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Breakdown of inhibitory effects induced by foot motor imagery on hand motor area in lower-limb amputees

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Abstract

Objective: Amputation of a limb induces plastic changes in motor cortex that modify the relationships between the missing limb and the remaining body part representations. We used motor imagery to explore the interactions between a missing lower limb and the hand/forearm cortical representations.
Methods: Eight right leg amputees and nine healthy subjects participated in the study. Focal transcranial magnetic stimulation was used to map out the hand/forearm muscle maps at rest and during imagined ankle dorsiflexion and plantarflexion.
Results: In healthy subjects, both motor imagery tasks strongly inhibited the map volume and contracted the map area of the hand muscles. By contrast, in amputees, imagined dorsiflexion and plantarflexion enhanced the map area and volume of the hand muscles. In the forearm muscle maps, both groups displayed a similar pattern of isodirectional coupling during both motor imagery tasks. Imagined dorsiflexion facilitated MEP amplitudes of the extensor and inhibited the flexor muscles of the upper limb. This pattern was reversed during imagined plantarflexion.
Conclusions: We argue that there exists an inhibitory relationship between the foot and hand motor cortices that ceases to exist after leg amputation.
Significance: The understanding of these functional mechanisms may shed light on the motor network underlying interlimb coordination.

Keywords: Connectivity; Leg amputation; Hand motor area; Motor imagery; Transcranial magnetic stimulation

1. Introduction

It is well known that the loss of one limb in both animals (Donoghue and Sanes, 1988; Sanes et al., 1990; Donoghue et al., 1990; Jacobs and Donoghue, 1991; Schieber and Deuel, 1997; Wu and Kaas, 1999) and humans (Hall et al., 1990; Cohen et al., 1991; Fuhr et al., 1992; Kew et al., 1994; Ridding and Rothwell, 1995; Pascual-Leone et al., 1996; Chen et al., 1998; Dettmers et al., 1999; Röricht et al., 1999; Schwenkreis et al., 2001; Schwenkreis et al., 2003; Irlbacher et al., 2002) induces a marked plastic reorganization within the primary motor cortex (M1). Generally, the cortical regions of unaffected muscles expand and invade those of absent muscles (Kaas and Qi, 2004; Nudo, 2006 for recent reviews). Furthermore, monkey amputation models have shown that the loss of a limb markedly modifies the horizontal connection pattern within M1 (Kaas and Qi, 2004). In addition, several transcranial magnetic stimulation (TMS) studies have shown

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that the amputation of an upper limb leads to a major cortical reorganization in the motor maps and an increase in the excitatory state of the amputated limb hemisphere (Cohen et al., 1991; Kew et al., 1994; Ridding and Rothwell, 1995; Pascual-Leone et al., 1996; Dettmers et al., 1999; Röricht et al., 1999; Schwenkreis et al., 2001; Irlbacher et al., 2002).

However, few studies have investigated the modifications that occur in M1 after amputation of a lower limb (Chen et al., 1998; Schwenkreis et al., 2003), and the question of whether the amputation of one leg induces a reorganization in the upper-limb cortical representations remains a matter of debate. Chen et al. (1998) reported a reduction of intracortical inhibitory circuits in the amputated limb hemisphere and suggested that the motor reorganization after amputation primarily takes place at the cortical level.

In addition, it is well known that forearm excitability in healthy subjects undergoes a specific modulation pattern during oscillations of the foot, which consists in increased H-reflex excitability of the hand flexors when the foot is plantarflexed (Baldissera et al., 1998; Baldissera et al., 2002; Cerri et al., 2003). It is thus conceivable that amputation of one leg may modify the hand/forearm motor representations as a consequence of a breakdown in the neural circuits underlying the complex interlimb coordination.

Motor imagery may be defined as being “a dynamic state during which the representation of a given motor act is internal rehearsal without any overt motor output” (Decety and Grezes, 1999) that shares several features with the executed movement, such as activation of the same motor cortical areas (Decety et al., 1989; Deiber et al., 1998; Jeannerod, 1994; Sirigu et al., 1995; Decety, 1996; Porro et al., 1996; Roth et al., 1996; Jeannerod and Frak, 1999; Gerardin et al., 2000; Sirigu and Duhamel, 2001; Ehrsson et al., 2003; Hanakawa et al., 2003; Carrillo-de-la-Pena et al., 2006). Moreover, TMS studies in healthy subjects have demonstrated that the dynamic pattern of corticomotor excitability changes during imagined movements is similar to that observed during executed movements (Abbruzzese et al., 1996; Abbruzzese et al., 1999; Yahagi et al., 1996; Kasai et al., 1997; Kiers et al., 1997; Rossi et al., 1998; Fadiga et al., 1999; Hashimoto and Rothwell, 1999; Rossini et al., 1999; Facchini et al., 2002; Stinear and Byblow, 2003; Vargas et al., 2004; Marconi et al., 2007).

Given the close relationship between imagined and executed movements, in the present study we used the ability of patients with an amputated leg to mentally represent movements to investigate the influence of the missing leg on the upper-limb cortical representations. Since cortical reorganization after amputation may have influenced the functional relationship between the upper and lower limbs, we hypothesised that the cortical excitability patterns induced by imagined ankle movements in the hand and forearm representations of amputees are different from those of healthy subjects.

