Journal of the Neurological Sciences 275 (2008) 51–59

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Long-term effects on motor cortical excitability induced by repeated muscle vibration during contraction in healthy subjects

Barbara Marconi [a,b,*], Guido M. Filippi [c], Giacomo Koch [b,d], Cristiano Pecchioli [b], Silvia Salerno [b,e], Romildo Don [b], Filippo Camerota [f], Vincenzo M. Saraceni [f], Carlo Caltagirone [b,d]

[a] European Brain Research Institute, Rita Levi-Montalcini Foundation 00143 Rome, Italy
[b] Laboratory of Clinical and Behavioral Neurology, IRCCS Santa Lucia Foundation, 00179 Rome, Italy
[c] Institute of Human Physiology, Catholic University of Rome, 00168 Rome, Italy
[d] Neurological Clinic, Department of Neurosciences, Tor Vergata University of Rome, 00133 Rome, Italy
[e] Department of Psychology, University of Palermo, 90128 Palermo, Italy
[f] Institute of Physical Medicine and Rehabilitation, 'La Sapienza' University of Rome, 00100 Rome, Italy

ARTICLE INFO

Article history:
Received 25 January 2008
Received in revised form 30 May 2008
Accepted 17 July 2008
Available online 29 August 2008

Keywords:
Motor cortex
TMS
Intracortical inhibition
Muscle vibration
Cortical plasticity
Neurorehabilitation

ABSTRACT

Objective: The effects of a novel repeated muscle vibration intervention (rMV; 100 Hz, 90 min over 3 consecutive days) on corticomotor excitability were studied in healthy subjects.
Methods: rMV was applied over the flexor carpi radialis (FCR) during voluntary contraction (experiment 1), during relaxation and during contraction without vibration (experiment 2). Focal transcranial magnetic stimulation (TMS) was applied before rMV and one hour, and one, two and three weeks after the last muscle vibration intervention. At each of these time points, we assessed the motor map area and volume in the FCR, extensor digitorum communis (EDC) and abductor digiti minimi (ADM). Short-interval intracortical inhibition (SICI) and facilitation (ICF) were tested for the flexor/extensor muscles alone.
Results: Following rMV under voluntary contraction, we observed a significant reduction in the FCR map volumes and an enhancement in the EDC. SICI was increased in the FCR and reduced in the EDC. These changes persisted for up to two weeks and occurred at the cortical level in the hemisphere contralateral to the side of the intervention.
Conclusion: We conclude that rMV, applied during a voluntary contraction, may induce prolonged changes in the excitatory/inhibitory state of the primary motor cortex. These findings may represent an important advance in motor disorder rehabilitation.

1. Introduction

In recent years, a growing body of evidence has demonstrated the capacity of the somatosensory cortex to undergo remodeling in response to various environmental changes, e.g. long periods of repeated sensory input [1]. Furthermore, alterations in sensory input have been shown to induce, in both animals and humans, a reorganization in the primary motor cortex [2–9]. Indeed, evidence strongly suggests that a period of pure sensory stimulation can affect motorcortical excitability [10–15]. Moreover, research on animals has provided the neuroanatomical substrate for these effects by discovering topographically and functionally specific corticocortical connections that link primary somatosensory (SI) and motor (M1) cortices [16,17]. Recent studies on humans have confirmed the functional relevance of these connections, highlighting the role played by somatosensory afferents combined with intrinsic motor cortical circuits in inducing plasticity in M1 [18,19].

Muscle vibration is a strong proprioceptive stimulus, which, at low amplitude, preferentially produces Ia afferent input [20–22]. Such a powerful input reaches both the SI [23,24] and M1 directly [25]. Data derived from non-human primates have shown that Group Ia afferent stimulation, induced by trains of low-amplitude vibration, affects the discharge of M1 cells, which indicates that motorcortical neuron activity is modulated by proprioceptive input arising from Ia afferents [26].

Interestingly, many previous transcranial magnetic stimulation (TMS) studies have shown that low amplitude vibration of a muscle is able to induce different changes in corticomotor excitability of the vibrated versus non-vibrated muscle [27–35].

These somatotopically-organized effects, observed in M1 after peripheral stimulation, may be ascribable to the afore-mentioned pattern of SI-M1 connections, which modify muscle representations that are "homotopic" relative to the stimulation site [36–38].

Moreover, in two recent studies, a repeated (90 min over 3 consecutive days) muscle vibration (rMV) intervention induced long-term changes in motor performance in both healthy subjects and patients [39,40]. These changes consisted of an improvement in

* Corresponding author. Laboratory of Clinical and Behavioral Neurology, IRCCS Santa Lucia Foundation, 00179 Rome, Italy. Tel./fax: +39 06 51501543.
E-mail address: b.marconi@hsantalucia.it (B. Marconi).

0022-510X/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2008.07.025

postural stability, in resistance to fatigue, as well as in the rise time of the maximal isometric force, which lasted for up to 2 weeks. One conceivable explanation for these results is that this intervention induces long-lasting neuroplastic changes in the network underlying motor control.

Furthermore, since several studies have highlighted the role played by the combination of somatosensory afferents and motor cortical circuit activity in driving plasticity in M1 [18,19], here we explore the long-term after-effects of a rMV protocol applied over the flexor carpi radialis (FCR) during voluntary contraction. We tested this hypothesis using TMS in healthy subjects before and at several time points after the last muscle vibration.

To the best of our knowledge, this is the first study in which the after-effects of muscle vibration have been investigated up to three weeks after the last intervention.

2. Materials and methods

2.1. Participants

Twenty-six healthy volunteers (eleven women and fifteen men; mean age: 35.1 ± 10.1 years) were enrolled for the purposes of this study, which was approved by the local ethics committee. All the volunteers were right-handed, as confirmed by the Edinburgh Handedness Inventory Scale [41], and all gave their written informed consent. None were aware of the aims of the study. All the experiments conformed to the *Declaration of Helsinki*. The volunteers were randomly assigned to one of the three experiments performed.

2.2. Transcranial magnetic stimulation

TMS was performed using two Magstim 200 stimulators (Magstim, 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, *Cz*, in the 10–20 International System) was taken. The coil was held with the handle pointing backwards and sideways, approximately 45° to the midline, to evoke anteriorly directed current in the brain. The optimal position (hot spot) for eliciting motor-evoked potentials (MEPs) from the target muscle, the FCR, was identified in each individual; the motor threshold at rest (RMT) was then assessed in a step-wise fashion and defined as the lowest stimulation intensity required to evoke MEPs larger than 50 μV in at least 50% of the trials [42]. Stimulation intensities are quoted as a percentage of maximal stimulator output. To map out the muscle representation, a 49-position grid (10.5 × 10.5 cm) was fixed on the subject's head and centred on the FCR hot spot (Fig. 1). The stimulator was set at 120% of the RMT and 5 stimuli were delivered to each grid point; the averaged MEPs were stored on disk and measured off-line according to standardized procedures. The motor cortex was examined in the rostral, dorsal, lateral and medial directions in steps of 1.5 cm until no further MEP could be elicited. A site was defined as excitable when TMS elicited at least two reproducible MEPs with an amplitude of at least 50 μV. Once mapping of the FCR muscle representation was completed, hot spot, RMT and motor map representations were also identified for the EDC and ADM muscles by means of the same experimental procedures as those used for the FCR.

