Primary motor cortex inhibition in spinal cord injuries

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Abstract

OBJECTIVES AND METHODS: Excitability changes in the primary motor cortex in 17 spinal-cord injured (SCI) patients and 10 controls were studied with paired-pulse transcranial magnetic stimulation. The paired pulses were applied at inter-stimulus intervals (ISI) of 2 ms and 15 ms while motor evoked potentials (MEP) were recorded in the biceps brachii (Bic), the abductor pollicis brevis (APB) and the tibialis anterior (TA) muscles.
RESULTS: The study revealed a significant decrease in cortical motor excitability in the first weeks after SCI concerning the representation of both the affected muscles innervated from spinal segments below the lesion, and the spared muscles rostral to the lesion. In the patients with motor-incomplete injury, but not in those with motor-complete injury, the initial cortical inhibition of affected muscles was temporarily reduced 2–3 months following injury. The degree of inhibition in cortical areas representing the spared muscles was observed to be smaller in patients with no voluntary TA activity compared to patients with some activity remaining in the TA. Surprisingly, motor-cortical inhibition was observed not only at ISI 2 ms but also at ISI 15 ms. The inhibition persisted in patients who returned for a follow-up measurement 2–3 years later.
CONCLUSION: The present data showed different evaluation of cortical excitability between patients with complete and incomplete spinal cord lesion. Our results provide more insight into the pathophysiology of SCI and contribute to the ongoing discussion about the recovery process and therapy of SCI patients.

INTRODUCTION

Spinal cord injury (SCI) entrains changes not only in the spinal cord but also in the brain. Brain reorganization has been demonstrated with functional imaging, often with conflicting results as to the character and topography of the changes (Kokotilo *et al.* 2009). Alternatively, central nervous system (CNS) reorganization can be investigated with electrophysiological methods measuring activity

ORIGINAL ARTICLE

in the excitatory and inhibitory circuits. A convenient method, well tolerated by patients, is transcranial magnetic stimulation (TMS). It consists in stimulating the primary motor cortex with a magnetic pulse across the skull bone causing excitation of cortico-spinal neurons via local inter-neurons (Amassian & Cracco 1987). The descending electrical activity can be detected by electromyography (EMG) as motor-evoked potentials (MEP) in skeletal muscles. With single-pulse TMS, SCI patients show higher MEP thresholds, longer latencies and durations than healthy subjects (Brouwer *et al.* 1992; Chang & Lien 1991), as well as modified cortico-spinal recruitment and facilitation of the motoneuron pool (Davey *et al.* 1999). Machida *et al.* (1991) have noted that weakness of voluntary muscle contraction correlated with delayed or absent responses to TMS but the relationship between electrophysiological findings and the clinical picture is not always clear (Brouwer *et al.* 1992; Ellaway *et al.* 2011).

A disadvantage of the single-pulse TMS technique is that it cannot distinguish between altered function at the spinal level and/or at the brain cortex (Kobayashi & Pascal-Leone 2003). To explore the cortical contribution of the cortico-spinal pathway excitability in SCI patients, it is more appropriate to use e.g. the paired-pulse TMS (pp-TMS) technique described by Kujirai *et al.* (1993) for the upper limbs and by Stokic *et al.* (1997) for the lower limbs. The pp-TMS consists of a combination of a sub-threshold conditioning stimulus (CS) with a supra-threshold test stimulus (TS) which can reveal different effects of cortical interneurones on cortical output, as mentioned below in the Methods section.

Since SCI patients showing clinical recovery make progress mainly within the first year following injury (Curt *et al.* 2008), it can be expected that intensive CNS remodeling takes place mainly at the acute and post-acute stage. Therefore, we recruited patients as soon as possible, within weeks following their accident. In certain patients, we were able to repeat the measurements 2–3 years later, for longitudinal comparison. We were interested in the relationship between the motor cortex excitability and the clinical motor handicap.

METHODS

Subjects

17 SCI patients (4 women, 13 men) and 10 age-matched healthy volunteers (4 women, 6 men) participated in the study (Table 1). All subjects gave their written informed consent with the experiment, and conform to the requirements of the Declaration of Helsinki. According to the American Spinal Injury Association (ASIA) impairment scale (AIS), 9 patients had a motor-complete lesion (AIS A and B; 2 women, 7 men) and 8 had a motor-incomplete lesion (AIS C and D; 2 women, 6 men). Following their injury, the patients had to undergo spinal surgery for the stabilization of their vertebral column. The first clinical and electrophysiological measurements were performed on average 43 days after the injury (Mt1) and again approximately 30 days later (Mt2). In 10 patients (5 motor-complete, 5 motor-incomplete) we were able to perform the measurements a third time 2–3 years later (Mt3).

Experimental paradigm

All measurements were performed with the subjects lying in a supine position.

First, the maximum voluntary contraction (MVC) of each investigated muscle was determined from three short isometric contractions. MVC was expressed as the mean peak EMG amplitude from the three trials. Then, we performed supramaximal electrical stimulation of nerves supplying the investigated muscles (maximum M-wave) to verify that there was no significant loss of peripheral motor fibers between measurements. After a pause of several minutes, single-pulse TMS was administered to determine the cortico-spinal recruitment in response to stimuli of a different intensity. Finally pp-TMS was performed to measure cortical excitability. Two measurements at an interval of about 30 days were performed in healthy controls and the two sets of data were not statistically different. Only data from Mt1 were used for comparison with patients.