2. Methods

2.1. Subjects

Eight amputee subjects (AS) (8 male; mean age: 49.2 ± 7.4 years) and nine healthy subjects (HS) (four women and five men; mean age: 46.1 ± 5.0 years) were enrolled in the study. In all the amputees, the right leg had been amputated above the knee from 7 to 18 years earlier (mean 10.3 ± 3.2 years). A prosthesis was used by all of them. The reasons for amputation were motor vehicle accident (6) and osteosarcoma (2). All the patients were normal at the neurological examination and none reported phantom limb experiences at the time of recruitment, as assessed by a standardized interview (Flor et al., 1995). None of the patients was receiving medication that could influence central nervous system (CNS) excitability. All healthy subjects and amputees were right-handed, as confirmed by the Edinburgh Handedness Inventory Scale (Oldfield, 1971). None were aware of the aim of the study and all gave their written informed consent to participate. The experimental procedures were approved by the local Ethics Committee and were performed according to the Declaration of Helsinki.

Subjects were comfortably seated in an armchair with both hands/forearms placed on a pillow in a pronated position and relaxed totally. They were asked to focus on a fixed point on the wall in front of them and were unable to see the equipment display. The amputee subjects were seated with their prosthesis flexed at 90° at the level of the knee joint.

2.2. Transcranial magnetic stimulation

TMS mapping was performed using a magnetic stimulator (Magstim 200, Magstim Company, Dyfed, UK) connected to a figure-of-eight-shaped coil (7 cm internal diameter). An adherent, inelastic cap was placed over the participant’s head, and the reference to an anatomical landmark (intersection of the interaural line and the nasion-inion connection, C_z,in the 10–20 International System) was taken. The coil was placed tangentially over the skull, with the handle pointing backwards and perpendicular to the presumed direction of the central sulcus, ~45° to the midline, to evoke anteriorly-directed current in the brain. The optimal location (hot spot) for eliciting motor-evoked potentials (MEPs) from the opponens pollicis (OP) was identified in each individual and its location marked in relation to C_z. Motor responses were simultaneously obtained from the abductor digiti minimi (ADM), the extensor digitorum communis (EDC) and the flexor carpi radialis (FCR). The motor threshold at rest (rMT) was determined at the optimal scalp position for activation. The rMT was defined as the lowest TMS intensity of magnetic stimulation required to evoke MEPs larger than 50 μV in at least 50% of the trials (Rossini et al., 1994) in all the aforementioned muscles (Marconi et al., 2007).

Stimulation intensities are quoted as a percentage of maximal stimulator output.

In order to map out the muscle representations, a grid of 49 positions, spaced 1.5 cm along both the medio-lateral and antero-posterior axes, was fixed on the subject's head (Fig. 1a). Once these procedures were completed, the maps for each muscle were obtained by stimulating each point of the grid lying over the motor strip. The motor cortex was examined in rostral, dorsal, lateral and medial directions until no further MEP could be elicited. For each scalp position, we recorded the motor responses evoked by five stimulations at 110% of the rMT. The mean of the peak-to-peak MEP amplitude recorded from each excitable scalp site was calculated, as were the area (number of scalp positions from which the MEPs were elicited) and volume (the sum of the averaged MEP amplitudes for each excitable scalp site) for all the cortical maps.

Both the amputated limb (AH) and the intact limb hemispheres (IH) were studied in all patients; in healthy subjects (HS), the left hemisphere (LH) alone was tested.

2.3. EMG recordings

A surface EMG was recorded from the OP, ADM, EDC and FCR muscles with silver–silver chloride electrodes taped in a belly-tendon montage. EMG signals were amplified with gain set at 3000 (Digitimer D360 amplifier; Digitimer Ltd, Welwyn Garden City, Herts, UK), band-pass filtered (30 Hz to 2 kHz), then recorded by a computer using SIGNAL software (Cambridge Electronic Design, Cambridge,UK) with a sampling rate of 5 kHz per channel. To ensure that relaxation was maintained during the imagery tasks, the tibialis anterior (TA) and gastrocnemius (GS) muscles were recorded in healthy subjects and in the intact side of amputees; in four amputee subjects electrodes were positioned over a muscle adjacent to the stump. An audio-feedback was used to ensure that total relaxation was maintained. Trials with voluntary EMG activity that might confound MEP measurements were excluded from the analysis.

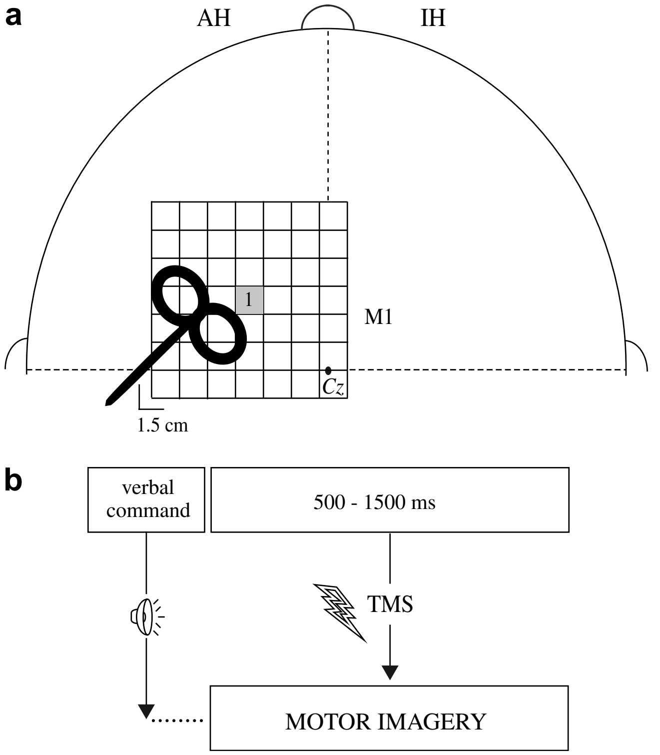

Fig. 1. (a) Schematic head with a grid of 49 positions (owing to limited space, only site 1 is indicated), spaced 1.5 cm along both the medio-lateral and antero-posterior axes lying over the motor cortex (M_1), superimposed over it to show the stimulated scalp sites. The C_z represents the intersection of the interaural line and the nasion-inion connection. (b) Schematic representation of the experimental procedure. Magnetic stimuli were delivered from 500 to 1500 ms after the verbal command.

