Enhanced motor function and its neurophysiological correlates after navigated low-frequency repetitive transcranial magnetic stimulation over the contralesional motor cortex in stroke

Shahid Bashir[a,b], Marine Vernet[a], Umer Najib[a], Jennifer Perez[a], Miguel Alonso-Alonso[a],
Mark Knobel[a], Woo-Kyoung Yoo[a], Dylan Edwards[a,c] and Alvaro Pascual-Leone[a,d,*]

[a]*Department of Neurology, Berenson-Allen Center for Noninvasive Brain Stimulation,
Beth Israel Deaconess Medical Center, Harvard Medical School, Brookline Avenue KS, Boston, MA, USA*
[b]*Faculty of Medicine, Department of Physiology, King Saud University, Riyadh, Saudi Arabia*
[c]*Department of Neurology and The Burke Medical Research Institute, Weill Cornell Medical College, NY, USA*
[d]*Institut Universitari de Neurorehabilitació Guttmann, Universidad Autónoma de Barcelona, Barcelona, Spain*

Abstract.
Background: The net effect of altered interhemispheric interactions between homologous motor cortical areas after unilateral stroke has been previously reported to contribute to residual hemiparesis. Using this framework, we hypothesized that navigated 1 Hz repetitive transcranial magnetic stimulation (rTMS) over the contralesional hemisphere would induce a stronger physiological and behavioural response in patients with residual motor deficit than in healthy subjects, because an imbalance in interhemispheric excitability may underlie motor dysfunction.
Methods: Navigated rTMS was conducted in 8 chronic stroke patients (67.50 ± 13.77 years) and in 8 comparable normal subjects (57.38 ± 9.61 years). We evaluated motor function (Finger tapping, Nine Hole Peg test, Strength Index and Reaction Time) as well as the excitatory and inhibitory function (resting motor threshold, motor evoked potential amplitude, intra-cortical inhibition and facilitation, and silent period) of the stimulated and non-stimulated motor cortex before and after navigated rTMS.
Results: rTMS induced an increase in excitability in the ipsilesional (non-stimulated) motor cortex and led to improved performance in the finger tapping task and pinch force task. These physiological and behavioral effects were more prominent (or robust) in the group of stroke patients than in the control group.
Conclusion: Navigated low-frequency rTMS involving precise and consistent targeting of the contralesional hemisphere in stroke patients enhanced the cortical excitability of the ipsilesional hemisphere and the motor response of the hemiparetic hand.

Keywords: Navigated rTMS, rehabilitation, plasticity, cortex excitability, intra-cortical inhibition, motor behavior

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*Corresponding author: Alvaro Pascual-Leone, M.D., Ph.D., Berenson-Allen Center for Noninvasive Brain Stimulation, Beth Israel Deaconess Medical Center, 330 Brookline Ave, Boston, MA 02215, USA. Tel.: +1 617 667 0203; Fax: +1 617 975 5322; E-mail: apleone@bidmc.harvard.edu.

1. Introduction

Cortical reorganization is known to occur during the post-stroke recovery process by various proposed mechanisms including interactions between the primary motor cortex in the ipsilesional and the contralesional hemisphere (Calautti et al., 2001; Duque et al., 2008; Grefkes et al., 2009; Murase et al., 2004; Talelli et al., 2006). In the context of experimental rehabilitative therapies in stroke, the model positing interhemispheric excitability imbalance and an important role of transcallosal connections provides a framework for hypotheses based on concepts: up-regulation of the excitability of intact portions of the ipsilesional motor cortex and down-regulation of the excitability of the contralesional motor cortex. Several ongoing clinical trials are based on this framework. The contralesional motor cortex is presumed to be disinhibited due to the lack of an inhibitory influence from the lesioned motor cortex, while at the same time, it exerts an excessive inhibitory influence on the lesioned motor cortex. Such excessive inhibitory influence on the ipsilesional hemisphere would impair recovery of function (Manganotti et al., 2002; Mansur et al., 2005; Murase et al., 2004).

Repetitive TMS (rTMS) is a non-invasive method for producing potent changes in cortical excitability promoting functional recovery in stroke patients via increased neuroplasticity (Ameli et al., 2009; Bashir et al., 2010; Malcolm et al., 2007; Mansur et al., 2005; Yozbatiran et al., 2009). The down regulation of the contralesional, disinhibited motor regions through neurostimulation is thought to balance the abnormal inhibitory influence on ipsilesional regions (Butefisch et al., 2008; Cramer et al., 2008; Schlaug et al., 2008). Pilot studies, using either rTMS (Ameli et al., 2009; Malcolm et al., 2007; Mansur et al., 2005; Yozbatiran et al., 2009) or transcranial direct current stimulation (tDCS) (Celnik et al., 2009; Fregni et al., 2005 and Hummel et al., 2005) have shown that these approaches can improve motor impairment, at least transiently.