Clinical evaluation

Clinical evaluation was performed on the day of electrophysiological measurements.

Manual muscle test (MMT) was performed on the biceps brachii (Bic), the abductor pollicis brevis (APB), and the tibialis anterior (TA) muscles. MMT was performed in a total of 51 muscles. In this paper, plegic and paretic muscles (force 0–4) are referred to as "affected" and muscles innervated from spinal segments above the lesion (force 5) as "spared". Nineteen muscles were spared rostral to the lesion and 32 were affected. Sixteen affected muscles were found in patients with motor-complete lesions and 16 in incomplete lesions. Depending on the extent of their motor and sensory deficit, the patients were assigned an ASIA score. The neurological level of injury was defined as the most caudal segment of the spinal cord with normal sensory and motor function on both sides of the body. Medical staff administering the clinical tests was not familiar with the TMS data collected by their collaborators and vice versa.

EMG Recording

EMG activity was recorded over the Bic, APB, and TA muscles using Ag-AgCl surface electrodes (1 cm in diameter). The electrodes were placed 2 cm apart in a bipolar arrangement, longitudinally over the muscle bellies. The EMG signal was amplified (× 3000), band-pass filtered (10–1000 Hz), digitized at a sampling rate of 5 kHz and stored in the Dantec Counterpoint electromyograph (Dantec, Skovlunde, Denmark) for off-line analysis. The rectified and smoothed EMG (100-ms time constant) was displayed on an oscilloscope to pro-

vide feedback about background muscle activity prior to TMS.

Supramaximal electrical stimulation of the peripheral nerves was realized to make sure that the measured MEP parameters reflected events in the central part of the cortico-muscular pathway. Hand-held surface electrodes were used to elicit M-waves in Bic (stimulation of the musculocutaneous nerve in the axillary fold), APB (stimulation of the median nerve at the wrist) and TA (stimulation of the common peroneal nerve in the popliteal fossa). The stimuli were rectangular pulses, 500 μs in duration, delivered by the stimulator of the Dantec electromyograph. The size of the M-wave was measured in terms of its peak-to-peak amplitude.

Transcranial magnetic stimulation

Excitability of the primary motor cortex contralateral to the recorded muscles was assessed using the pp-TMS technique, as described by Kujirai *et al.* (1993). The pp-TMS consists of a combination of a sub-threshold CS and supra-threshold TS which can expose different effects of cortical interneurones on cortical output. The effect of the paired pulse on MEP amplitude depends on the length of the inter-stimulus interval (ISI) and the intensity of each stimulus (Ilic *et al.* 2002). The CS can reduce the capacity of TS to produce a descending potential in the cortico-spinal tract if it precedes the TS by less than 5 ms. This inhibitory effect, measurable as a reduction of MEP amplitude, has been named short intra-cortical inhibition (SICI). If the ISI lies between 5 and 20 ms, the CS increases the effect of the TS and the phenomenon is known as intra-cortical facilitation (ICF).

A double-cone coil with loop diameters of 110 mm was used to elicit MEP in all muscles investigated. With this coil, MEP could be elicited from plegic or paretic muscles where stimulation with a figure-of-eight coil was inefficient or uncomfortable because of high stimulation intensities. First, we verified in the upper limbs of healthy subjects that cortical excitability data obtained with both types of coil were comparable. The coil was powered by two magnetic stimulators (Mag-Stim 2002) connected through a Bistim module (The Magstim Company Ltd., Wales, U.K.).

The optimal site for TMS stimulation was chosen as the coil position from which an MEP of maximal amplitude could be elicited at minimal stimulation intensity. The coil, with current flowing in the antero–posterior direction at the centre of its root (inducing postero-anterior current in the cortex), was moved in small steps over the scalp to mark the motor "hot spot". In all subjects, the "hot spot" was situated over the contralateral central sulcus region for muscles of the upper extremity (medial for Bic and lateral for APB) while for TA it was situated about 5 cm posterior to the vertex slightly contralateral to the sagittal mid-line.

In each subject, we started TMS by determining adequate stimulation intensities. Resting motor threshold (RMT) was defined as the minimum output of the stimulator that induced a reliable MEP in at least five out of ten trials when the muscle was completely relaxed. Active motor threshold (AMT) was identified similarly during a tonic contraction of the target muscle (20% MVC). Despite visual and acoustic EMG feedback, it was difficult for some SCI subjects to maintain a constant level of contraction and we tolerated a fluctuation 15–25%. The instruction to activate the target muscle was also given to patients who had no voluntary control of their plegic muscles. Subsequently, we determined the TS intensity for pp-TMS. Magnetic stimuli at intensities of 90, 110, 130, and 150% AMT were administered to the primary motor cortex in a random order while the target muscles were active at 20% MVC. Peak-to-peak amplitude values of three MEPs at each intensity were averaged and plotted against the stimulus intensity (expressed as a percentage of AMT). In this way, we obtained the initial part of a cortico-spinal recruitment curve to make sure that CS was subthreshold (80% AMT) and TS produced MEPs sensitive to facilitation or inhibition. We verified that maximal MEP amplitude was never reached at 130% AMT but did not attempt to evoke maximal MEPs since the required intensities were often beyond tolerance. MEP peak-to-peak amplitude and latency, defined as the time between the stimulus artifact and the onset of MEP, were determined visually off-line from three individual sweeps at stimulus intensity 150% AMT.