2.4. Study design

Motor imagery (MI) tasks were used to investigate the corticomotor excitability of the hand and forearm muscle representations during imagined foot movements.

Amputees and healthy subjects were examined under the following conditions: (a) complete mental and muscular relaxation (rest), (b) imagined right ankle dorsiflexion (MI DF) and (c) plantarflexion (MI PF). Before testing started, participants physically performed both dorsiflexion and plantarflexion. The amputee subjects practiced the two tasks with the intact side (left ankle), and therefore we asked them to transform these movements in an internal representation to perform mentally with the amputated side. All participants practiced until they felt confident with the imagined movements and were able to retain an imagery pattern similar to that physically performance. During MI DF and MI PF, all the participants were instructed to imagine the movement in a first person perspective by recalling the feelings and sensations they experienced when they had performed the movement, using the same force, speed, and repetition rate (about 0.3 c/s), upon the verbal command "imagine the movement". The ease or difficulty of imagined movements was rated by all participants using a 7 point scale, ranging from very hard (1) to very easy (7). Performing the motor imagery tasks was similarly difficult for amputees and healthy subjects.

Magnetic stimuli were delivered upon a verbal command by the experimenter. Using dedicated software (PsyScope), a PC triggered the Magstim 200 U with a random delay ranging between 500 and 1500 ms (in steps of 250 ms) following the experimenter's verbal command given through a headphone microphone (Plantronics Audio 320 Stereo PC Multimedia Headset). The experimental paradigm has been summarized in Fig. 1b. The subjects and patients were asked not to think about any movement during the interval between two consecutive stimuli.

2.5. Data analysis

The following neurophysiological parameters were studied for amputees and healthy subjects and averaged across subjects:

(1) Mean motor threshold at rest (rMT).
(2) Mean map area, defined as the number of scalp positions whose stimulation evoked MEPs in that muscle. The motor map area for each muscle was defined as the number of "active" sites of all the participants divided by the number of participants.
(3) Mean map volume. The mean MEP amplitude for each scalp site was first calculated, and then the amplitudes of all the positions were summed up to obtain the map volume. Finally, the mean value of each site was calculated and averaged for all the participants; a site was only included in the averaged maps if present in all the subjects.
(4) The center of gravity (CoG) of the motor maps. CoG was defined as the map location representing amplitude-weighted center of the area of excitability. CoGs for each upper-limb muscle map were calculated according to Wassermann et al. (1992): the lateral coordinate (x-coordinate) of the CoG was computed by multiplying the lateral coordinate at each position by its amplitude-weight and summing over all positions. The antero-posterior coordinate (y-coordinate) was computed by the same method.

A parametric statistical analysis was used to take into account all the sources of variation. We used analysis of variance (ANOVA) for repeated measures as the main statistical procedure, in which *group* (AS versus HS) was considered as the between-subject factor, while *task* and *muscle* were considered as within-subject factors. We performed two separate 3-way ANOVAs (*group* × *task* × *muscle*) to assess the significant differences across map area and, subsequently, map volume of all the muscles. For amputee subjects, in order to compare the amputated limb (AH) and intact limb hemispheres (IH), two 3-way ANOVAs (*hemisphere* × *task* × *muscle*) for the map area and volume were performed. Finally, separate ANOVAs for each muscle (*hemisphere* × *task* × *axis*) were performed to assess the significant differences among map CoGs. Post hoc comparisons (Tukey's test) were performed when the interaction was statistically significant. The assumption of sphericity, which was checked by means of Mauchly's test, was not significant; no correction was applied to the degrees of freedom. Student's t-test was used when two means were compared. Throughout the statistical analysis, the p value level of significance was set at 0.05.

3. Results

3.1. Effects of foot motor imagery on the upper-limb muscle maps: Amputees (AS) versus healthy subjects (HS).

At rest, the mean rMT values were 56.6 ± 5.9% in the amputated limb hemisphere (AH) of patients, and 59.0 ± 5.3% in the LH of healthy subjects (HS) (two-tailed t test $p = 0.045$). In addition, we found a significant medialization of the x (medio-lateral) coordinate of the CoG at rest in the AH for all upper-limb muscles when compared with HS (all $p < 0.05$) (see Table 1). These results clearly emerge from the disparity in the two-dimensional (2-D) maps shown in Fig. 3 (AS) and Fig. 4 (HS). The y (antero-posterior) coordinate of the CoG did not differ significantly between AH and LH of healthy subjects. Additionally, no significant differences were observed in the CoG across tasks in both groups of subjects, though a slight further shift toward the midline was observed for all upper-limb muscle maps in the AH during imagined ankle dorsiflexion (MI DF) and plantarflexion (MI PF) (Fig. 3).

The 3-way ANOVA applied on the map area revealed a significant difference in the factors *group* [$F(1,15) = 66.64$; $p = 0.002$] and *muscle* [$F(3,45) = 6.85$; $p = 0.018$], as well as in the interactions *group* × *muscle* [$F(3,45) = 13.07$; $p = 0.008$], *task* ×*muscle* [$F(6,90) = 2.81$; $p = 0.027$] and *group* × *task* × *muscle* [$F(6,90) = 2.93$; $p = 0.029$]. All the muscle map areas were significantly larger at rest in AS (OP, $p = 0.022$; ADM, $p = 0.019$; EDC, $p = 0.008$; FCR, $p = 0.015$) than in HS (Fig. 2a). The post hoc comparisons revealed that in HS alone the OP and ADM map sizes were significantly smaller in both MI DF ($p = 0.007$ and $p = 0.018$, respectively) and MI PF ($p = 0.001$ and $p = 0.016$, respectively) than at rest (Fig. 2a) This reduction in map size induced by both imagery tasks on the OP muscle observed in HS clearly emerged from the disparity in the two-dimensional (2-D) maps shown in Fig. 3 (AS) and Fig. 4 (HS) (given the similarity of the hand muscle pattern, ADM was omitted). In addition, the map area of the EDC during MI DF and of the FCR during MI PF were significantly larger (all $p < 0.05$) than at rest in both AH and HS (Fig. 2a).