However, reports addressing effects of 1 Hz rTMS on contralesional motor cortex are limited and it is still unclear how rTMS over the contralesional hemisphere might modulate the excitability of the ipsilesional hemisphere. One study only showed that 1 Hz rTMS on contralesional hemisphere decreased short-interval intracortical inhibition (SICI) in primary motor cortex of both hemispheres (Manganotti et al., 2002). In the present study, we aimed to further explore the excitation/

inhibition balance of both hemispheres after a similar procedure.

Although the role of coil positioning techniques has been evaluated in several studies (Herwig et al., 2003; Moisa et al., 2009; Rusjan et al., 2010; Ruohonen et al., 2010; Sack et al., 2007, and Sparing et al., 2008), the effects of navigated rTMS on motor function and neurophysiology in stroke has not been fully explored. In navigated rTMS, the location of stimulation can be defined accurately relative to various anatomical structures, allowing stimulation of brain areas with greater spatial accuracy and reproducibility than conventional TMS method in healthy subjects (Bashir et al., 2011). Most of these studies have shown that neuronavigation increased spatial precision of the stimulation target. Navigated rTMS is thus believed to lead to greater effects and is increasingly used worldwide. Another aim of the study was to verify that navigated rTMS was indeed leading to meaningful improvements of motor function and physiology.

Finally, a third aim of the study was to compare the effects of navigated rTMS between stroke patients and control subjects. Indeed, many rTMS protocols are first tested in healthy volunteers before being translated towards rehabilitation protocols in patients. However, different baseline physiology, e.g., in terms of interhemispheric balance in the case of stroke patients, might modulate rTMS effects, which are believed to be strongly state-dependent.

2. Materials and methods

2.1. Subjects

Eight patients (mean age: 67.50 ± 13.77 years) and eight control subjects of comparable age (mean age: 57.38 ± 9.61 years) participated in this study. The investigation was carried out according to the latest version of the Declaration of Helsinki and was approved by the Beth Israel Deaconess Medical Center's Institutional Review Board. All participants gave their written informed consent prior to enrollment in the study.

The participants exhibited normal cognitive status, as indexed by their Mini Mental State Examination (MMSE; Folstein et al., 2001) scores (normal range: 28–30). Furthermore, neurological examination of the subjects revealed no abnormal signs that were suggestive of any underlying neurological or psychological condition other than stroke. The partic-

Table 1
Demographic characteristics of the stroke patients and
age-matched controls

	Controls	Patients	*p* value
n	8	8	
Age (mean $\pm$ SD)	57.38 ± 9.61	67.50 ± 13.77	0.188
Gender (M:F)	3 : 5	4 : 4	0.619
MMSE (mean $\pm$ SD)	30.00 ± 0.00	28.50 ± 2.27	0.200

n: number of subjects; SD: standard deviation; M: male; F: female;
MMSE: Mini Mental State Examination.

ipants were not taking any medication known to affect motor cortical excitability at the time of the study and had no contraindications to receive TMS (Rossi et al., 2009). The demographic and clinical characteristics of the sample are shown in Table 1. Further information about the stroke patients is included in Table 2. In each participant, navigated rTMS was performed in a single session lasting approximately 4-5 hours.

2.2. Experimental set-up

Prior to TMS, all subjects underwent high-resolution T1-weighted structural MRI scanning. Imaging data were fed to the navigation software (eXimia 3.1, Nexstim Ltd, Helsinki Finland) for automatic 3D brain reconstruction, and the reconstructed images were used to guide navigation and deliver TMS. Nexstim 59 mm (mean winding diameter) focal figure-of-eight TMS coils were used. We used a TMS coil delivering monophasic pulses (type 201514P) for the paired-pulse stimulation paradigm and a TMS coil delivering biphasic pulses (type 201383P) otherwise. During stimulation, surface electromyography (EMG) was recorded and continuously monitored online (ME 6000, Mega Electronics Ltd, Kuopio, Finland) using pre-gelled, disposable Ag/AgCl electrodes (10 mm diameter). Active electrodes were attached to the skin overlying the first dorsal interosseus (FDI) muscle and reference electrodes were placed over the metacarpophalangeal joints. The EMG signals were filtered (8–500 Hz), amplified, displayed and stored for off-line analysis. The TMS system delivered trigger pulses that synchronized the TMS and EMG systems. The measurement protocol has been previously described in detailed (Bashir et al., 2011; Säisänen et al., 2008).

