 67.9 ± 4.5% MSO; both near 125% AMT). The background EMG was lower in the SCI subjects (27.1 ± 1.3 \( \mu \)V vs. 62.4 ± 7.6 \( \mu \)V; \( P < 0.00001 \)) when controlling for voluntary effort.

Fig. 1A displays representative SICI data from an SCI subject and an uninjured control. Most individuals showed a U-shaped relationship between the intensity of the CS and the amount of inhibition, which was generally centered on 80% of AMT (Fig. 1B and C). In the SCI subjects, the CS had a significant effect on the size of the MEP (ANOVA: \( F(6,107) = 2.88, P = 0.012 \)). The MEP was significantly reduced by a CS of 80% AMT (marked by grey circle in Fig. 1B; 12.2 ± 4.2% inhibition; \( P = 0.010 \)) and the stronger CS of 110% AMT tended to facilitate the MEP in line with the recruitment of short-interval intracortical facilitation (\( P = 0.053 \)). In contrast, test MEPs in the control subjects were suppressed over a larger range of CS intensities from 60% to 90% AMT and were facilitated at 120% AMT (see grey triangles in Fig. 1C; ANOVA: \( F(8,81) = 9.93, P < 0.00001 \)).

Fig. 1D shows the average recruitment profile of SICI in both subject groups plotted as a function of AMT, which was 62.5 ± 3.4% and 53.7 ± 4.2% MSO in the SCI and control subjects, respectively (arrows in Fig. 1E). Although the MEPs were modulated in a similar U-shaped pattern, the average SICI (measured from 70% to 90% AMT) was significantly smaller in the SCI subjects (7.6 ± 3.9%) compared to the uninjured controls (29.4 ± 5.4%; \( P = 0.0030 \)). Likewise, the same CS reduced the MEP area by 14.1 ± 6.6% in the SCI subjects and by 40.0 ± 6.8% in the uninjured controls (\( P = 0.018 \)). Because incomplete SCI increases AMT (Davey et al., 1998; Smith et al., 2000a), the SICI recruitment curves were re-plotted as function of the % MSO intensities used (see Section 2.7) to control for the absolute CS intensities applied to the cortex. When doing so, the U-shaped SICI recruitment curve for the SCI group was shifted to the right relative to the uninjured controls (Fig. 1E). The absolute CS intensities (in % MSO) producing SICI>50% (SICI measurements that were >50% of the group mean, see Section 2.7) were greater in the SCI subjects compared to controls (KS-test: \( P = 0.0098 \), data not shown). Neither MEPmax, the TS intensity or the level of background EMG was correlated to an individual's average SICI (all \( r \) values >0.49 and all \( P > 0.10 \)). Similarly, there was no difference in the average SICI between SCI subjects taking and not taking baclofen, a GABA-B receptor agonist that alleviates spasticity (8 limbs ON baclofen and 10 limbs OFF baclofen; \( t \)-test: \( P = 0.40 \)).

3.2. Experiment 2: Effect of CS on spinal excitability

Because higher CS intensities (in terms of MSO) were needed to produce SICI in the SCI subjects, we examined if spinal factors might have contributed to this difference. That is, a part of the MEP suppression during SCI could be mediated by CS-activated descending pathways that in turn activate spinal inhibitory circuits, with the former having a higher threshold in the SCI subjects. To test this, the TA H-reflex, a measure of spinal excitability, was conditioned by sub-threshold TMS. The synchronous arrival of corticospinal and afferent volleys at the motoneuron pool resulted in facilitation of the H-reflex (near \( -3 \) ms, Fig. 2A), while slightly longer intervals (i.e. \( -2 \) to 1 ms,.

Fig. 2. Effect of sub-threshold TMS on the TA H-reflex. (A) The effect of a sub-threshold CS (at 90% of AMT) on the H-reflex in 3 uninjured control subjects. The H-reflex stimulus was delivered before (negative intervals) or after (positive intervals) the conditioning TMS pulse. The arrows represent the conditioning-test interval that was chosen for the 3 representative subjects (i.e., where the H-reflex was first inhibited/disfacilitated). (B) The effect of the CS on the group H-reflex (closed triangles; intervals chosen between \(-2\) and \(1\) ms) and similar sized MEP (open triangles). \( P < 0.05 \).
see arrows in Fig. 2A) revealed an abrupt reduction in the H-reflex consistent with the presence of spinal inhibition (similar to that shown by Iles and Pisini, 1992; Nielsen et al., 1993). In most individuals, the H-reflex (0.29 ± 0.08 mV in amplitude) was inhibited/disfacilitated when sub-threshold TMS was delivered at an interval of ~2 to 1 ms. However, when averaged across subjects, none of the CS intensities inhibited the group H-reflex (0.29 ± 0.08 mV in amplitude) during SICI (P = 0.013).