For pp-TMS, the CS intensity was set at 80% AMT and the TS intensity at 130% AMT. The patients were asked to pre-contract their muscles at 20% MVC. Healthy subjects were tested both with pre-contraction and at rest. The two magnetic stimuli were delivered through the same coil at ISIs of 2 ms and 15 ms to measure SICI and ICF. Five single-test stimuli and five paired CS-TS stimuli were applied at random order, at each of the two ISIs. Peak-to-peak MEP amplitude was measured on each individual sweep and the values were averaged. The amplitude of the conditioned MEPs was expressed as a percentage of the mean size of the test MEPs.

Statistical analysis

Clinical scores were compared using the independent samples t-test (2-sided, equal variances not assumed). One-way ANOVA with factor "muscle condition" was used to test for differences in single-pulse MEP parameters (RMT, AMT, latency, amplitude) and in the excitability of the primary motor cortex. Three "muscle conditions" were defined for each muscle (Bic, APB, TA): 1 – affected (paretic or plegic), 2 – spared (rostral to the lesion), and 3 – controls. If a significant variance in mean values was detected, the Dunett's T3 test was applied to specify which "muscle condition" was responsible for the difference. The independent samples t-test (2-sided, equal variances not assumed) was performed to test for differences in cortical excit-

ability between spared muscles in subjects with paretic TA and in subjects with plegic TA. This test was also used to compare cortical excitability of affected muscles between motor-completely and incompletely injured subjects, at Mt1, Mt2 and Mt3. For this analysis, the affected Bic, APB and TA data were grouped together, after verification with ANOVA that there was no difference in their relative excitability neither at ISI 2 ms nor at ISI 15 ms at any point in time. The level of statistical significance was set at 0.05.

RESULTS

Clinical evaluation

In subjects with motor-incomplete lesion, all affected muscles (16/16) increased their force by at least 1 point between Mt1 and Mt2 while in patients with motor-complete lesion the increase by 1 point was observed only in 25% (4/16) of the affected muscles. Between Mt2 and Mt3, the improvement was 45% (5/11) in motor-incompletely lesioned and 30% (3/10) in motor-completely lesioned. The ASIA score increased by 31±5 (mean ± SE) between Mt1 and Mt2 in patients with motor-incomplete SCI, which was significantly more (p=0.0005) than in patients with motor-complete lesion (4±1.3). Between Mt2 and Mt3 the score increase was 17±5.5 for incompletely lesioned patients and 5±1.8 for completely lesioned, which was not significantly different (p=0.081). The MMT and ASIA data show better clinical recuperation in motor-incompletely injured, occurring mainly in the early period.

Transcranial magnetic stimulation

Single-pulse TMS

The MEP values obtained with single-pulse TMS were presented in Table 2. The mean RMT was not significantly different between the "muscle conditions" (as defined in the section Statistical analysis) for any muscle. The AMT in affected TA was significantly higher than controls at all three measurements (pMt1=0.025, pMt2=0.011, pMt3=0.0072). The latency was significantly longer in the affected APB and TA compared to controls at all three measurements

Tab.1. SCI subjects' characteristics.

No.	Sex	Age	Day after injury			Cause of injury	NLI	AIS	MMT			Medication
			Mt1	Mt2	Mt3				Bic	APB	TA	
1	M	64	49	77	983	paragliding	C4	C	2-3-3	0-2-2	2-4-4	citalopram, metamizol, zolpidem, alprazolam, promethazine, baclofen, mirtazapine
2	M	44	28	64	682	car accident	C4	C	4-5-5	0-1-3	0-3-3	citalopram, nimesulide, clonazepam, tramadol
3	F	21	59	90	1253	car accident	C5	B	3-4-5	0-0-0	0-0-0	mirtazapine, alprazolam, zolpidem, metamizole
4	M	26	67	95	NA	car accident	C5	B	3-4	0-0	0-0	citalopram, baclofen, tizanidine, tetrazepam, alprazolam, diclofenac
5	M	36	49	80	NA	jump into water	C5	D	4-5	2-3	3-4	citalopram, gabapentin, tizanidine, propiverine, clonazepam
6	M	33	19	59	1007	motorbike acc.	C6	D	5-5-5	5-5-5	0-3-4	citalopram, alprazolam, zolpidem
7	M	35	50	84	861	car accident	C6	D	4-5-5	1-3-4	1-2-4	citalopram, clonazepam, baclofen, gabapentin
8	M	27	68	104	1065	car accident	C7	A	5-5-5	2-2-3	0-0-0	citalopram, alprazolam
9	M	22	45	86	820	car accident	C7	A	2-4-5	0-0-0	0-0-0	alprazolam, mirtazapine
10	F	18	40	65	NA	car accident	T5	D	5-5	5-5	2-4	citalopram, alprazolam, clonazepam
11	M	27	17	44	NA	car accident	T6	A	5-5	5-5	0-0	citalopram, baclofen, nimesulide, metamizole
12	M	34	23	47	1178	car accident	T11	A	5-5-5	5-5-5	0-0-0	citalopram, propiverine
13	F	45	32	75	NA	fall from height	L1	D	5-5	5-5	2-4	metamizole
14	F	19	34	62	766	fall from height	L1	A	5-5-5	5-5-5	0-1-1	citalopram, oxybutynine
15	M	20	42	71	NA	car accident	L1	A	5-5	5-5	0-0	citalopram, tramadol, nimesulide
16	M	23	31	71	1278	tram accident	L3	D	5-5-5	5-5-5	2-4-5	citalopram
17	M	21	79	119	NA	car accident	L3	A	5-5	5-5	0-0	citalopram, bromazepam, gabapentin, diazepam