Intracortical excitability in the FCR and ECD muscles was investigated using the paired-pulse protocol [43], with a supra-threshold test stimulus preceded by a sub-threshold conditioning magnetic stimulus. Seven different interstimulus intervals (ISI) were tested: ISIs of 1, 2, 3 and 5 ms were measured to study short-interval intracortical inhibition (SICI), while ISIs of 7, 10 and 15 ms were measured to study intracortical facilitation (ICF). SICI and ICF were recorded separately for the two aforementioned muscles. The intensity of the conditioning stimulus (CS) was set at 70% of the RMT. In each experimental session, the intensity

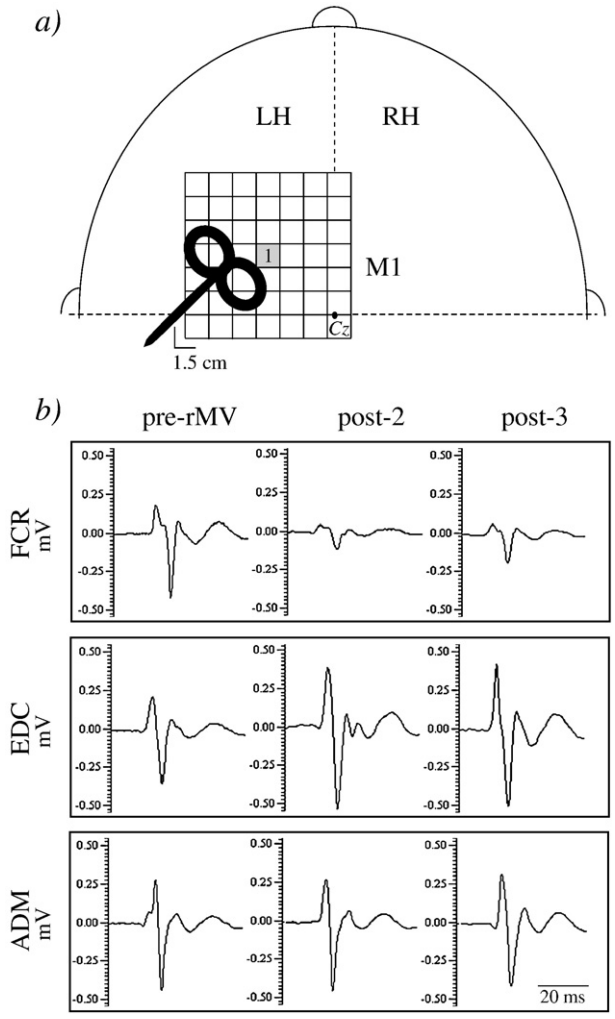

Fig. 1. a) Schematic head with a grid of 49 positions, spaced 1.5 cm along both the medio-lateral and antero–posterior axes, lying over the motor cortex (M1) that shows the stimulated scalp sites. The *Cz* represents the intersection of the interaural line and the nasion–inion connection. b) The figure shows MEPs obtained from the FCR, EDC, and ADM at the hot spot (grey square) of a representative subject; the motor responses (MEPs) were acquired before the intervention (pre-rMV), and one (post-2) and two (post-3) weeks after the end of the intervention. The MEP amplitudes decrease in the FCR muscle and increase in the EDC at post-2 and post-3. Given the similarity of the pattern displayed by the muscles at post-1, this time point is not shown; since the MEP amplitude at post-4 was virtually equal to that at pre-rMV, the pre-rMV data have not been shown either. No MEP amplitude changes are visible for the ADM muscle.

of the test stimulus (TS) was adjusted to evoke a MEP amplitude of ~0.7 mV. SICI and ICF were investigated in blocks of trials consisting of several randomly intermixed conditions (TS alone or preceded by the CS at different interstimulus intervals), each presented 10 times. The time between two consecutive trials was always 5 ± 20% s. During the TMS experiments, subjects were comfortably seated in an armchair with both forearms pronated and positioned on a pillow.

2.3. EMG recordings and H reflex

A surface EMG was recorded from the FCR, EDC and ADM 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 means of a computer using SIGNAL software (Cambridge Electronic Design, Cambridge, UK) with a sampling rate of 5 kHz per channel. An audio-feedback was used

to ensure that total relaxation was maintained. Trials with voluntary EMG activity that might confound the MEP measurements were excluded from the analysis.

The *H* reflex was recorded from the FCR muscle with surface electrodes. It was elicited by electrical stimulation of the median nerve at the antecubital fossa with a 1 ms square-wave constant current (5 s interval). The *M* wave was elicited during supramaximal stimulation of the nerve; the stimulation intensity was varied to obtain the maximal *H* wave amplitude (H_{max}). The amplitudes of the maximum motor responses (M_{max}) and H_{max} were measured peak to peak; 10 responses were averaged for each subject and the H/M ratio was calculated. The EMG signal was amplified (1 mV/D) and band-pass filtered (2 to 10,000 Hz) with a Medtronic Key-point electromyography, before being stored on a personal computer for off-line analysis.

2.4. rMV intervention

Low-amplitude rMV at a fixed frequency of 100 Hz was applied over the target muscle, i.e. the FCR, by means of a specific commercial device (Cro®System, NEMOCO srl, Italy), *previously used in similar protocols* [39,40]. Briefly, the instrument consists of an electromechanical transducer, a specific mechanical support and an electronic control device. The transducer was positioned over the FCR muscle in a point approximately corresponding to its maximal transversal size. The vibration was applied by means of a small probe with a 10 mm diameter directly over the FCR muscle belly. The transducer was driven to produce sinusoidally modulated forces ranging between 7 and 9 N. The vibration amplitude ranged from 0.05 to 0.1 mm, which was sufficient to drive Ia spindle afferents [20], to avoid muscle fiber injury [44] and subthreshold for the tonic vibration reflex (TVR) [45,46]. The evaluation of displacement was performed by means of an infrared sensor that detected an infrared LED orthogonally placed on the lateral face of the vibrator tip. Moreover, since vibration of a contracted muscle is more likely to induce TVR [46,47], we verified that TVR was absent by means of continuous EMG monitoring [48].

The mechanical support allowed soft tissues overlying the muscle–tendon complex to be progressively compressed so as to minimize superficial tissue compliance.

During the rMV, subjects were invited to assume a comfortable position on a chair, with their forearm positioned on a table and the hand supinated. The muscle vibration intervention was applied over 3 consecutive days, 3 times a day, with each application lasting 10 min. The 3 applications on the same day were separated by a 60 s interval, during which the stimulus was removed and the subject's muscles could relax.

2.5. Main experiment (Exp. 1): long-term effects of rMV under voluntary contraction condition

Ten subjects were asked to maintain a voluntary isometric wrist flexion during the rMV intervention (*VC* = vibration + contraction) at 20% of the maximal voluntary contraction (MVC), as assessed by visual EMG feedback.

The following neurophysiological parameters were studied and averaged across subjects:

1) Motor threshold at rest (RMT) for the FCR, EDC and ADM.
2) Map area, defined as the number of scalp positions whose stimulation evoked MEPs in that muscle. The mean motor map area for each muscle was defined as the number of "active" sites in all the participants divided by the number of participants. The map area was obtained from the FCR, EDC and ADM.
3) Map volume. The mean MEP amplitude for each scalp site was first calculated, and the amplitudes of all the positions were then summed to obtain the map volume. Lastly, the mean value for 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. The map volume was obtained from the FCR, EDC and ADM.
4) SICI/ICF in the FCR and EDC.
5) H reflex from the FCR muscle (6/10 subjects).