The subjects sat in a comfortable recliner and held their hands in a supine position on their laps while measurements were recorded. They were instructed to remain silent during the study to avoid speech-induced modulation of cortical excitability.

The subjects were also monitored for drowsiness and asked to keep their eyes open throughout the experiment, which was checked regularly by the experimenter. Muscle relaxation was controlled via continuous online visual EMG monitoring, and re-checked offline via visual inspection for absence of background activity.

2.3. Determination of the optimal site and motor threshold

In each session, the motor cortical output was mapped carefully to determine the optimal representation of the FDI muscle on both hemispheres in both groups. The site that evoked the MEPs of the highest amplitude (henceforth, the hot spot) was identified. Then, resting motor threshold (RMT) was determined as the minimum TMS intensity that produced at least five motor evoked potentials (MEPs) equal or greater than $50 \mu V$ peak-to-peak amplitude out of 10 consecutive stimuli delivered at an inter-stimulus interval of 5–10 s.

2.4. TMS

2.4.1. Single-pulse
Following determination of RMT, 10 single stimuli 5–10s apart were delivered to the hot spot at an intensity of 120% of RMT to determine baseline MEP amplitude and latency.

2.4.2. Paired pulse paradigm
The paired pulse protocol followed the design by Kujirai et al. (1993) with two conditioning-test pulse interstimulus intervals (ISI): 3 ms to assess SICI and 12 ms to assess ICF. RMT was determined as described above but this time the monophasic pulse was used. The conditioning stimulus was applied at an intensity of 90% of resting motor threshold, the intensity of the test stimulus 120% of resting motor threshold, and these intensities remained constant in all paired-pulse TMS trials. Conditioned MEP amplitude (i.e., recorded after a paired-pulse) was compared to unconditioned MEP amplitude (i.e., recorded after a test stimulus alone). The interval between pairs of stimuli was at least 4–7s to avoid carry-over effects.

2.4.3. Cortical silent period
The contraction force was measured using a pinch grip simultaneously with both hands in a series of brief (4–7s) maximal isometric efforts (maximum

Table 2
Clinical characteristics of the stroke patients

Patient	Age/ Gender	Time since stroke*	MMSE	Lesioned Hemisphere	BI	MRS	SIC	FMS UL	FMS LL
1	71/M	2.25	30	LH	85	3	200	47	63
2	84/M	2.10	30	RH	90	3	200	34	78
3	68/F	4.25	25	RH	55	4	160	24	25
4	58/M	1.04	30	RH	65	3	160	28	20
5	41/F	2.15	30	RH	85	3	215	42	73
6	78/M	3.23	28	RH	95	3	190	42	69
7	62/F	2.80	25	RH	70	4	175	30	25
8	78/F	2.6	30	LH	95	3	190	48	29
Mean	67.50	2.55	28.5	–	80.00	3.25	186.25	36.87	47.75
SD	13.77	0.87	2.27	–	14.88	0.46	19.78	9.09	25.05

*In years; SD: standard deviation; M: male; F: female; MMSE: Mini Mental State Examination; LH: left hemisphere; RH: right hemisphere; BI: Barthel Index; MRS: Modified Rankin Scale; SIC: Stroke Impact Scale; FMS UL: Fugl Mayer Scale Upper Limb; FMS LL: Fugl Mayer Scale Lower Limb.

voluntary contraction, MVC). To assess the ipsilateral and the contralateral silent period (SP), force level was set to 20% of MVC. The muscle contraction was controlled by the subject with online visual force monitoring at the 20%MVC target. TMS-pulses were delivered during active tonic muscle contraction of approximately 4–7 s duration, and the subjects were instructed to maintain muscle contraction for at least 2 s after the stimulus was delivered. The TMS intensity was set at 120% of the resting motor threshold.

2.4.4. rTMS intervention

All subjects received a train of 1200 stimuli of 1 Hz rTMS at 90% RMT to the right M1 in normal subjects, and the contralesional M1 in stroke patients, delivered over the optimal site for MEPs in the contralateral FDI muscle (hot spot). Repetitive TMS in the 1 Hz range has previously been shown, in most subjects, to suppress excitability of the motor cortex in the targeted hemisphere and increase excitability in the contralateral motor cortex (reviewed by Fregni and Pascual-Leone, 2007; Kobayashi and Pascual-Leone, 2003). In the navigated rTMS intervention procedure, the cortex and hot spot were visualized in real-time by the navigated brain stimulation software to aid the delivery of rTMS (Fig. 1B). The coil was hand-held, and no mechanical coil support was employed during the stimulation procedures, allowing the experimenter to continually steady the coil within the target boundaries across the procedure.