M – male, F – female; Mt1, Mt2, Mt3 – 1st, 2nd and 3rd measurement sessions; NLI – neurological level of injury; C, T, L – cervical, thoracic and lumbar spinal cord with segment number; AIS – ASIA impairment scale, MMT – manual muscle test; Bic – biceps brachii, APB – abductor pollicis brevis, TA – tibialis anterior. NA – data not available

(APB: pMt1=0.0016, pMt2<0.0001, pMt3=0.0054; TA: pMt1=0.042, pMt2=0.049, pMt3=0.01). MEP amplitude was significantly lower in the affected APB and TA (APB: pMt1<0.0001, pMt2=0.002, pMt3=0.014; TA: pMt1<0.0001, pMt2<0.0001, pMt3=0.0024). In general, the affected muscles showed a larger variability in MEP parameters. The trend towards higher motor threshold, longer latency and lower amplitude was not observed in the spared muscles.

Paired-pulse TMS

Paired-pulse TMS in control subjects revealed cortical inhibition at ISI 2 ms and neither inhibition nor excitation at ISI 15 ms. In SCI subjects, pp-TMS at ISI 2 ms and ISI 15 ms produced MEP inhibition both in the affected and the spared muscles (Figure 1). At Mt1, a significant difference in cortical excitability among normal, spared and affected muscles was found for Bic at ISI 2 ms (pBic=0.003) and for Bic, APB and TA at ISI 15 ms (pBic<0.0001, pAPB=0.0006, pTA=0.0003). The Dunett T3 test revealed that affected and spared muscles were significantly inhibited compared to the control muscles. There was no significant difference in cortical excitability between spared and affected muscles (Figure 2). The same comparison made one month later (Mt2) gave similar results: at ISI 2 ms, the

Tab. 2. MEP parameters measured with single-pulse TMS (means and standard errors).

			Bic		APB		TA	
			Mean	SE	Mean	SE	Mean	SE
RMT (% MSO)	Mt1	Control	42.8	2.3	42.7	2.4	47.6	1.6
		Spared	41.0	2.3	44.5	4.7	NA	NA
		Affected	39.3	5.6	55.0	7.9	56.7	7.5
	Mt2	Spared	41.6	1.8	43.8	4.4	NA	NA
		Affected	39.6	5.1	52.8	7.8	55.2	8.5
	Mt3	Spared	38.0	3.3	37.0	5.5	NA	NA
		Affected	44.4	1.7	46.4	5.3	58.6	5.9
AMT (% MSO)	Mt1	Control	36.2	2.2	37.0	2.0	40.4	1.5
		Spared	32.4	2.2	38.4	4.2	NA	NA
		Affected	33.2	4.4	46.9	6.3	58.6	7.3
	Mt2	Spared	34.6	2.4	37.1	4.5	NA	NA
		Affected	34.2	4.3	46.2	6.5	62.6	6.9
	Mt3	Spared	32.0	3.6	32.0	4.0	NA	NA
		Affected	38.4	2.5	40.7	5.1	56.9	5.5
Latency (ms)	Mt1	Control	13.5	0.3	22.2	0.5	32.2	0.7
		Spared	13.5	0.4	22.4	0.6	NA	NA
		Affected	12.8	0.4	38.5	5.5	49.2	7.7
	Mt2	Spared	13.7	0.3	22.3	0.5	NA	NA
		Affected	13.2	0.2	35.6	3.0	50.4	7.5
	Mt3	Spared	13.8	0.5	21.9	1.2	NA	NA
		Affected	14.2	0.6	43.4	7.6	50.4	6.6
Amplitude (mV)	Mt1	Control	3.3	0.3	3.5	0.4	0.8	0.0
		Spared	3.5	0.3	3.1	0.5	NA	NA
		Affected	3.7	1.4	0.6	0.3	0.2	0.0
	Mt2	Spared	3.6	0.5	2.9	0.8	NA	NA
		Affected	3.7	1.1	0.7	0.3	0.3	0.1
	Mt3	Spared	3.2	0.7	3.5	1.2	NA	NA
		Affected	3.4	1.2	1.1	0.7	0.4	0.1

RMT – resting motor threshold; AMT – active motor threshold; MSO – maximal stimulator output; Mt1, Mt2, Mt3 – 1st, 2nd and 3rd measurement sessions; Bic - biceps brachii, APB - abductor pollicis brevis, TA - tibialis anterior; NA – data not available