Recordings were performed at the following time points: before the intervention (pre-rMV) and one hour (post-1), and one (post-2), two (post-3) and three (post-4) weeks after the end of muscle vibration (performed on the day 3). Both the right ipsilateral hemisphere (RH) and the left contralateral hemisphere (LH), relative to the side of the rMV intervention (i.e. right FCR), were studied in all the subjects to calculate the area and volume. The LH alone was studied in all the subjects to determine SICI/ICF.

2.6. Control experiment (Exp. 2): effects of rMV under muscular relaxation. Effects of contraction without vibration

Two additional conditions were assessed in this experiment. In one group of 5 subjects, the vibratory stimulus was applied with the wrist totally relaxed (*VR* = vibration + relaxation) and the hand resting on the table surface; audio-feedback EMG was used to ensure that total relaxation was maintained. In another group, 5 subjects were asked to maintain a voluntary isometric wrist flexion using the same procedure as in experiment 1, but did not receive the vibratory stimulus (*CTR* = contraction without vibration); visual EMG feedback was used to ensure that 20% of the MVC was maintained. The RMT, motor map area and volume and SICI/ICF were recorded as in experiment 1, at the same time points. The LH alone was studied in this control experiment.

2.7. Control experiment (Exp. 3): short-term effects of single MV under voluntary contraction condition

A further six subjects participated in this experiment. Here, the short-term after effects of a single (10 min) muscle vibration (sMV) intervention on the FCR were tested. The RMT, MEP amplitude, SICI (ISI 3 ms) and ICF (ISI 10 ms) were measured over the FCR and EDC muscles. Recordings were performed at the following time points: before the intervention (pre-sMV) and 15, 30 and 45 min after the end of the intervention, which was performed during wrist flexion. The LH alone was studied in this control experiment.

2.8. Data analysis

For the RMT, area and volume, the effects of the rMV intervention were quantified by measuring the peak-to-peak MEP amplitudes of all the muscles to single pulse TMS offline. The relative inhibition and facilitation for SICI/lCF were expressed as the ratio between the CS and test MEP size.

We applied analysis of variance (ANOVA) for repeated measures as the main statistical procedure, in which factors were always considered as within-subject.

Exp. 1. Three-way ANOVAs (*hemisphere* × *muscle* × *time*) were used to assess the effects of rMV on the RMT, area and volume, relative to the time of intervention (5 levels) for the FCR, EDC and ADM muscles. Further 3-way ANOVAs (*muscle* × *ISI* × *time*) were undertaken to determine SICI/ICF in the FCR and EDC. Moreover, 1-way ANOVA, with *time* as the within factor, was performed to assess possible spinal cord excitability changes after the rMV intervention by measuring the H/M ratio in the FCR of the *VC* group.

Exp. 2. Two-way (muscle × time) and 3-way ANOVAs (muscle × ISI × time) were performed separately for the *VR* and *CTR* groups on the same neurophysiological parameters as those studied in Exp. 1 to rule out the possibility of long-term effects induced by either vibration (*VR* group) or contraction (*CTR* group) alone.

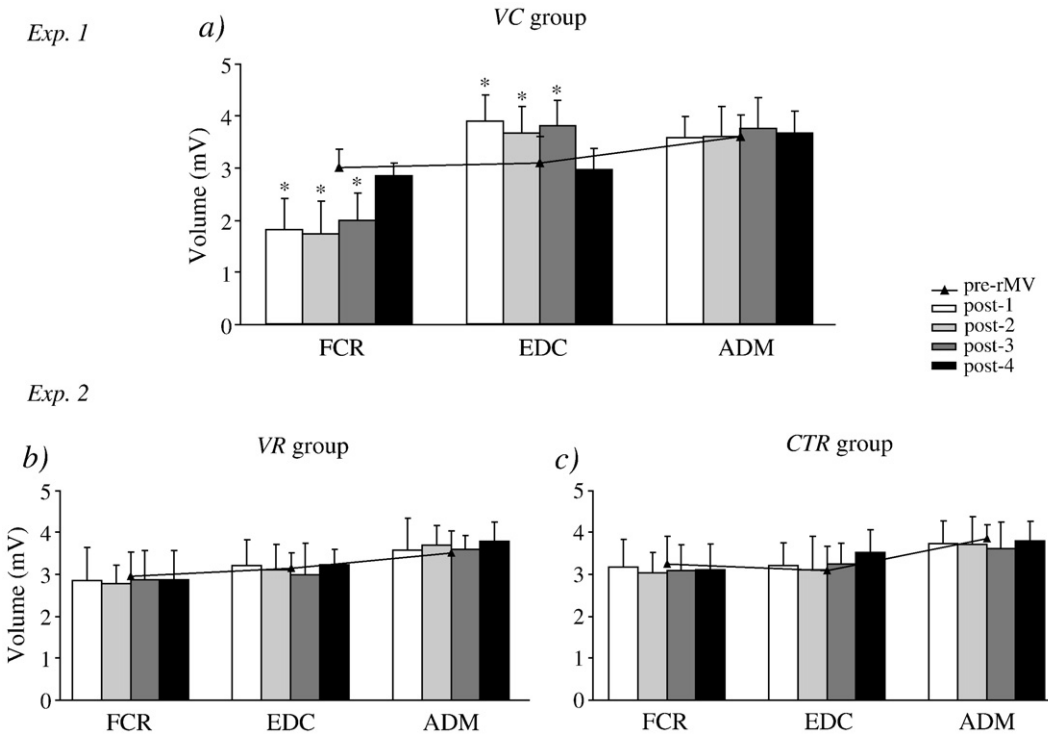

Fig. 2. Histograms (mean values±SD) of all the muscle map volumes (FCR, EDC and ADM) in the *VC* (a), *VR* (b) and *CTR* (c) groups, before the intervention (pre-rMV, line with triangles) and one hour (post-1), and one (post-2), two (post-3) and three (post-4) weeks after the end of the intervention. The FCR map volumes significantly decrease while the EDC map volume increases at post-1, post-2 and post-3, when compared with pre-rMV, in the *VC* group alone. At post-4, both the flexor and extensor MEP amplitudes return to their baseline values (pre-rMV). No significant change in map volume was observed for the ADM muscle. Lastly, there are no significant differences in the map volumes of either the *VR* or *CTR* groups in any of the muscles (all $p>0.05$) at any of the time points when compared with pre-rMV. Asterisks indicate significant differences from the baseline values (pre-rMV) (see Results for details).