Data analysis on motor cortical output revealed that, at rest, map volumes were significantly more enhanced in all the muscles in AS (OP, $p = 0.014$; ADM, $p = 0.001$; EDC, $p = 0.002$; FCR, $p = 0.010$) than in HS (Fig. 2b). Moreover, the 3-way ANOVA revealed that both main factors *group* [$F(1,15) = 45.82$; $p = 0.005$] and *muscle* [$F(3,45) = 17.60$; $p = 0.014$], as well as the interactions *group* × *task*[$F(2,30) = 19.16$; $p = 0.012$], *group* x *muscle* [$F(3,45) = 11.23$; $p = 0.018$], *task* × *muscle* [$F(6,90) = 42.03$; $p = 0.001$] and *group* × *task* × *muscle* [$F(6,90) = 5.97$; $p = 0.015$], were significant. At the post hoc comparisons, both the MI DF and MI PF tasks significantly increased the OP ($p = 0.012$ and $p = 0.030$, respectively) and ADM ($p = 0.040$ and $p = 0.009$, respectively) map volume in AS, if compared with rest (Fig. 2b). By contrast, in HS, the MI DF and MI PF significantly reduced the map volumes of both the OP ($p = 0.010$ and $p = 0.031$, respectively) and ADM ($p = 0.025$ and $p = 0.019$, respectively), if compared with rest (Fig. 2b). The contrasting effects induced by both imagery tasks on the hand muscles in the two groups can be clearly seen by comparing the 2-D maps shown in Fig. 3 (AS) and Fig. 4 (HS). Furthermore, MI DF significantly increased the volume of the EDC (AS, $p = 0.001$; HS, $p = 0.010$) and decreased that of the FCR

Table 1
Mean values (cm ± SD) of CoG for the different muscle maps at rest in the AH and IH of amputees (AS), and in left hemisphere (LH) of healthy subjects (HS)

Amputee subjects(AS)				Healthy subjects (HS)	
AH		*IH*		*LH*	
x axis	*y* axis	*x* axis	*y* axis	*x* axis	*y* axis
OP					
4.0 ± 1.6	1.8 ± 2.2	4.5 ± 1.4	1.6 ± 1.8	4.7 ± 2.1	1.7 ± 1.3
ADM					
3.8 ± 1.5	1.6 ± 1.9	4.3 ± 1.0	1.7 ± 1.5	4.4 ± 1.8	1.5 ± 0.9
EDC					
3.0 ± 1.3	2.0 ± 2.7	3.6 ± 2.1	1.9 ± 1.7	3.8 ± 1.1	2.0 ± 1.4
FCR					
2.9 ± 1.9	2.2 ± 2.8	3.7 ± 1.8	2.3 ± 1.2	3.6 ± 1.1	2.2 ± 1.6

Bold type indicates a significantly medial shift in the x coordinate in the AH compared with both IH and HS for all muscles.

(AS, $p = 0.008$; HS, $p = 0.041$) in both AS and HS (Fig. 2b). This pattern of cortical excitability was inverted by MI PF, which significantly enhanced the FCR map volume (AS, $p = 0.001$; HS, $p = 0.003$) and reduced the EDC (AS, $p = 0.001$; HS, $p = 0.021$) (Fig. 2b). The reciprocal pattern of corticomotor facilitation, observed in EDC