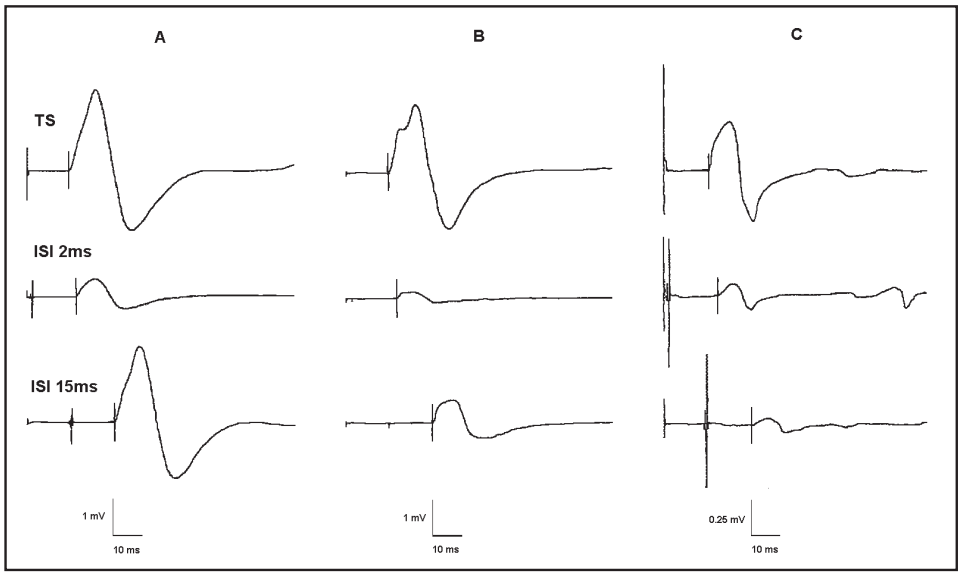

Fig. 1a–c. Biceps brachii MEPs (single sweeps) in a healthy control subject (A), a paraplegic subject (B), and a C4-lesioned subject (C) at the first measurement. Upper row: MEPs generated with single-pulse test stimulus (TS). Middle and lower row: MEPs generated with paired-pulse TMS at ISI of 2 ms and 15 ms, respectively. Vertical lines: stimulation artifacts and MEP onset. Note the inhibition not only at ISI 2 ms but also at ISI 15 ms in the affected and the spared muscles in the patients.

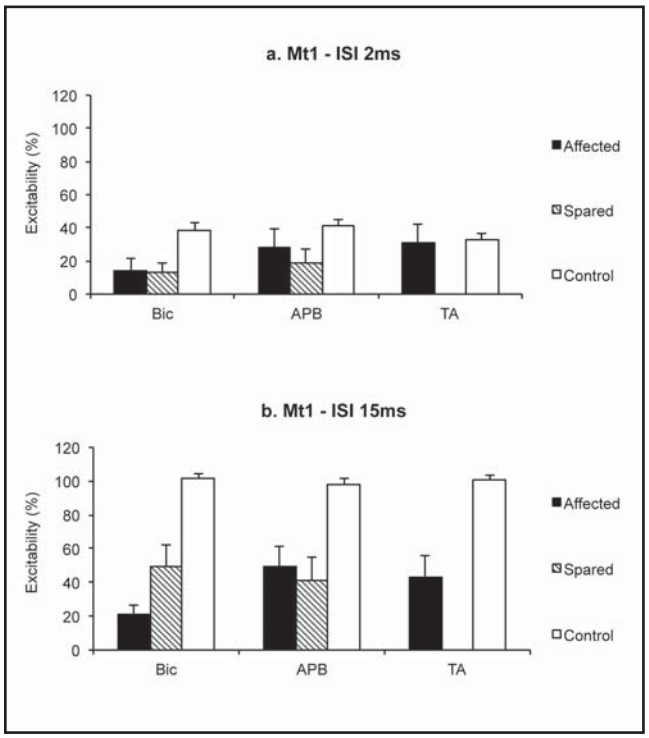

Fig. 2a–b. Primary motor cortex excitability at Mt1 (mean values and standard errors). (a) At ISI 2 ms, the inhibition in the biceps area is significantly stronger for affected and spared muscles compared to controls. (b) At ISI 15 ms, the Bic, APB and TA cortical area shows a significantly lower excitability than in controls.

variance control-spared-affected was found significant only in Bic (pBic=0.043). At ISI 15 ms, the variance was significant for all muscles (pBic<0.0001, pAPB=0.0007, pTA=0.0053). Again, significantly stronger inhibition was found between affected muscles vs. controls and spared muscles vs. controls while affected muscles were not different from spared ones.