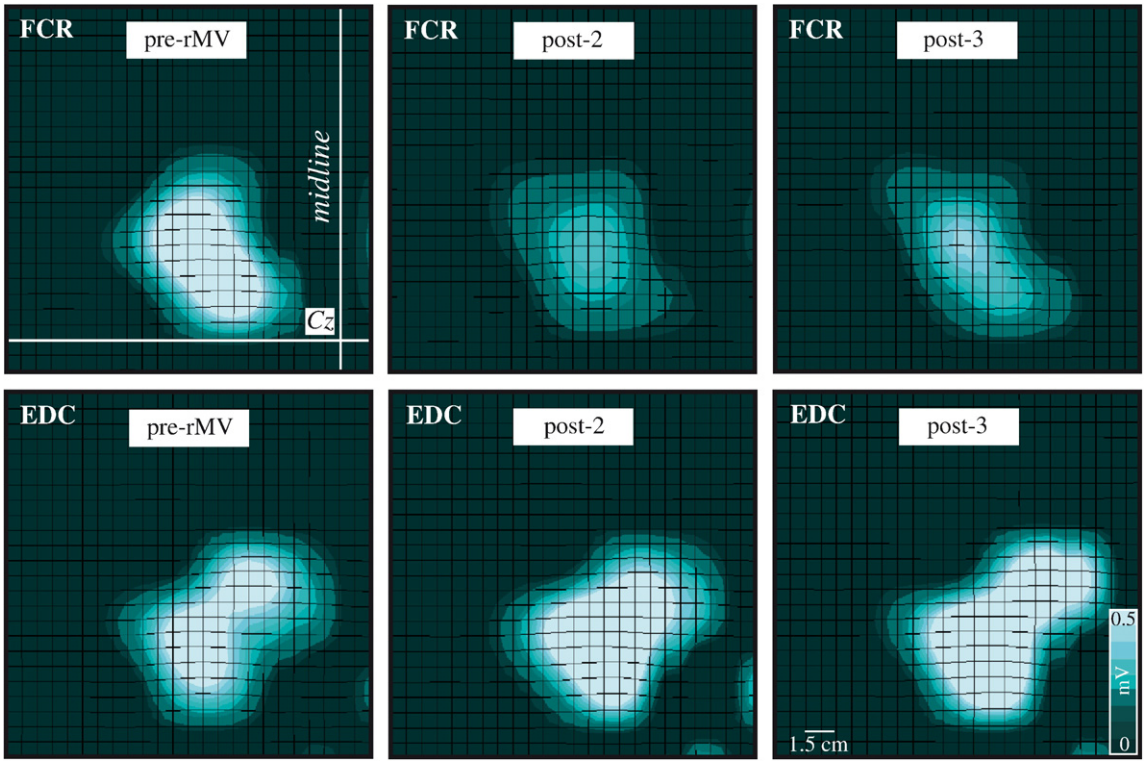

Fig. 3. Two-dimensional (2-D) cortical maps of the FCR and EDC muscles before and at the different time points after rMV in the *VC* group. Map volumes of the FCR and EDC muscles are shown at the baseline (pre-rMV), at post-2 and post-3. An evident decrease in the FCR map volume can be seen at post-2 and post-3, while the EDC map volume pattern is reversed at the same time points. The post-1 time point has not been shown because the data from this time point parallel those at post-2 and post-3. The post-4 (not shown) map volumes are virtually the same as the pre-rMV map volumes. The color coded palette of each map ranges from dark (0 mV) to light green (0.5 mV). The scale bar used is 1.5 cm for both the *x* and *y* axes. The other conventions are the same as those in Fig. 1.

Exp. 3. Two-way (muscle×time) and 3-way ANOVAs (muscle×ISI×time) were undertaken to assess the MEP amplitudes and SICI/ICF in the FCR and EDC muscles, relative to the time of intervention (4 levels).

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.

The p value level of significance throughout the statistical analysis was set at 0.05, considering Bonferroni correction.

3. Results

3.1. Main experiment (Exp. 1): long-term effects of rMV on corticomotor excitability during contraction

The mean RMT obtained from the hemisphere contralateral to the rMV (LH) before the intervention was 45.2±5.6%, 41.4±7.9% and 36.1±6.2% for the FCR, EDC and ADM, respectively. There were no significant differences in the RMT between the two hemispheres. ANOVA did not reveal any significant differences in the RMT after rMV of the FCR at any of the time points considered ($p>0.05$) for any of the afore-mentioned muscles. Moreover, ANOVA showed that the mean map areas observed at pre-rMV (FCR=6.6±1.3; EDC=7.5±2.8; ADM=9.2±1.9) did not change at any of the subsequent time points ($p>0.05$) in any of the muscles. The mean amplitudes of the H and M waves in the FCR muscle at pre-rMV were 0.9±0.2 mV and 8.1±0.2 mV, respectively (H/M ratio=0.11±0.03). ANOVA showed that, after rMV, the H/M amplitude ratio did not change at any of the time points either ($p>0.05$).

3.1.1. Persisting effects of rMV on cortical map volume

Data analysis of the map volumes revealed that the main factors *hemisphere* [F (1,9)=8.42; $p=0.018$], *muscle* [$F(2,18)=19.92$; $p<0.001$] and *time* [$F(4,36)=24.08$; $p<0.001$], as well as their interactions *hemisphere*×*muscle* [$F(2,18)=16.62$; $p<0.001$], *hemisphere*×*time* [$F(4,36)=23.40$; $p<0.001$], *muscle*×*time* [$F(8,72)=35.47$; $p<0.001$] and *hemisphere*×*muscle*×*time* [$F(8,72)=10.51$; $p<0.001$], were all significant. *Post-hoc* comparisons revealed that the FCR motor map volume was significantly reduced at post-1 ($p=0.011$), post-2 ($p=0.029$) and post-3 ($p=0.032$) when compared with pre-rMV (Fig. 2-*a*). This reduction in map volumes induced by rMV on the FCR can also be observed in the two-dimensional (2-D) maps shown in Fig. 3. Given the similarity between the results at post-1 and those at post-2 and -3, the post-1 results have not been shown. By contrast, the results obtained from the EDC muscle revealed that the motor map volumes were significantly increased at post-1 ($p=0.030$), post-2 ($p=0.014$) and post-3 ($p=0.010$) when compared with pre-rMV (Fig. 2-*a*). The increase in map volumes induced by rMV on the EDC muscle can also be observed in the 2-D maps shown in Fig. 3. Neither the reduction observed in the FCR motor maps nor the increase observed in the EDC maps was maintained at post-4 (Fig. 2-*a*). Data relative to the ADM muscle map volume did not reveal any significant differences in any of the main factors either.

3.1.2. Persisting effects of rMV on short-interval intracortical inhibition (SICI)

The 3-way ANOVA (muscle×ISI×time) performed to assess differences in SICI/ICF after rMV of the FCR showed that the main factors muscle [$F(1,9)=57.30$; $p<0.001$], ISI [$F(6,54)=90.45$; $p<0.001$] and time [$F(4,36)=66.95$; $p<0.001$], as well as their interactions muscle×ISI [$F(6,54)=25.59$; $p<0.001$], muscle×time [$F(4,36)=96.36$; $p<0.001$], ISI×time [$F(24,216)=78.05$; $p<0.001$] and muscle×ISI×time [$F(24,216)=80.33$; $p<0.001$], were significant. *Post-hoc* comparisons revealed that SICI for the FCR muscle was significantly increased for the 2 ms (all $p<0.001$), 3 ms (all $p<0.01$) and 5 ms (all $p<0.01$) ISIs at post-1 ($p=0.002$), post-2 ($p=0.008$) and post-3 ($p=0.009$) when compared with pre-rMV (Fig. 4). By contrast, SICI for the EDC muscle was significantly reduced for the 2 ms and 3 ms (all $p<0.01$) ISIs at post-1 ($p=0.009$), post-2 ($p=0.002$) and post-3 ($p=0.007$) when compared with pre-rMV (Fig. 4).