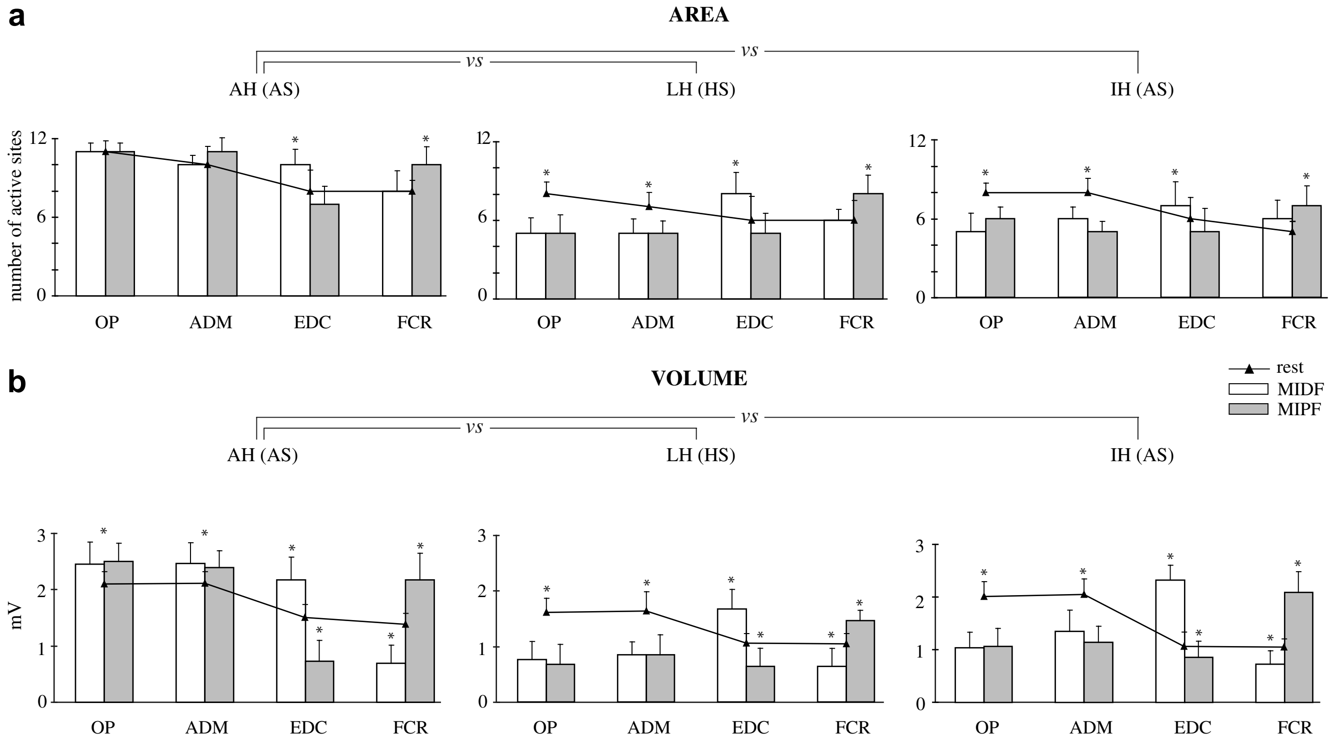

Fig. 2. Histograms (mean values ± SD) of the muscle map area (a) and volume (b) in both amputated limb (AH) and intact limb hemispheres (IH) of amputees (AS) and left hemisphere (LH) of healthy subjects (HS), obtained at rest (line with triangles), during MI DF (white bars) and MI PF (grey bars). The comparison between the two groups of subjects (AS *vs* HS) revealed an increase in both map area and volume of all the muscles at rest in AH (AS), if compared with LH (HS). The MI DF and MI PF tasks reduced both the area and volume of the hand muscles (OP and ADM) in HS. During both MI DF and MI PF, an increase in map volume was observed in OP and ADM muscles of AH. Both groups exhibited an enhancement in the EDC muscle map volume during MI DF, together with a decreased FCR map volume and this pattern was reversed during MI PF. The comparison between the two hemispheres (AH vs IH) of amputee subjects disclosed an increase in both map area and volume of all the muscles at rest in AH, if compared with the IH. Both MI DF and MI PF tasks reduced area and volume of the hand muscles (OP and ADM) in IH, while an increase in volume was observed in OP and ADM maps of AH. Both hemispheres (AH and IH) displayed an increase in the EDC map volume during MI DF together with a decrease in the FCR map volume. This pattern was reversed during MI PF. We have not, for the sake of simplicity, shown all the significance differences between the different values (see Section 3 for details).