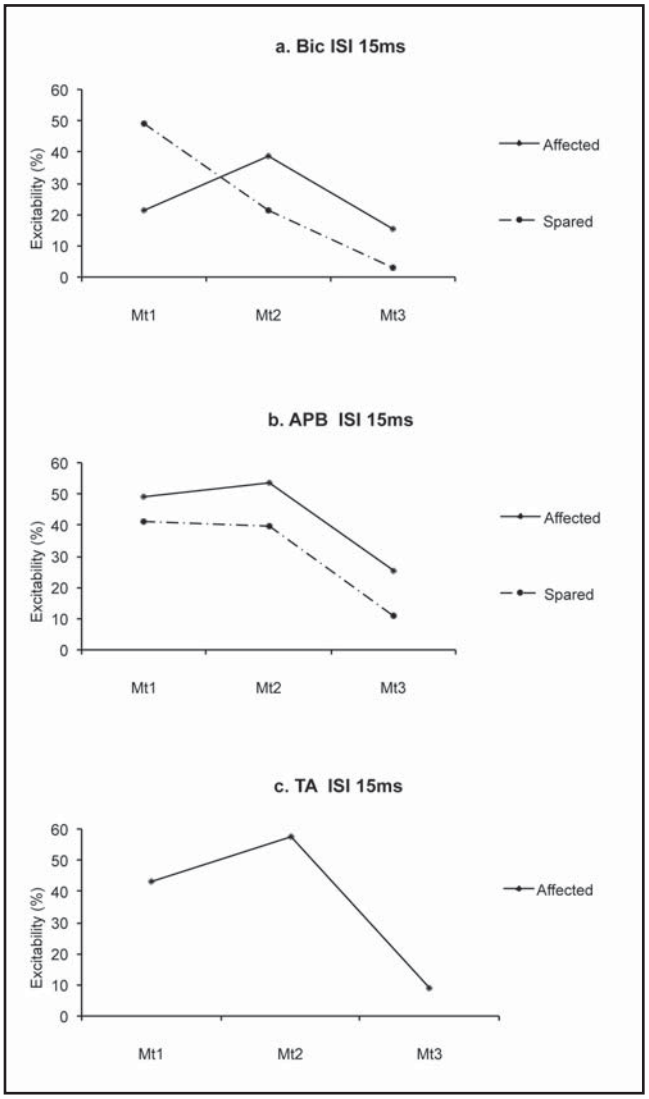

Fig. 3a–c. Evolution of primary motor cortex excitability (mean values) measured at ISI 15 ms. Bic, APB and TA areas are under inhibition at Mt1. Note the rising trend for the affected muscles and the falling trend for the spared muscles between Mt1 and Mt2, and the falling trend for all muscles between Mt2 and Mt3.

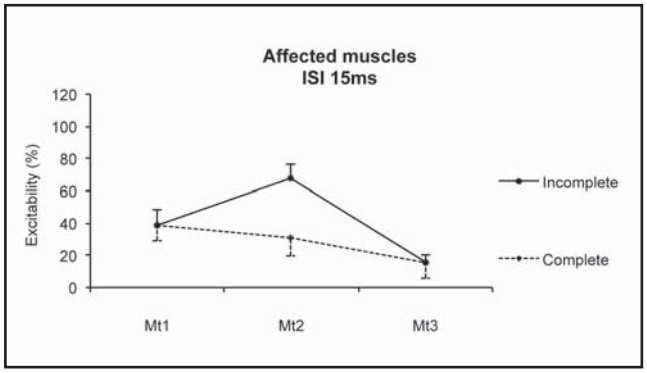

Fig. 4. Primary motor cortex excitability (mean values and standard errors) of affected muscles, measured at ISI 15 ms. Note the significant difference between motor-completely injured and motor-incompletely injured patients at Mt2.

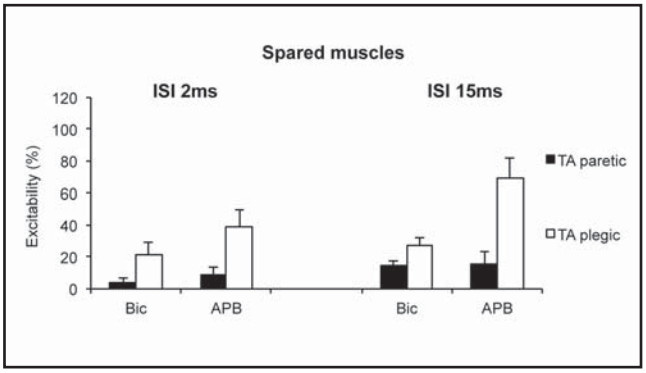

Fig. 5. Primary motor cortex excitability (mean values and standard errors) of spared muscles at Mt2, measured at ISI 2 ms and at ISI 15 ms. The inhibition is stronger in patients with paretic TA compared to patients with plegic TA.

Searching for longitudinal information about cortical excitability following SCI, trends between Mt1, Mt2 and Mt3 were compared. At ISI 2 ms, the initial MEP inhibition revealed no clear trend for any muscle between Mt1 and Mt2 (not shown). At ISI 15 ms, the spared muscles showed increasing cortical inhibition between Mt1 and Mt2, while an opposite trend (decreasing inhibition) was detected in the affected muscles (Figure 3). Between Mt2 and Mt3, increasing inhibition was seen in all muscles (affected and spared), significant in TA both at ISI 2 ms (p=0.015) and ISI 15 ms (p=0.005).

Inspection of individual data showed that the reduction of inhibition of the affected muscles between Mt1 and Mt2 concerned only motor-incompletely lesioned subjects (Figure 4). The difference in inhibition from motor-completely lesioned at Mt2 was nearly significant for ISI 2 ms (p=0.06) and significant for ISI 15 ms (p=0.015).

The extent of the spinal lesion had an influence on the excitability of cortical areas representing the spared muscles rostral to the lesion (Figure 5). Biceps cortical excitability in 5 patients with plegic TA was compared with 5 patients with paretic TA. Similarly, APB cortical excitability was compared in 4 patients with plegic and 4 with paretic TA. There was more cortical inhibition in the Bic or APB area if the TA was paretic compared with when the TA was plegic. This tendency at Mt1 became statistically significant at Mt2 (pBic ISI 2 ms=0.043, pAPB ISI 2 ms=0.044, pBic ISI 15 ms=0.042, pAPB ISI 15 ms=0.018). Only 2 patients meeting the criteria of plegic TA and never-affected upper arm muscles were present at Mt3, making statistical conclusions impossible.