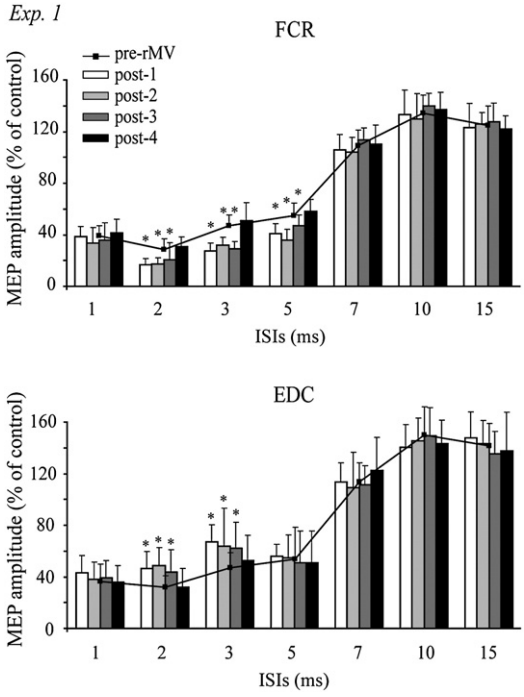

Fig. 4. Short-interval intracortical inhibition (SICI) and facilitation (ICF) in the FCR and EDC muscles of the *VC* group before and at the different time points after rMV (Experiment 1). The interstimulus intervals (ISIs in ms) are shown on the horizontal axis, while the vertical axis shows the size of the conditioned test response expressed in relation to the size of the unconditioned test response (mean values±SD). There is a significant increase in SICI (ISIs of 2, 3 and 5 ms) in the FCR muscle at post-1, post-2 and post-3 when compared with pre-rMV (line with squares). By contrast, there is a significant decrease in SICI (ISIs of 2 and 3 ms) in the EDC muscle post-1, post-2 and post-3 when compared with pre-rMV. SICI relative to both flexor and extensor muscles returns to its baseline value at post-4. No changes in ICF emerge for any of the muscles at any of the time points. The other conventions are the same as those in Fig. 2.

No significant effect was observed in ICF (7, 10 and 15 ms ISIs) for either the FCR or EDC muscles. Neither the increase nor the reduction following rMV in the flexor and extensor SICI, respectively, was maintained at post-4 (Fig. 4).

3.2. Control experiment (Exp. 2): effects of rMV during muscular relaxation and effects of contraction without vibration

The mean RMT obtained from the LH of the *VR* group before rMV were 45.8±6.2%, 42.3±5.2% and 37.4±5.7% for the FCR, EDC and ADM, respectively. ANOVA did not reveal any significant differences in the RMT after rMV of the FCR at any of the time points considered ($p>0.05$) for any of the afore-mentioned muscles. Moreover, ANOVA showed that the mean map areas observed at pre-rMV (FCR=5.8±3.2; EDC=7.4±3.8; ADM=9.9±3.8) did not change at any of the subsequent time points ($p>0.05$) in any of the muscles.

The mean RMT obtained from the LH of the *CTR* group before voluntary contraction were 47.3±5.3%, 44.4±5.4% and 35.4±5.9% for the FCR, EDC and ADM, respectively. ANOVA did not reveal any significant differences in the RMT of the FCR at any of the time points considered ($p>0.05$) for any of the afore-mentioned muscles. Moreover, ANOVA showed that the mean map areas observed before voluntary contraction (FCR=6.4±4.2; EDC=6.9±2.7; ADM=10.1±4.2) did not change at any of the subsequent time points ($p>0.05$) in any of the muscles. The data

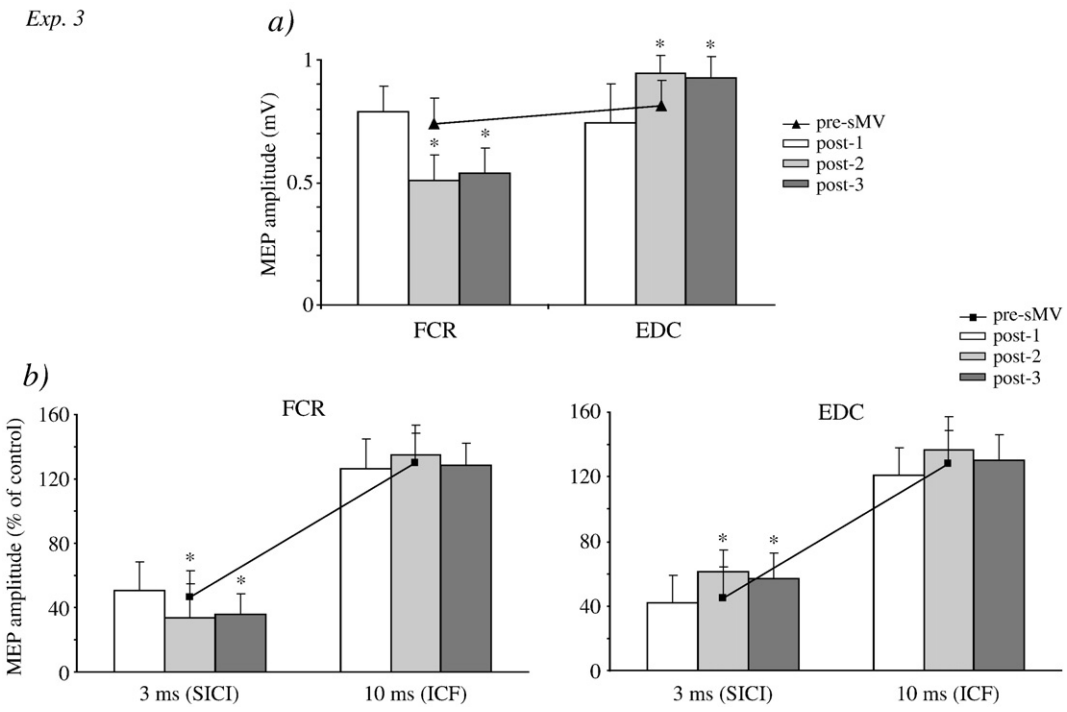

Fig. 5. MEP amplitudes (a), SICI and ICF (b) obtained from the hot spot of the FCR and EDC muscles before and at the different short time points after sMV (Experiment 3). There is a significant decrease in the FCR MEP amplitudes 30 (post-2) and 45 min (post-3) after sMV when compared with pre-sMV (line with squares). By contrast, there is a significant increase in the EDC MEP amplitudes at post-2 and post-3 when compared with pre-sMV. The SICI (ISI 3 ms) significantly increases in the FCR at post-2 and post-3 when compared with pre-sMV, but decreases in the EDC at the same time points. No changes in ICF emerge for either the flexor or extensor muscles at any of the time points after sMV. The other conventions are the same as those in Fig. 4.

relative to the map volume of both the *VR* and *CTR* groups are shown in Fig. 2. The 2-way ANOVAs (muscle×time) performed on both experimental conditions did not reveal any significant differences in map volumes in either the *VR* (Fig. 2-*b*) or *CTR* (Fig. 2-*c*) at any of the time points (all $p>0.05$). Moreover, the 3-way ANOVAs (muscle×ISI×time) performed separately on the *VR* and *CTR* groups did not detect any significant differences in either SICI or ICF in any of the muscles studied at any of the time points (all $p>0.05$).