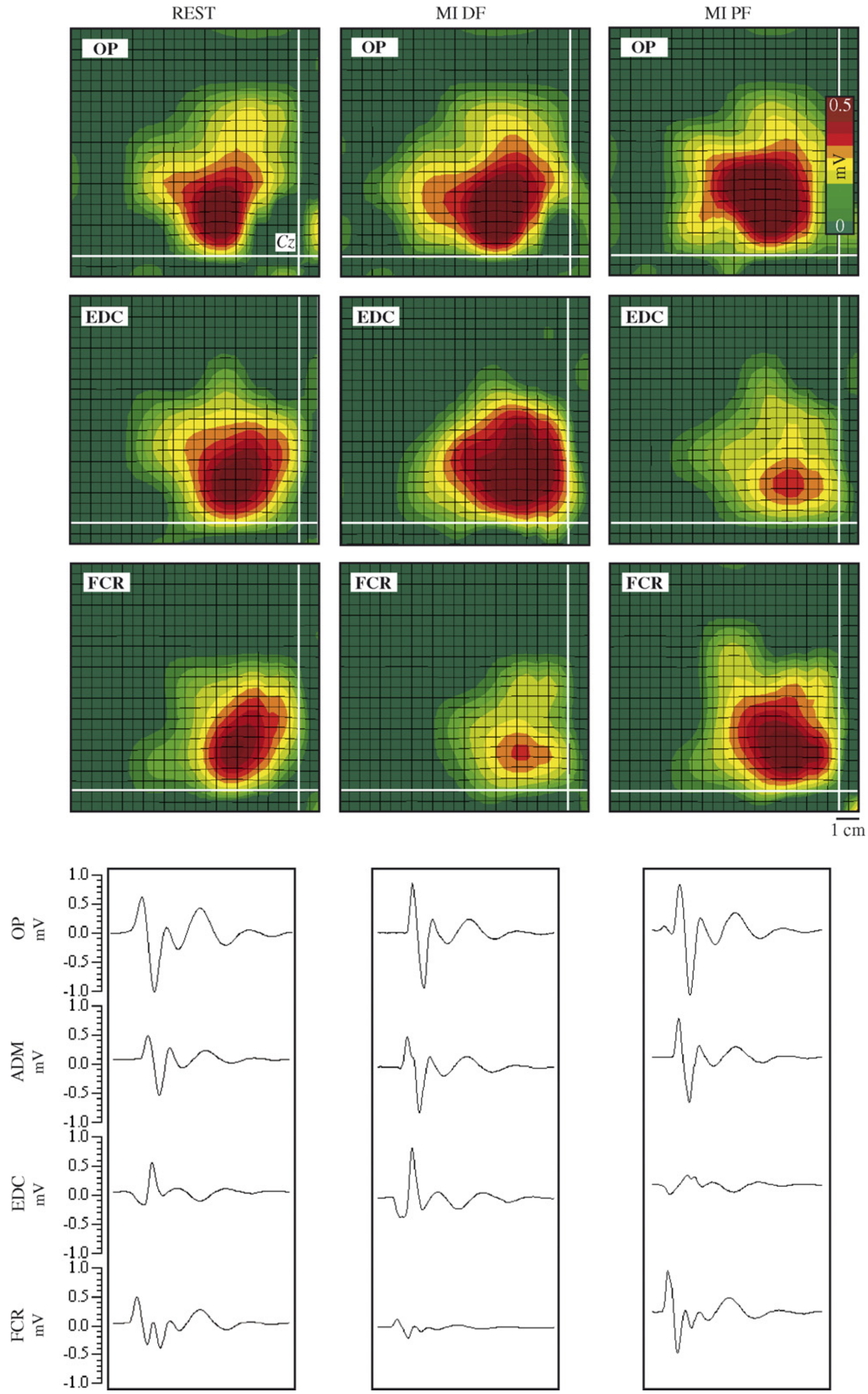

Fig. 3. Two-dimensional (2-D) maps of the OP, EDC and FCR muscles, during rest, MI DF and MI PF obtained from amputee subjects. Given the similarity of the pattern displayed by the two hand muscles, the ADM has been omitted. The MI DF and MI PF tasks increased both the OP map area and volume, if compared with rest. An evident motor cortical facilitation was observed in the EDC muscle map volume during MI DF, together with an inhibition in the FCR. This pattern was inverted during MI PF. A noticeable medialization of the whole area in all experimental conditions (rest, MI DF and MI PF) was observed together with an additional medial shift observed during both motor imagery tasks. The color code palette of each map ranges from dark green (0 mV) to dark red (0.5 mV). The scale bar used is 1 cm for both *x* and *y* axes. The insets below show the original MEPs obtained from a representative amputee subject. The facilitation pattern observed in the forearm muscles (EDC and FCR) motor responses is inverted during MI DF and MI PF, which is in keeping with the 2-D cortical map reconstructions and resembles that of observed healthy subjects (Fig. 4).

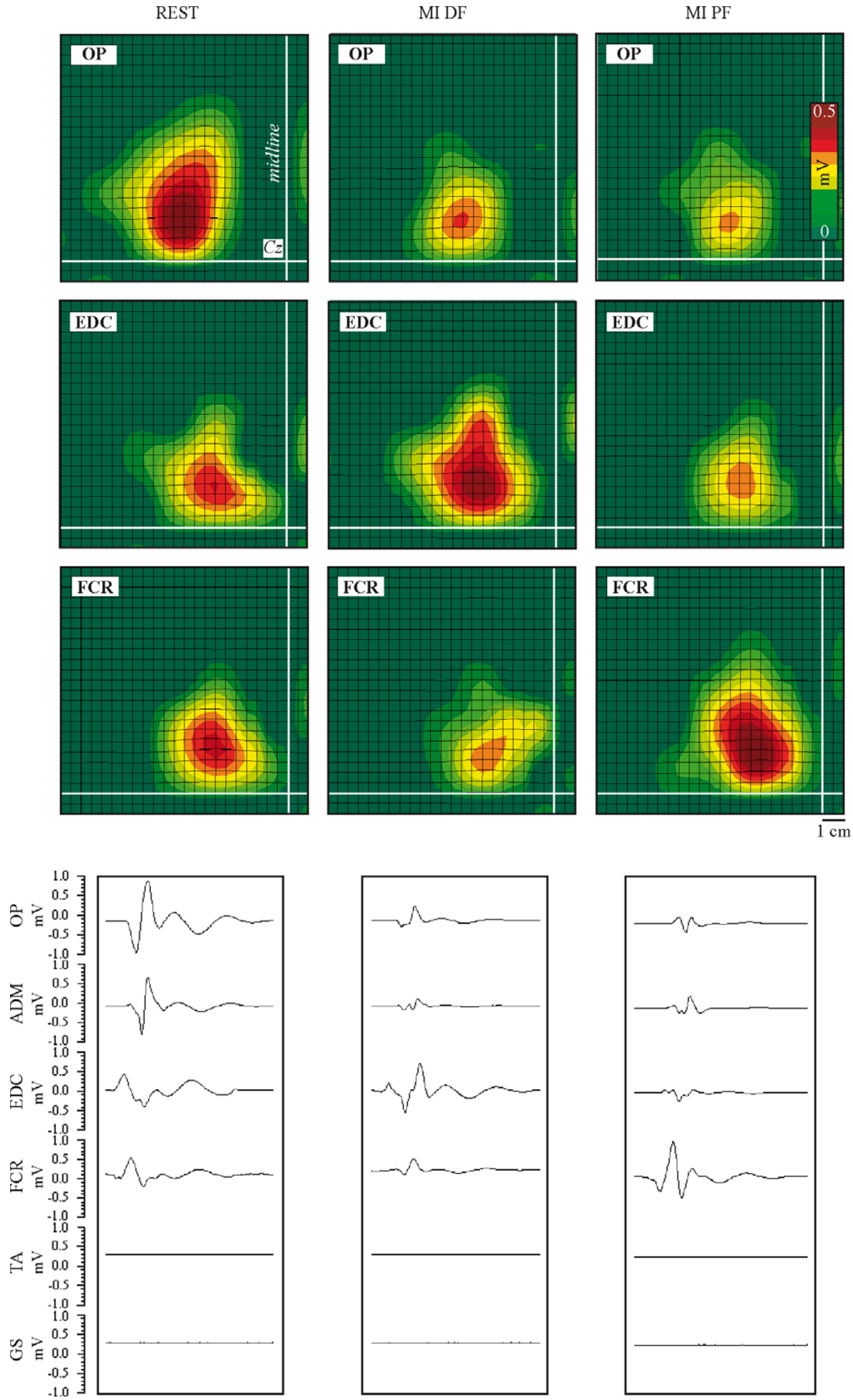

Fig. 4. The same 2-D maps as in Fig. 3 obtained from healthy subjects. The MI DF and MI PF tasks markedly reduced both the OP map area and volume, if compared with rest. An evident motor cortical facilitation was observed in the EDC map volume during MI DF, together with an inhibition in the FCR muscle. This pattern was inverted during MI PF. The MEP amplitudes in both hand muscles (OP and ADM) were markedly reduced during MI DF and MI PF as evident in the insets below which show the original MEPs acquired from a representative healthy subject. The other conventions are the same as those in Fig. 3.

and FCR during MI DF and MI PF, was similar in AS and HS, as is evident from the 2-D maps shown in Fig. 3 (AS) and Fig. 4 (HS).