2.5. Behavioral tasks

To assess the functional significance of possible neurophysiological differences before and after rTMS, the participants completed a battery of tests to evaluate the motor function of their right and left hands. The behavioral tasks were administered immediately before the neurophysiological measurements, preceding the rTMS intervention, and the same tasks were completed immediately after the neurophysiological measurements following rTMS intervention. The tasks were presented in the order in which they are described below, with the right hand tested before the left hand (Fig. 1A).

2.5.1. Nine-hole peg test

The time required to insert nine pegs into a pegboard and then remove the pegs from the pegboard was recorded across 3 trials. A 1-minute break was given between each trial.

2.5.2. Pinch grip strength

Average pinch grip strength (kg force) was measured according to a previously described protocol that exhibits good validity and test-retest reliability (Mathiowetz et al., 1984). The subjects grasped a pinch key with the pad of their thumb opposed against the lateral aspect of the middle phalanx of the index finger. For each assessment, three pinch grip trials, lasting 10 seconds each, were performed and averaged. One minute of rest was allowed between trials.

2.5.3. Finger tapping task

The subjects were asked to tap a button with their index finger of each hand as quickly as possible for the duration of 10 seconds. The tapping assessment comprised 5 trials. A 15-second rest period was allowed between most trials, and there was a 1-minute break between the third and fourth trials.
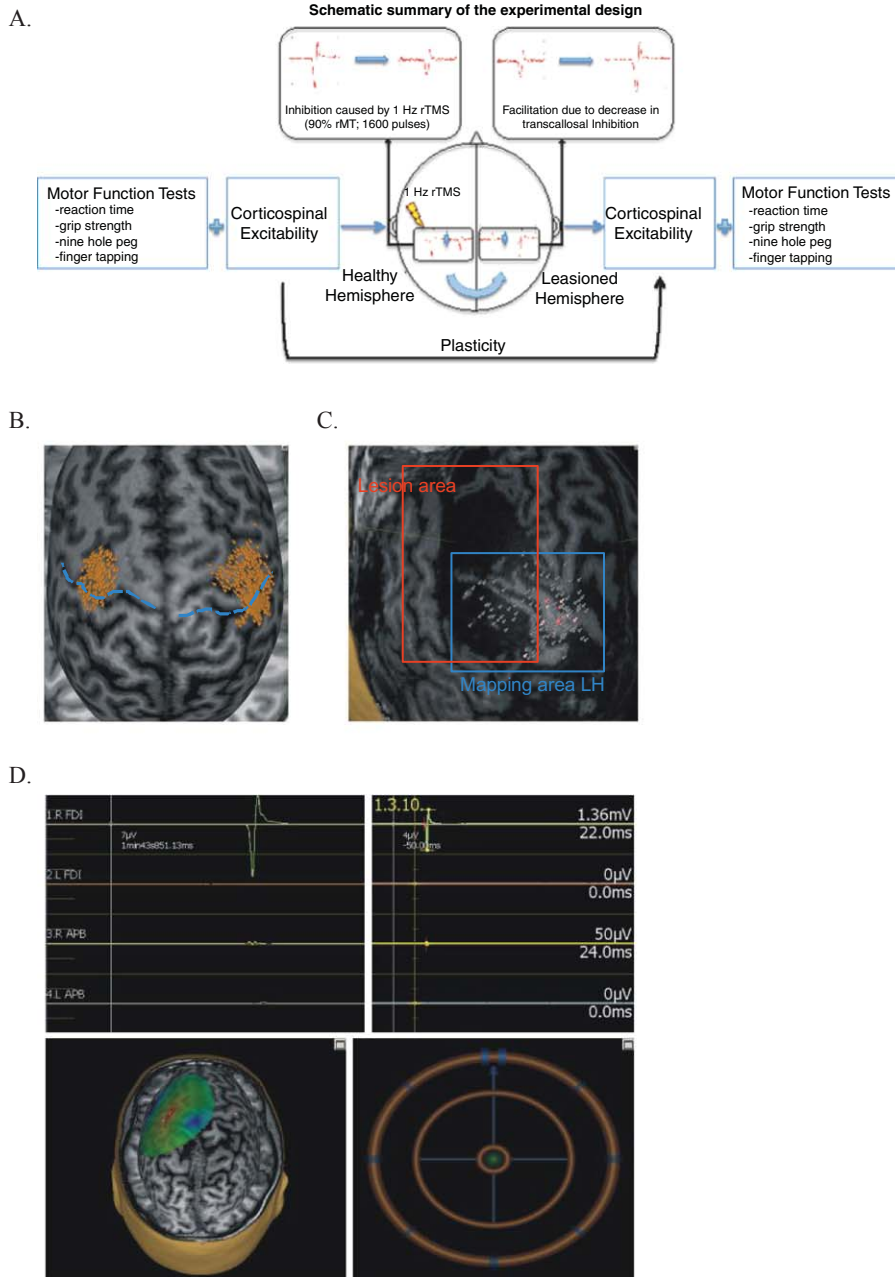
Fig. 1. A. Time course of the experiment. Navigated repetitive transcranial magnetic stimulation (rTMS) was applied over the primary motor cortex hand area of the contralesional hemisphere (and the right hemisphere in controls) at a frequency of 1 Hz and a stimulus intensity of 90% of the resting motor threshold (RMT) for approximately 27 min. The measurements performed to assess motor function (9-hole peg, pinch grip strength, finger tapping and reaction time tasks) and neurophysiology (motor threshold, motor evoked potential, intracortical inhabitation and facilitation and silent period measurements) were conducted both before and after rTMS. B. A sample screenshot from one subject illustrating the area mapped in each hemisphere to identify the optimal motor location (hot spot) for the first dorsal interosseus (FDI) muscle, relative to the central sulcus (blue dashed line). The central location of each stimulus pulse is visualized as a small ball; the head of the ball shows the orientation of the coil for each single pulse. C. A sample screenshot from one patient illustrating the area mapped in the lesion hemisphere. D. A screenshot of the online screen illustrating a single evoked response in the FDI and its peak-to-peak amplitude and onset latency (top, left and right two different scales); the relative strength of the electric field, which is color-coded (red corresponds to high values, and blue corresponds to low values, bottom left); the position feedback indicator, providing real-time feedback on the location, roll, pitch and yaw for consistent and reliable targeting (bottom right). The brain is "peeled" to a depth of 25 mm; i.e., the visualized stimulation surface is located at this depth from the scalp.