3.3. Control experiment (Exp. 3): short-term effects of sMV on corticomotor excitability during contraction

The data of this experiment were obtained before, and 15, 30 and 45 min after the application of a 10-minute MV (sMV). The mean RMT obtained from the LH before sMV was 46.3±4.9% and 44.6±6.5% for the FCR and EDC, respectively. ANOVA did not reveal any significant differences in the RMT between pre-sMV and any of the subsequent time points studied (15, 30 and 45 min after the end of intervention) (all $p>0.05$). The data relative to the MEP amplitude before and after the sMV are shown in Fig. 5-*a*. The 2-way ANOVA (muscle×time) performed to detect any differences in single MEPs after sMV of the FCR showed that both the main factors muscle [$F(1,5)=32.66$; $p=0.002$] and time [$F(3,15)=9.11$; $p=0.001$], as well as their interaction muscle×time [$F(3,15)=55.47$; $p<0.001$], were significant. *Post-hoc* comparisons revealed that the MEP amplitude of the FCR significantly decreased 30 ($p=0.022$) and 45 min ($p=0.044$) after the end of the sMV intervention. By contrast, the MEP amplitude of the EDC significantly increased 30 ($p=0.022$) and 45 min ($p=0.044$) after the end of the sMV intervention (Fig. 5-*a*).

The 3-way ANOVA (muscle×ISI×time) performed to detect any differences in SICI/ICF after sMV of the FCR showed that the main factors muscle [$F(1,5)=20.58$; $p=0.006$], ISI [$F(1,5)=70.56$; $p<0.001$] and time [$F(3,15)=6.61$; $p=0.004$], as well as their interactions muscle×ISI [$F(1,5)=23.63$; $p=0.004$], muscle×time [$F(3,15)=7.69$; $p=0.002$] and muscle×ISI×time [$F(3,15)=12.81$; $p<0.001$], were significant. *Post-hoc* comparisons revealed that SICI for the FCR muscle (3 ms) significantly increased 30 ($p=0.042$) and 45 min ($p=0.004$) after the end of the sMV intervention when compared with pre-sMV (Fig. 5-*b*). By contrast, SICI for the EDC muscle was significantly reduced 30 ($p=0.015$) and 45 min ($p=0.018$) after the end of the sMV intervention when compared with pre-sMV (Fig. 5-*b*). No significant effect was observed in ICF (10 ms) for either the FCR or EDC muscles after sMV when compared with pre-sMV.

4. Discussion

The present experiments show that a repeated muscle vibration (rMV) intervention, applied during a voluntary contraction of the target muscle (i.e. FCR), induced long-lasting cortical excitability changes in M1 contralateral to the side of the intervention that persist up to two weeks in healthy subjects. These effects consist of a significant reduction in the cortical map volume of the FCR in concomitance with an increase in the map volume of the antagonist muscle (EDC). In addition, SICI increased in the FCR and decreased in the EDC. There was no effect on ICF in either the flexor or extensor muscles. No changes were observed in the map volume of the hand muscle (ADM), which suggests that the rMV intervention induced a specific activation in the cortical representation of the target muscle. Moreover, three weeks after the end of the rMV intervention, all the neurophysiological parameters had returned to their pre-intervention values. In addition, although rMV did not affect the H reflex, the possibility that other subcortical changes might have had an additional effect on MEP amplitude modulation cannot be ruled out.

Although our findings confirm the well-known capability of vibratory stimulation to modify corticomotor excitability [27–35], if compared with findings from previously published studies, they contain important novel elements as well as some discrepancies.

The most important novel element in the present study is the remarkable persistence of the corticomotor excitability changes induced by our protocol.

Moreover, whereas the intervention in our study inhibited the cortical representation of the target muscle, the majority of previous studies have shown that a short period of muscle vibration applied to a target muscle increased motor-evoked potentials and decreased SICI in the vibrated muscle, and had the opposite effect on the neighbouring and/or antagonist non-vibrated muscles [28–31,33].

We believe that the particular characteristics of the protocol used in the present study may in part account for these discrepancies.

4.1. Sensorimotor combined activation as a possible mechanism underlying the long-lasting after-effects on corticomotor excitability

The main finding of this study are the noticeable long-term after-effects (up to 2 weeks) induced in corticomotor excitability by our protocol; as described above, it is characterized by repeated periods of sensory stimulation (over three days) as well as by a constant voluntary contraction of the target muscle during the intervention. These features are peculiar to the protocol used in the present study, since the majority of previously published studies have applied muscle vibration to relaxed hand and/or forearm muscles [27–35].

In addition, the vibration frequency was set at 100 Hz, i.e. within a range (75–120 Hz) that seems to be particularly effective on the central nervous network underlying motor control [49]. Interestingly, data from animal research have shown that both vibration exposure time and frequency are closely related to long-term potentiation/depression (LTP/LTD) [50–52]. Moreover, it is noteworthy that a number of findings support the notion that M1 plasticity is driven by the combined activity of somatosensory afferents and intracortical motor circuits [18,19] and that this interaction plays a crucial role in cortical reorganization [53]. These modifications may be ascribed to the specific functional pattern of connections linking SI and M1 homotopic sites [16,17]. In this regard, it has been demonstrated that the main sensory inputs to the motor cortex terminate on interneurons in the superficial cortical layers II and III, which have both excitatory and inhibitory effects on the corticofugal cells of layers V and VI [54–58], thus suggesting that SI stimulation influences the pyramidal tract neurons in different ways.

In the light of these findings, we suggest that the prolonged activation of the descending volley, induced by the ongoing muscle contraction with ascending volleys, might explain, at least in part, the long-lasting changes in corticomotor excitability of the target muscle (i.e. inhibition) as well as of its functional antagonist (i.e. facilitation).

A possible neurophysiological correlate, underlying the prolonged after-effects, may be changes in the strength of the afore-mentioned corticocortical connections, following a Hebbian-like mechanism of synaptic plasticity.

4.2. Peculiar features of our protocol as possible mechanisms underlying the long-lasting inhibitory changes in the target muscle

Another interesting finding that emerged from the present experiments is that our rMV intervention reduced the amplitude of MEPs, and therefore map volume, in the target flexor (FCR) muscle and facilitated motor responses and map volume in the antagonist extensor muscle (EDC). Moreover, SICI increased in the FCR and decreased in the EDC.

By contrast, most previous TMS studies have shown a different (i.e. inverse) MEP modulation. Indeed, muscle vibration was shown to increase MEP amplitude and decrease SICI in the target muscle, but suppress motor responses and increase SICI in all the non-vibrated muscles [27–31,33,34].

In particular, in studies in which MEPs were recorded from both extensor/flexor forearm muscles [28,29], muscle vibration increased MEPs in the vibrated muscle and simultaneously depressed motor responses in the non-vibrated/antagonist muscle. The latter phenomenon might result from a reciprocal mechanism of inhibition as well as from the inhibitory action of muscle afferents on corticospinal output to the antagonist muscle [59].

However, some characteristics of the protocol applied in the present study (e.g. the very long vibration exposure time) may partially account for the differences in the results between our study and previous studies. Indeed, the muscle vibration intervention in our study was applied over 3 consecutive days, 3 times a day, (each application lasting 10 min) during contraction of the target muscle (Experiment 1). By contrast, most previously published studies applied a far shorter period of muscle vibration, lasting a few seconds [28,30,31,60] or repeated cycles of 15 min (2 s on, 2 s off) [33,34].

Interestingly, the application of prolonged vibratory stimulation (up to 60 min) to the wrist flexor results in a persistent increase (up to 30 or 60 min) in the MEP amplitude, motor area and volume of the antagonist extensor muscle [32,35]. However, whereas Steyvers et al. [32] reported an additional increase in corticomotor excitability of the vibrated muscle in their study, Forder-Cordero et al. [35] did not observe any significant changes in the vibrated muscle.