3.2. Effects of foot motor imagery on the upper-limb muscle maps: amputated (AH) versus intact limb hemisphere (IH).

At rest, the mean rMT values were significantly lower in the AH (56.6 ± 5.9%) than in the IH (60.1 ± 6.1%) (two-tailed *t* test $p = 0.018$). Moreover, we found a significant medialization of the *x* (medio-lateral) coordinate of the CoG at rest for all upper-limb muscles in the AH compared with the IH (all $p < 0.05$) (see Table 1).

Data analysis performed to assess differences in map areas between amputated limb (AH) and intact limb hemispheres (IH) revealed that both main factors *hemisphere* [$F(1,7) = 45.73$; $p = 0.006$] and *muscle* [$F(3,21) = 4.01$; $p = 0.020$], as well as the interactions *hemisphere × muscle* [$F(3,21) = 9.00$; $p = 0.002$] and *hemisphere × task × muscle* [$F(6,42) = 2.97$; $p = 0.025$], were significant. The post hoc comparisons showed that, at rest, the map areas of all the muscles were significantly larger (OP, $p = 0.012$; ADM, $p = 0.001$; EDC, $p = 0.008$ and FCR, $p = 0.041$) in the AH than in the IH (Fig. 2a). In addition, the hand muscle map areas were very similar in both MI DF and MI PF tasks in the AH (Fig. 2a). By contrast, in the IH, the MI DF and MI PF significantly reduced both the OP ($p = 0.005$ and $p = 0.012$, respectively) and ADM ($p = 0.001$ and $p = 0.021$, respectively) map areas, if compared with rest. Moreover, the map areas of the EDC during MI DF and of the FCR during MI PF were also slightly, though significantly, enlarged in the IH, if compared with the rest condition (all $p < 0.05$) (Fig. 2a).

Data analysis undertaken to assess differences in map volumes between hemispheres (AH vs IH) revealed that the main factors *hemisphere* [$F(1,7) = 41.43$; $p = 0.007$] and *muscle* [$F(3,21) = 10.53$; $p = 0.018$], as well as the interactions *hemisphere × task* [$F(3,21) = 16.18$; $p = 0.009$], *hemisphere × muscle* [$F(3,21) = 17.13$; $p = 0.010$], *task × muscle* [$F(6,42) = 36.25$; $p = 0.008$] and *hemisphere × task × muscle* [$F(6,42) = 4.42$; $p = 0.033$], were significant. Post hoc comparisons showed that in the AH, at rest, the map volumes were greater than in the IH in all muscles: OP ($p = 0.035$), ADM ($p = 0.002$), EDC ($p = 0.008$) and FCR ($p = 0.042$) (Fig. 2b). Furthermore, motor cortical output of the hand muscles was found to be greater during MI DF and MI PF in the AH (MI DF: OP, $p = 0.002$ and ADM, $p = 0.011$; MI PF: OP, $p = 0.019$ and ADM, $p = 0.012$) if compared with the rest (Fig. 2b). By contrast, the hand muscle map volumes were significantly smaller in the IH during both motor imagery tasks (MI DF: OP, $p = 0.001$; ADM, $p = 0.031$, and MI PF: OP, $p = 0.008$; ADM, $p = 0.005$), if compared with the rest (Fig. 2b). In addition, during MI DF both hemispheres displayed a significantly increased EDC motor cortical output together with a decrease in FCR map volume, if compared with the rest; this pattern was reversed during MI PF (all $p < 0.05$) (Fig. 2b).

4. Discussion

The present study shows that imagined ankle movements markedly reduce the map size and inhibit the hand cortical representation in healthy subjects. Furthermore, imagined dorsiflexion increased the MEP amplitude of the upper-limb extensor, and concurrently inhibited the flexor muscle. This cortical excitability pattern was reversed during the imagined plantarflexion movements. A clear breakdown in the inhibitory effects induced by ankle motor imagery on the hand cortical representation occurred in amputees. Indeed, instead of reducing the area and volume of the hand cortical maps, imagined dorsiflexion and plantarflexion enhanced the excitability of the hand motor region. Additionally, during the aforementioned motor imagery tasks, amputees displayed an inhibition/facilitation pattern in the forearm muscle cortical maps that closely resembled that of healthy subjects. Our results support earlier findings showing that ankle movement modifies excitability in the upper-limb region (Baldissera et al., 2002; Borroni et al., 2004), and go beyond by demonstrating a functional inhibitory relationship between foot and hand motor cortical representations that collapses after amputation of a leg.

4.1. Inhibitory relationship between foot and hand motor cortical representations

An important finding of our study is that there exists an inhibitory relationship between the foot and hand motor cortical representations in healthy subjects, and that this functional relationship collapses in the amputated limb hemisphere following amputation of a leg.