DISCUSSION

This study revealed a significant increase of cortical inhibition occurring in the first weeks after SCI and persisting for at least two years. The increase concerned not only affected muscles but also spared muscles rostral to the lesion. In motor-incompletely injured patients, the initial cortical inhibition of affected muscles was temporarily reduced about 2–3 months after injury. The excitability of cortical areas representing spared muscles rostral to the lesion was related to the degree of TA impairment.

Single-pulse TMS

Our patients showed post-injury evolution typical of those observed in the SCI population (Curt *et al.* 2008). Both the MMT and the ASIA scores showed better recovery in patients with incomplete – as opposed to patients with complete – lesion. In the early post-injury period, it was difficult to record MEPs in the affected muscles, especially in the TA. In some motor-completely injured subjects, the TA MEPs were still missing at the second session and in some they never reappeared. The fact that we were sometimes also able to elicit MEPs in patients with clinically motor-complete SCI confirms the presence of axons capable of conduction descending through the lesion (Gianutsos *et al.* 1987). In addition to high threshold, MEPs in affected muscles also showed longer latencies, smaller amplitudes and often a polyphasic form, as compared to healthy subjects. These results are in concert with other studies reporting altered MEP parameters in SCI (Clarke *et al.* 1994; McKay *et al.* 2005; Brouwer *et al.* 1992; Davey *et al.* 1998).

We checked that the M-wave amplitudes in our SCI patients were comparable to those of normal subjects and they did not change over time (results not shown). Therefore, it is improbable that the changes responsible for the altered MEP took place in the peripheral nerves.

Paired-pulse TMS

To our knowledge, only three studies have used pp-TMS to investigate cortical excitability of muscles below the spinal lesion. Shimizu *et al.* (2000) stimu-

lated the hand primary motor cortex of a woman with SCI related to her Machado-Joseph disease (fall due to ataxia). The authors reported motor cortical reorganization or hyperexcitability because they found significant MEP inhibition only at ISI 3 ms while in healthy controls the inhibition was significant at ISIs of 1, 2, 3 and 5 ms. Facilitation was found at ISI 10 ms in both the patient and controls. Saturno *et al.* (2008) used pp-TMS to study cortical excitability of a hand muscle in one patient with low cervical ischemic myelopathy. No MEP inhibition was observed at ISIs 2 and 3 ms, while a significant facilitation was seen at 5 and 10 ms (not at 15 ms). The lack of inhibition at short ISIs suggested a reduced inhibitory activity in the motor cortex. In agreement with these reports, Roy *et al.* (2011) found reduced SICI over a range of CS intensities in hand and leg muscles of patients with incomplete SCI.

It is interesting to compare the TMS results with information obtained with functional brain imaging, keeping in mind that the two methods do not measure the same cortical events. Using fMRI, Jurkiewicz *et al.* (2007) studied wrist extension movements in quadriparetic SCI patients, 1, 3, 6, and 12 months after injury. In the early post-SCI period, little activation within the primary motor cortex was present but a progressive enlargement in the activation volume was seen during motor recovery (Jurkiewicz *et al.* 2007; Duggal *et al.* 2010). Conversely, SCI subjects with poor recovery showed a reduced volume of activation (Jurkiewicz *et al.* 2010) which is consistent with previous studies in chronic SCI patients (Turner *et al.* 2001; Sabbah *et al.* 2002; Cramer *et al.* 2005). These results suggest that early after SCI the primary motor cortex corresponding to the weak body parts decreases its activity and its later activity is related to clinical outcome. The decrease of the activation volume can be due to a reduction of the absolute number of cortical neurons but it may also reflect their reduced excitability, as suggested by our pp-TMS results.

We were surprised to see that, in the present study, the inhibition concerned not only the motor cortex representations of the affected muscles but also those of unaffected muscles rostral to the spinal lesion. A review of TMS literature dealing with spared muscles in SCI provides conflicting results. Some authors (applying single-pulse TMS) suggest expansion and enhanced excitability of the cortico-spinal pathways reaching the spared muscles, while some do not (Levy *et al.* 1990; Topka *et al.* 1991; Streletz *et al.* 1995; Brouwer & Hopkins-Rosseel, 1997; Laubis-Herrmann *et al.* 2000; Lotze *et al.* 2006). Using pp-TMS, Krause *et al.* (2007) found no difference in the cortical excitability of the spared first dorsal interosseus muscle between thoracic-SCI patients and healthy subjects (the patients showed longer CSP with single-pulse TMS). Functional imaging studies focused on spared muscles provide equally varied findings. Both presence and absence of expansion and/or shift of cortical representations of spared muscles have been reported (Bruehlmeier *et al.* 1998; Curt *et al.* 2002; Mikulis *et al.* 2002; Lotze *et al.* 2006).