Along this vein, it seems plausible that differences in the protocols applied in the various studies (e.g. vibration exposure time, muscle condition, time point at which the TMS investigation started) may dramatically change the effects observed in target muscle cortical excitability, promoting a shift toward facilitation or inhibition.

Therefore, since it has been demonstrated that the maintenance of a minimal voluntary contraction down-regulates inhibitory neurons [61], we may speculate that the "switching off" of intracortical inhibitory circuits creates a different (i.e. more facilitated) neurophysiological substrate on which Ia afferents may operate.

Moreover, since we found a very similar pattern to that observed in the main experiment even when a single muscle vibration lasting 10 min was applied (Experiment 3), it seems reasonable to suggest that the inhibitory effects induced by a repeated intervention may consolidate the changes induced by a single intervention over a longer period of time.

Finally, although the mechanisms underlying the afore-described cortical excitability changes are not yet clear, it is conceivable that the extraordinarily long exposure time to stimulation, combined with the maintenance of a contraction during the intervention, plays a crucial role in inducing the prolonged inhibition observed in the vibrated muscle.

One alternative explanation may be a shift toward inhibition (i.e. a temporal profile of the GABA-ergic circuits) [62,63] that may occur at some point after the end of stimulation. Further studies, based on a very strict, prolonged time course, are warranted to investigate this hypothesis.

4.3. Possible clinical implications

In the present study, we demonstrate that rMV induces a significant, long-lasting increase in SICI of the target muscle (FCR) and an inverse pattern in its functional antagonist (EDC), which might reflect a parallel up- and down-regulation of the GABA-ergic circuits in cortical areas controlling the pair of antagonists.

Although the mechanisms underlying intracortical inhibitory systems are not yet clear [64], there is evidence that SICI is altered in several pathological conditions, such as stroke [65] and amputees [3,8], that are characterized by the suppression of SICI and a shift toward enhanced excitation. Interestingly, increased SICI has been shown to parallel motor functional recovery after stroke [66]. In addition, it has been demonstrated that muscle-specific modulation of intracortical inhibition may play a role in preventing unintended muscle activation during task performance [67,68].

To sum up, since cortical output has been shown to be dependent on the balance between excitatory/inhibitory systems [69], our data, which show that it is possible to promote prolonged somatosensory-driven changes in the cortical inhibitory state, open interesting perspectives in the functional recovery of motor disorders due to a breakdown in the excitatory/inhibitory balance (e.g. in post-stroke

spasticity), although further studies are warranted to verify this hypothesis.

Acknowledgment

This work was supported by a Regione Lazio REG/09 grant.