3.3. Experiment 3: Effect of matched TS intensity on SICI

To examine whether the absolute TS intensities (in terms of % MSO) were responsible for the high CS intensities needed to produce SICI in the SCI subjects, comparable TS intensities were also tested in the control subjects. When a strong TS intensity was used in the controls (79.4 ± 2.5% MSO; ~160% of AMT), the U-shaped SICI recruitment curve was shifted to the right and was centred on a CS intensity of 100% AMT (Fig. 3A). With matched TS intensity (and background EMG equal to 25.8 ± 2.2 μV), the average SICI in the controls (24.2 ± 5.4%) was still considerably greater than in the SCI subjects (t-test: P = 0.021; Fig. 3A). Notably, when the CS intensities in % AMT were converted to % MSO values, both SICI recruitment curves became centred on similar conditioning MSO intensities and peaked near 50% MSO (Fig. 3B). Similar results are also shown using the normalized histogram which characterizes the incidence of SICI > 50% at the different conditioning MSO intensities (Fig. 3C). In addition, as determined using the K–S test, the recruitment of SICI > 50% by the conditioning MSO intensities was not statistically different between the groups (Fig. 3D; P = 0.69). This signifies that incomplete SCI did not alter the relative recruitment pattern of SICI.

3.4. Experiment 4: SICI in the hand

Fig. 4A shows that the U-shaped relationship between the CS intensity and the amount of SICI was also preserved in the hand motor cortex of SCI subjects (open symbols, data from 8 FDI muscles, 4 subjects). Again, the size of the test MEP recorded in the FDI muscle occurred along the steep portion of the recruitment curve (at ~0.5 MEPmax: 0.39 ± 0.10 mV) and was similar to the size of the test MEP recorded in the TA muscle (0.41 ± 0.04 mV). Average SICI in the FDI muscle (23.3 ± 7.0%) was significantly greater than the inhibition (7.6 ± 3.9%) observed in the ankle flexor (closed symbols, P = 0.047). When SCI was compared using MEP areas, the average SICI in the hand (27.2 ± 4.5%) only tended to be greater than in the leg (14.1 ± 6.6%; P = 0.22).

When compared to control values (set as 100%), residual corticospinal function (MEPmax: ~23% in FDI and ~48% in TA) and volitional strength (MVC: ~22% in FDI and ~33% in TA) tended to show greater impairment in the FDI muscle. The latency of the FDI and TA MEPs in the SCI subjects were significantly delayed by 8.2 and 9.4 ms, respectively (from 17.3 and 27.5 ms in controls, bottom

![Fig. 3. Effect of matched TS intensity on SICI in the TA muscle. (A) Comparison of SICI using matched TS intensity in the SCI subjects (closed circles; ~78% MSO) and the uninjured controls (open triangles; ~79% MSO). (B) Curves in (A) plotted in terms of MSO (calculated from the average AMT) to show which CS intensities yielded inhibition. Mean AMT for controls (49.2% MSO) was significantly lower than SCI subjects (62.5% MSO; P = 0.014). (C) Histogram of the CS intensities (in % of MSO) that produced SICI > 50% in the SCI subjects (black bars) and the uninjured controls (white bars). (D) Cumulative fraction of SICI > 50% induced by the CS from 30% to 70% MSO in the SCI subjects (solid line) and the controls (dotted line). The data in (D) was taken from the region in (C) where the two histograms overlapped.](https://example.com/figure3.png)
of boxes in Fig. 4B; all P < 0.005). Likewise, the MEP duration (length of boxes) was prolonged compared to the size-matched MEPs collected in the control subjects (all P < 0.05).