The opinion differences regarding motor cortical changes following SCI are probably related to SCI etiology, level and completeness of lesion, time elapsed since injury, etc. Differences in TMS experimental paradigm can also contribute to different outcomes. Ridding & Rothwell (1997) reported that cortical map areas and the slope of the MEP recruitment curves in patients with ischemic arm anaesthesia or amputation increased at rest but not during voluntary activity. Similarly, Lotze *et al.* (2006) found smaller MEP amplitudes in SCI patients than in controls if spared upper limb muscles were pre-contracted, as opposed to rest. Brouwer & Hopkins-Rosseel (1997) found no evidence of motor area alteration among their SCI patients with TMS of pre-contracted spared muscles.

In the present study, we opted for stimulating the cortex with muscle pre-contraction for two reasons. First, the facilitation allowed the recording of MEPs in SCI that were small or absent in the relaxed state. Second, by monitoring the background EMG, we controlled for the level of cortico-spinal excitation at the moment of stimulation. We observed that contraction of the target muscle in our healthy subjects abolished ICF at ISI 15 ms, compared with the relaxed state (Kujirai *et al.* 1993; Ridding *et al.* 1995). It is possible that muscle activation is also the reason why our results in SCI patients differ from some pp-TMS studies performed in a relaxed state (Schimizu *et al.* 2000; Saturno *et al.* 2008; Krause *et al.* 2007). However, this argument is not applicable in the case of the recent study by Roy *et al.* (2011) who found reduced SICI in the cortex representing affected hand or leg muscles in SCI patients maintaining a tonic contraction. Although the conditions of their experiment resemble those in the present study, it has been demonstrated that even very small differences in CS and TS intensities or ISI can influence SICI or ICF (Sanger *et al.* 2001; Daskalakis *et al.* 2002; Hanajima *et al.* 2003; Peurala *et al.* 2008; Wagle-Shukla 2009; Garry & Thomson 2009; Saisanen *et al.* 2011). Additionally, it should also not be excluded that the difference in time lapse since injury contributed to different outcomes of the two studies.

We encountered cortical inhibition not only at ISI 2 ms, as expected, but also at ISI 15 ms. Therefore, we tested whether the inhibition at ISI 15 ms was caused artificially by the shape of the double-cone coil. The coil generates maximal stimulation at its central part but its wings could have caused unwanted stimulation of the distant cortex with an inhibitory effect on the area of interest. However, we observed similar inhibition when checking with a focal figure-of-eight coil whose wings are not in contact with the scalp. It should also be noted that we did not observe inhibition at ISI 15 ms with the double-cone coil in control subjects. Therefore, the inhibition at ISI 15 ms was not related to the stimulation technique.

Given the global character of the cortical inhibition (inhibition at ISI 2 ms and ISI 15 ms both in affected and non-affected muscles) one should also consider the possible influence of a general condition the patients were experiencing in connection to SCI. Our patients underwent physiotherapy according to Vojta (Vojta & Peters 1992), Kolar (Kolar & Kobesova 2010) or Bobath (Bobath 1990), received neuro-medication (Ziemann 2011) and experienced increased fatigue (Benwell *et al.* 2006). The spinal injury was perceived by some as painful which could have influenced cortical excitability (Dubé & Mercier 2011).

Assuming that the marked primary motor cortex inhibition seen in this study is a genuine physiological response to the spinal lesion, we present several reflections on its existence: It is possible that the inhibition may, for example, represent a cortical mechanism counterbalancing spinal hyperexcitability setting in towards the end of the spinal shock (Little *et al.* 1999; Mailis & Ashby 1990; Calancie *et al.* 1993; Bennett *et al.* 2004; Gorassini *et al.* 2004), related to such negative phenomena as uncontrollable clonus of a limb or painful muscle spasms. On the other hand, hyperreflexia may have some beneficial effect on functional recovery (Pearson 2001). Interestingly, the transient reduction of cortical inhibition of affected muscles in our motor-incompletely injured patients coincided in time (Mt2) with an increase of muscle tone and the return of some voluntary control. Our Mt3 data suggest that 2–3 years following SCI, the primary motor cortex is under strong inhibition coinciding with little clinical improvement.

The fact that the cortical inhibition concerned also the spared muscles rostral to the lesion may be related to the extent of expansion of the spared cortex toward the de-efferented cortex, as reported by others (Levy *et al.* 1990; Topka *et al.* 1991; Bruehlmeier *et al.* 1998). If such expansion occurred in our patients, the inhibition in the expanding areas could have helped maintain the total motor drive to spared muscles within usual limits. In the present study, the cortical inhibition of spared muscles was less pronounced in patients with plegic TA than in patients with some voluntary control over the leg muscle. The difference became statistically significant at Mt2, which was also the period of reduced inhibition in the areas representing affected muscles in motor-incompletely lesioned subjects. It is possible that the amount of excitatory activity in the motor areas innervating the affected muscles influences the neighbouring spared-muscle areas, e.g. through lateral inhibition.

CONCLUSION

The present study provides new insight into the early excitability changes in the primary motor cortex following SCI. It shows that following SCI, the excitability in the primary motor cortex is generally decreased. In incompletely lesioned subjects, however, a transient period of inhibition release exists that may be critical for recovery. Our findings may contribute to the discussion about the SCI recovery process and its influencing by therapy. The results are particularly relevant to the emerging therapeutic use of repetitive TMS (Belci *et al.* 2004; Kuppuswamy *et al.* 2011; Gamboa *et al.* 2011).

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