One tentative explanation for the foot-hand inhibitory relationship we observed in healthy subjects is that this interaction becomes functionally more relevant during tasks requiring interlimb coordination. For instance, precision hand motor tasks are more difficult to perform during walking (Zehr and Duysens, 2004; Ivanenko et al., 2005). Moreover, recent studies on the operational principles of motor cortical function support the notion that the modulation of excitation/inhibition in motor cortex may be a crucial mechanism in the selection of movement-related muscle synergies as a dynamic process (Capaday, 2004). On the basis of these evidences, we suggest that the inhibitory relationship, activated during ankle movement in healthy subjects, may reduce the output gain of the neuronal circuits underlying the hand complex motor repertoires in order to simplify the construction of motor behavior (d'Avella et al., 2003).

Furthermore, the inter-hemispheric asymmetries observed in the area and volume of the cortical maps, following the loss of a leg, parallel those found in earlier TMS studies performed on upper limb amputees, in which a marked motor map reorganization characterised the amputated limb hemisphere (Hall et al., 1990; Cohen et al., 1991; Kew et al., 1994; Ridding and Rothwell, 1995; Pascual-Leone et al., 1996; Ziemann et al., 1998; Dettmers et al., 1999; Röricht et al., 1999; Schwenkreis et al., 2001; Irlbacher et al., 2002). Additionally, in accordance with previous findings (Cohen et al., 1991; Chen et al., 1998), the rMT in our study was reduced in the amputated limb hemisphere if compared with both the intact side in patients and the motor cortex in healthy subjects. In this regard, previous paired-pulse TMS studies reported reduced activity in intracortical inhibitory GABA circuits following the loss of a limb (Chen et al., 1998; Dettmers et al., 1999) that could contribute to the modifications in the cortical excitability observed in the current study.

Animal amputation models suggest that loss of GABAergic inhibition in the motor cortex could lead to a “permissive period” in which axons grow and new connections are formed (Kaas and Qi, 2004; Nudo, 2006 for recent reviews). These changes in connectivity and/or in connection strength may also place the hand cortical regions beyond the control of inhibitory influences.

Furthermore, there are no reports of direct anatomical connections between the hand and foot cortical representations in M1 (Huntley and Jones, 1991), nor are there strong projections from the hand and foot cortical areas towards homologous regions in the contralateral M1 (Pandya and Vignolo, 1971; Jenny, 1979; Jones et al., 1979; Gould et al., 1986; Johnson et al., 1989; Rouiller et al., 1994). Therefore, other crucial nodes, such as the premotor and/or supplementary areas, may play an important role in the corticocortical network underlying interlimb coordination (Ehrsson et al., 2000; Debaere et al., 2001). At this regard, Byblow et al. (2007) recently reported that functional connectivity between dorsal premotor area and M1 facilitated isodirectional movements of hand and foot in healthy subjects, suggesting that this circuit might contribute to modulate the excitability of M1 during interlimb coordination.

4.2. Are cortical rearrangements explained by the territorial “invasion”?

Another interesting finding in our study is that lower-limb amputation not only enhanced the motor response in the upper-limb cortical maps, but also induced a significant medialization of the whole area in all experimental conditions (rest, MI DF and MI PF). Moreover, an additional medial shift was observed in the upper-limb motor maps in amputees during imagined dorsiflexion and plantarflexion; though not significant, this shift does suggest that such tasks may further activate the cortical network previously devoted to the missing lower-limb.

However, it is not clear whether the enlarged cortical area and the directional shift of the motor maps reflect a true expansion of the upper-limb representations into regions previously devoted to the leg (Jacobs and Donoghue, 1991; Schieber and Hibbard, 1993; Donoghue et al., 1996; Huntley, 1997; Sanes and Donoghue, 1997), since this phenomenon might be the mere consequence of a decrease in the motor threshold of pre-existing unmasked silent synapses (Cohen et al., 1991; Ridding and Rothwell, 1995; Röricht et al., 1999). In addition, further studies are warranted to address the possible rearrangements in the cortical territories that lie between the upper and lower limbs’ motor cortical representations.

Whatever the reasons underlying these changes are, the reorganization we observed in M1 after the loss of a leg does not seem to obey the simple law of “invasion” of the former distal leg area by the stump, but points to a more extensive rearrangement of the cortical territories that probably modifies the relationships between the missing limb and the remaining body part representations. These modifications may contribute to the remodelling of new anatomo-functional motor architecture that is dependent on the surviving elements of the network and on their ability to function efficiently (Rossini and Pauri, 2000; Talelli et al., 2006).

4.3. Is interlimb coordination based on a hardwired network?

We also found that the cortical excitability changes, observed in amputees during imagined dorsiflexion and plantarflexion, spared the forearm muscles, in which a reciprocal inhibition/facilitation pattern was maintained. These data are in agreement with those reported in previous works, which identified a reciprocal pattern of corticomotor excitability linking the upper and lower limbs (Baldissera et al., 2002). Why the isodirectional facilitation pattern between the upper and lower limbs is not modified in amputees remains unclear. One conceivable explanation for this mechanism may come from numerous previous studies suggesting that the isodirectional coupling between ipsilateral limbs seems to be hardwired in the CNS (Baldissera et al., 1982; Baldissera et al., 1991; Baldissera et al., 2000; Kelso and Jeka, 1992; Carson et al., 1995; Jeka and Kelso, 1995; Swinnen et al., 1995; Serrien and Swinnen, 1998). Consequently, interlimb reciprocal interactions may be less susceptible to plastic rearrangements after the loss of a limb.

In conclusion, our study demonstrates that the loss of a leg induces a functional reorganization in the upper-limb motor cortical maps. In particular, we identified an inhibitory relationship between the foot and hand motor cortical representations that breaks down after the amputation of a lower limb.

A better knowledge of these physiological mechanisms may shed light on the neural mechanisms underlying interlimb coordination and, consequently, help to develop new strategies in neurorehabilitation.

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