References

[1] Feldman DE, Brecht M. Map plasticity in somatosensory cortex. Science 2005;310:810–5.
[2] Recanzone GH, Allard TT, Jenkins WM, Merzenich MM. Receptive-field changes induced by peripheral nerve stimulation in SI of adult cats. J Neurophysiol 1990;63:1213–25.
[3] Cohen LG, Bandinelli S, Findley TW, Hallett M. Motor reorganization after upper limb amputation in man. A study with focal magnetic stimulation. Brain 1991;114:615–27.
[4] Jacobs KM, Donoghue JP. Reshaping the cortical motor map by unmasking latent intracortical connections. Science 1991;251:944–7.
[5] Ridding MC, Rothwell JC. Reorganisation in human motor cortex. Can J Physiol Pharm 1995;73:218–22.
[6] Pascual-Leone A, Peris M, Tormos JM, Pascual AP, Catala MD. Reorganization of human cortical motor output maps following traumatic forearm amputation. Neuroreport 1996;7:2068–70.
[7] Nudo RJ, Milliken GW, Jenkins WM, Merzenich MM. Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. J Neurosci 1996;16:785–807.
[8] Chen R, Corwell B, Yaseen Z, Hallett M, Cohen LG. Mechanisms of cortical reorganization in lower-limb amputees. J Neurosci 1998;18:3443–50.
[9] Ridding MC, Rothwell JC. Afferent input and cortical organisation: a study with magnetic stimulation. Exp Brain Res 1999;126:536–44.
[10] Classen J, Liepert J, Wise SP, Hallett M, Cohen LG. Rapid plasticity of human cortical movement representation induced by practice. J Neurophysiol 1998;79:1117–23.
[11] Hamdy S, Rothwell JC, Aziz Q, Singh KD, Thompson DG. Long-term reorganization of human motor cortex driven by short-term sensory stimulation. Nat Neurosci 1998;1:64–8.
[12] Ridding MC, Brouwer B, Miles TS, Pitcher JB, Thompson PD. Changes in muscle responses to stimulation of the motor cortex induced by peripheral nerve stimulation in human subjects. Exp Brain Res 2000;131:135–43.
[13] Ridding MC, McKay DR, Thompson PD, Miles TS. Changes in corticomotor representations induced by prolonged peripheral nerve stimulation in humans. Clin Neurophysiol 2001;112:1461–9.
[14] Kaelin-Lang A, Luft AR, Sawaki L, Burstein AH, Sohn YH, Cohen LG. Modulation of human corticomotor excitability by somatosensory input. J Physiol 2002;540:623–33.
[15] Charlton CS, Ridding MC, Thompson PD, Miles TS. Prolonged peripheral nerve stimulation induces persistent changes in excitability of human motor cortex. J Neurol Sci 2003;208:79–85.
[16] Ghosh S, Brinkman C, Porter R. A quantitative study of the distribution of neurons projecting to the precentral motor cortex in the monkey (*M. fascicularis*). J Comp Neurol 1987;259:424–44.
[17] Huerta MF, Pons TP. Primary motor cortex receives input from area 3a in macaques. Brain Res 1990;537:367–71.
[18] Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 2000;123:572–84.
[19] Ridding MC, Taylor JL. Mechanisms of motor-evoked potential facilitation following prolonged dual peripheral and central stimulation in humans. J Physiol 2001;537:623–31.
[20] Brown MC, Engberg I, Matthews PB. The relative sensitivity to vibration of muscle receptors of the cat. J Physiol 1967;192:773–800.
[21] Matthews PBC. Mammalian muscle receptors and their central action. London: Edward Arnold; 1972.
[22] Roll JP, Vedel JP, Ribot E. Alteration of proprioceptive messages induced by tendon vibration in man: a microneurographic study. Exp Brain Res 1989;76:213–22.
[23] Heath CJ, Hore J, Phillips CG. Inputs from low threshold muscle and cutaneous afferents of hand and forearm to areas 3a and 3b of baboon's cerebral cortex. J Physiol 1976;257:199–227.
[24] Hore J, Preston JB, Cheney PD. Responses of cortical neurons (areas 3a and 4) to ramp stretch of hindlimb muscles in the baboon. J Neurophysiol 1976;39:484–500.
[25] Jones EG, Porter R. What is area 3a? Brain Res Rev 1980;203:1–43.
[26] Fourment A, Chennevelle JM, Belhaj-Saif A, Maton B, Fourment A, Chennevelle JM, et al. Responses of motor cortical cells to short trains of vibration. Exp Brain Res 1996;111:208–14.
[27] Kossev A, Siggelkow S, Schubert M, Wohlfarth K, Dengler R. Muscle vibration: different effects on transcranial magnetic and electrical stimulation. Muscle Nerve 1999;22:946–8.
[28] Siggelkow S, Kossev A, Schubert M, Kappels HH, Wolf W, Dengler R. Modulation of motor evoked potentials by muscle vibration: the role of vibration frequency. Muscle Nerve 1999;22:1544–8.
[29] Rosenkranz K, Altenmuller E, Siggelkow S, Dengler R. Alteration of sensorimotor integration in musician's cramp: impaired focusing of proprioception. Clin Neurophysiol 2000;111:2040–5.
[30] Rosenkranz K, Pesenti A, Paulus W, Tergau F. Focal reduction of intracortical inhibition in the motor cortex by selective proprioceptive stimulation. Exp Brain Res 2003;149:9–16.
[31] Rosenkranz K, Rothwell JC. Differential effect of muscle vibration on intracortical inhibitory circuits in humans. J Physiol 2003;551:649–60.
[32] Steyvers M, Levin O, Van Baelen M, Swinnen SP. Corticospinal excitability changes following prolonged muscle tendon vibration. Neuroreport 2003;14:1901–5.
[33] Rosenkranz K, Rothwell JC. The effect of sensory input and attention on the sensorimotor organization of the hand area of the human motor cortex. J Physiol 2004;561:307–20.
[34] Rosenkranz K, Rothwell JC. Differences between the effects of three plasticity inducing protocols on the organization of the human motor cortex. Eur J Neurosci 2006;23:822–9.
[35] Forner-Cordero A, Steyvers M, Levin O, Alaerts K, Swinnen SP. Changes in corticomotor excitability following prolonged muscle tendon vibration. Behav Brain Res 2008;190:41–9.
[36] Chen R, Corwell B, Hallett M. Modulation of motor cortex excitability by median nerve and digit stimulation. Exp Brain Res 1999;129:77–86.
[37] Classen J, Steinfelder B, Liepert J, Stefan K, Celnik P, Cohen LG, et al. Cutaneomotor integration in humans is somatotopically organized at various levels of the nervous system and is task dependent. Exp Brain Res 2000;130:48–59.
[38] Tamburin S, Manganotti P, Zanette G, Fiaschi A. Cutaneomotor integration in human hand motor areas: somatotopic effect and interaction of afferents. Exp Brain Res 2001;141:232–41.
[39] Brunetti O, Filippi GM, Lorenzini M, Liti A, Panichi R, Roscini M, et al. Improvement of posture stability by vibratory stimulation following anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 2006;14:1180–7.
[40] Fattorini L, Ferraresi A, Rodio A, Azzena GB, Filippi GM. Motor performance changes induced by muscle vibration. Eur J Appl Physiol 2006;98:79–87.
[41] Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9:97–113.
[42] Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol 1994;91:79–92.
[43] Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. J Physiol 1993;471:501–19.
[44] Necking LE, Lundström R, Dahlin LB, Lundborg G, Thornell LE, Fridén J. Tissue displacement is a causative factor in vibration-induced muscle injury. J Hand Surg 1996;21:753–7.
[45] Lance JW. The reflex effects of muscle vibration. Proc Aust Assoc Neurol 1966;4:49–56.
[46] Hagbarth KE, Eklund G. The effects of muscle vibration in spasticity, rigidity, and cerebellar disorders. J Neurol Neurosurg Psychiatry 1968;31:207–13.
[47] Marsden CD, Meadows JC, Hodgson HJ. Observations on the reflex response to muscle vibration in man and its voluntary control. Brain 1969;92:829–46.
[48] Mottram CJ, Maluf KS, Stephenson JL, Anderson MK, Enoka RM. Prolonged vibration of the biceps brachii tendon reduces time to failure when maintaining arm position with a submaximal load. J Neurophysiol 2006;95:1185–93.
[49] Steyvers M, Levin O, Verschueren SM, Swinnen SP. Frequency-dependent effects of muscle tendon vibration on corticospinal excitability: a TMS study. Exp Brain Res 2003;151:9–14.
[50] Sakamoto T, Porter LL, Asanuma H. Long-lasting potentiation of synaptic potentials in the motor cortex produced by stimulation of the sensory cortex in the cat: a basis of motor learning. Brain Res 1987;413:360–4.
[51] Linden DJ. Long-term synaptic depression in the mammalian brain. Neuron 1994;12:457–72.
[52] Bear MF, Malenka RC. Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol 1994;4:389–99.
[53] Sailer A, Molnar GF, Cunic DI, Chen R. Effects of peripheral sensory input on cortical inhibition in humans. J Physiol 2002;544:617–29.
[54] Caria MA, Kaneko T, Kimura A, Asanuma H. Functional organization of the projection from area 2 to area 4 gamma in the cat. J Neurophysiol 1997;77:3107–14.
[55] Kosar E, Waters RS, Tsukahara N, Asanuma H. Anatomical and physiological properties of the projection from the sensory cortex to the motor cortex in normal cats: the difference between corticocortical and thalamocortical projections. Brain Res 1985;345:68–78.
[56] Porter LL, Sakamoto T, Asanuma H. Morphological and physiological identification of neurons in the cat motor cortex which receive direct input from the somatic sensory cortex. Exp Brain Res 1990;80:209–12.
[57] Kaneko T, Caria MA, Asanuma H. Information processing within the motor cortex. I. Responses of morphologically identified motor cortical cells to stimulation of the somatosensory cortex. J Comp Neurol 1994;345:161–71.
[58] Kaneko T, Caria MA, Asanuma H. Information processing within the motor cortex. II. Intracortical connections between neurons receiving somatosensory cortical input and motor output neurons of the cortex. J Comp Neurol 1994;345:172–84.
[59] Bertolasi L, Priori A, Tinazzi M, Bertasi V, Rothwell JC. Inhibitory action of forearm flexor muscle afferents on corticospinal outputs to antagonist muscles in humans. J Physiol 1998;511:947–56.
[60] Rosenkranz K, Williamon A, Butler K, Cordivari C, Lees AJ, Rothwell JC. Pathophysiological differences between musician's dystonia and writer's cramp. Brain 2005;128:918–31.
[61] Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, et al. Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. J Physiol 1998;508:625–33.
[62] Davies CH, Davies SN, Collingridge GL. Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. J Physiol 1990;424:513–31.

[63] Kang Y, Kaneko T, Ohishi H, Endo K, Araki T. Spatiotemporally differential inhibition of pyramidal cells in the cat motor cortex. J Neurophysiol 1994;71:280–93.
[64] Chen R. Interactions between inhibitory and excitatory circuits in the human motor cortex. Exp Brain Res 2004;154:1–10.
[65] Talelli P, Greenwood RJ, Rothwell JC. Arm function after stroke: neurophysiological correlates and recovery mechanisms assessed by transcranial magnetic stimulation. Clin Neurophysiol 2006;117:1641–59.
[66] Bütefisch CM, Netz J, Wessling M, Seitz RJ, Homberg V. Remote changes in cortical excitability after stroke. Brain 2003;126:470–81.
[67] Liepert J, Classen J, Cohen LG, Hallett M. Task-dependent changes of intracortical inhibition. Exp Brain Res 1998;118:421–6.
[68] Stinear CM, Byblow WD. Role of intracortical inhibition in selective hand muscle activation. J Neurophysiol 2003;89:2014–20.
[69] Sanger TD, Garg RR, Chen R. Interactions between two different inhibitory systems in the human motor cortex. J Physiol 2001;530:307–17.