4. Discussion

The present study shows that the reduction of a test MEP response by a sub-threshold CS, referred to as SICI, is reduced in subjects with incomplete SCI compared to uninjured controls. This is in agreement with previous reports of SICI measured in two SCI subjects using a single CS intensity (Shimizu et al., 2000; Saturno et al., 2008). When the absolute TS intensity (in % MSO) was matched between the two groups, the absolute CS intensities producing peak SICI were similar, even though SICI remained reduced in the SCI subjects. Moreover, the relative modulation of the SICI recruitment curves at the incrementing CS intensities, as measured by SICI>50%, was also very similar between the SCI subjects and controls. We discuss that although the relative excitability profile of cortical SICI networks is unchanged after SCI, the effective inhibition of the MEP is reduced, either from cortical and/or sub-cortical mechanisms.

4.1. Why the recruitment properties for SICI is unaffected by SCI

From the first experiment, we noticed that when SICI was assessed using a TS near 125% of AMT in both groups, the actual CS intensities (in % MSO) that suppressed the test MEP were significantly higher in the SCI subjects than in the controls. This such a result agrees with reports using the technique of TMS-induced EMG suppression after SCI (Davey et al., 1998; Smith et al., 2000a). To identify the origin of this change, we first examined whether the increased threshold to inhibit the test MEP could be mediated by spinal inhibitory circuits activated by the damaged corticospinal pathway that required higher CS intensities to activate. Such a hypothesis seemed viable in light of the fact that motor cortex stimulation can activate inhibitory spinal interneurons to lower/hind limb muscles, even at sub-threshold CS intensities (see Fig. 2A; Cowan et al., 1986; Iles and Pisini, 1992; Nielsen et al., 1993; Nielsen and Petersen, 1995; Preston et al., 1967). However, unlike that observed for the MEP, a robust U-shaped pattern of H-reflex inhibition in the TA muscle was not produced by the CS, suggesting that the activation of spinal inhibitory pathways by the damaged corticospinal tract did not mediate the increase in threshold for SICI in the SCI subjects.

Because SICI depends on the interplay of the CS and TS intensity (Ilić et al., 2002), we subsequently examined whether the high TS intensity (~78% MSO) needed to evoke an appreciable test MEP in the SCI subjects could explain why SICI was evoked at higher CS intensities compared to controls. When we also used a high TS intensity in the control subjects (~79% MSO), the SICI recruitment curve (in % MSO) was shifted to the right (compare Figs. 1E and 3B) so that the peak SICI was aligned to the SCI data, suggesting that a high TS intensity contributed to inducing SICI at the higher CS intensities in the SCI subjects. Moreover, the induction of SICI>50% at the different CS intensities was similar (Fig. 3C and D), signifying that the relative excitability profiles of the inhibitory cortical networks were not altered by the spinal cord lesion.

4.2. Rationale for comparing CS and TS intensities in terms of % MSO

Because AMT occurred at ~63% MSO in the SCI subjects compared to 49–54% MSO in the uninjured controls, we rationalized that it would be useful to examine the actual MSO intensities used in the experiments since it provides a more similar comparison of motor cortex activation in both groups. For this reason, it is relevant to convert the group’s CS intensities from % AMT to % MSO. Likewise, using TS intensities set by the group’s average % MSO value should activate a more similar volume of motor cortex compared to intensities set by a percentage of the AMT. Thus, comparing group data at similar absolute stimulation intensities may be useful to show similarities (or detect differences) in SICI between two populations.

4.3. SICI is reduced by SCI

4.3.1. Possible cortical mechanisms

Considering that SICI networks preferentially inhibit higher-threshold, later I wave inputs (I2 waves or later), and not I1 waves (Di Lazzaro et al., 2001; Nakamura et al., 1997; Di Lazzaro et al., 1998; Hanajima et al., 1998), what then might have contributed to the weak SICI in the SCI subjects given that I2 and later wave inputs were likely recruited by the strong TS intensities? Although the relative excitability profile of the cortical SICI networks appears unchanged after SCI, the effective inhibition they impart onto cortical neurons may be reduced. After SCI, GABAergic inhibition is reduced in the spinal cord (Boullenguez et al., 2010; Norton et al., 2008), and a similar phenomenon may be occurring in the motor cortex. Alternatively, or in addition, the excitability of networks producing short-interval intracortical facilitation may also increase.
after SCI as a mechanism to enhance activation of residual corticospinal tract pathways. A preserved activation threshold to the CS but a reduced post-synaptic inhibition from inhibitory cortical networks could explain why the SICI recruitment curves in the SCI subjects occurred at similar CS intensities but were reduced in magnitude compared to controls.

4.3.2. Possible sub-cortical mechanisms

MEPs in leg muscles are usually contingent on the presence of at least four descending waves (i.e., D, I1, I2 and I3) consistent with the idea that MEPS require appropriate spatio-temporal summation at the motoneuron pool (Houlden et al., 1999). After incomplete SCI, MEPS are reduced because the amplitudes of these descending waves are likewise reduced. In addition, the descending waves are more desynchronized, i.e., chronodispersed, after SCI due to partial loss of axons and from demyelination (Liu et al., 1997; Nashmi and Fehlings 2001) so that the temporal dispersion of the corticospinal volley would also bring fewer motoneurons to firing threshold (Brouwer et al., 1992; Alexeeva et al., 1997; Calancie et al., 1999). Therefore, it is conceivable that a reduction in the amplitude of the descending I waves by SICI will have a reduced effect on a desynchronized corticospinal volley below the injury and ultimately, a reduced effect on the spatio-temporal summation at the motoneuron pool. Evidence supporting the downstream effect of desynchronization in the corticospinal tract on motoneuron recruitment has been shown in patients with pure hereditary spastic paraparesis, a condition that usually prolongs the central motor conduction time. In these individuals, the temporal modulation of the soleus H-reflex by TMS is much reduced because of the temporally-diffuse corticospinal inputs (SerranoV et al., 2008). Although to our knowledge there is no direct evidence to suggest that impaired corticospinal transmission hampers the ability to inhibit or facilitate the MEP, analogous to that shown for the H-reflex, it may be interesting to examine this idea in more detail.

In addition to axonal dysfunction of the descending corticospinal tract, there is recent evidence of peripheral motor axon dysfunction after SCI (Lin et al., 2007; Van De Meent et al., 2010), indicating that both central and peripheral pathways can contribute to the abnormal modulation of the MEP. Coincidentally, muscle fibre conversion also occurs after SCI (reviewed in Biering-Sorensen et al., 2009; Stein et al., 1992), adding to the complexity of comparing corticospinal recruitment and amplitude of SICI between groups, even when controlling for the MEP amplitude as a percentage of the maximal M-wave (see Lackmy and Marchand-Pauvert, 2010).

4.3.3. SICI in leg and hand muscles after SCI

Based on the peak-to-peak MEP amplitude, SICI was on average threefold larger in the hand than in the leg (23.3% vs. 7.6% inhibition, respectively). These findings suggest that SICI after incomplete SCI is more readily elicited in an intrinsic hand muscle than in an ankle flexor, even when the upper limb corticospinal projections were more severely compromised. Here, we discuss a few factors that might explain these findings.

Firstly, impaired neuronal conduction in the corticospinal tract may have contributed to these differences in SICI. Although the prolongation of the MEP latency was similar in both muscles (i.e., 8.2 and 9.4 ms), it is nonetheless plausible that the descending volleys to the leg motoneurons were more weakly synchronized given the longer central pathway (i.e., the longer the volleys had to travel, the more asynchronous they became). Secondly, the relative representation of direct corticomotoneuronal vs. indirect corticospinal connections may have contributed to the difference in SICI (see Abbuzzese et al., 1999). However, in keeping with the fact that both ankle flexor and distal hand muscles possess many direct corticomotoneuronal connections (Brouwer and Ashby, 1992), and strength differences in SICI have not always been reported in hand, arm, foot, thigh and trunk muscles in control subjects (Chen et al., 1998), such an explanation is currently less likely. Thirdly, the recruitment of a different proportion of fast and slow TA and FDI motoneurons by the TS may also account for the differences in the SICI amplitude between the two muscles (see Houlden et al., 1999; Di Lazzaro et al., 2001; Nakamura et al., 1997; Di Lazzaro et al., 1998; Hanajina et al., 1998), suggesting that this influence was minimal.

5. Conclusion

The present study shows that although the relative excitability profile of SICI networks is unchanged after incomplete SCI, the effective inhibition that these neurons impart on the muscle MEP is reduced. It is possible, then, that the inability to effectively terminate voluntary contractions, which leads to unwanted muscle activity, may in part be related to the diminished SICI that occurs after spinal cord injury.

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