2.5.4. Simple reaction time task

The subjects were instructed to press the space bar with their index finger as fast as possible in response to an on-screen stimulus, and the time (ms) between stimulus onset and response was recorded. For each assessment, two trials of 20 stimuli each were presented to the participant.

2.6. Data and statistical analyses

To determine the MEP in response to TMS, continuous EMG data were sampled in 350 ms epochs, 50 ms before and 300 ms after each TMS pulse. FDI muscle responses (latency and MEP) were analyzed using MegaWin software (Mega Electronics Ltd, Kuopio, Finland). Corticospinal excitability was assessed by measuring the peak-to-peak amplitude of MEPs in the contralateral FDI muscle in response to single TMS pulses. To minimize the variability of TMS-induced single-pulse responses, the largest and smallest amplitude MEP responses were excluded from the analysis. The baseline and post-rTMS MEP amplitude and latencies were determined as the mean response across eight trials for each subject. For SICI and ICF, the size of the conditioned MEPs was expressed as a percentage of the unconditioned MEPs.

All behavioral measures were assessed in each hand both before and after rTMS and averaged across all of the trials performed by each participant.

All statistical tests were two-tailed, with statistical significance defined at $p < 0.05$. All statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago IL).

The effects of navigated rTMS were evaluated through a repeated measures analysis of variance (ANOVA) with the HEMISPHERE (stimulated and non-stimulated) as a within-subjects factor and the GROUP (stroke patients and healthy controls) as a between-subjects factor.

3. Results

The subjects did not report any adverse effects during the course of the study.

3.1. Corticospinal excitability measures

Cortical excitability measures indicate the relative changes ($\Delta = $[post-pre]/pre) of all the
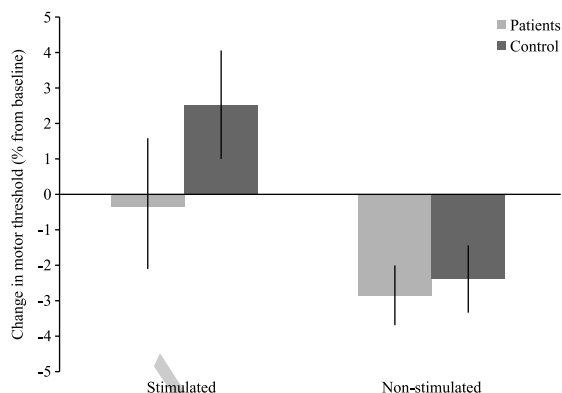

Fig. 2. Relative changes of the RMT induced by rTMS ($\Delta = $[post-pre]/pre) in each hemisphere and each group. Error bar: standard error.

neurophysiological parameters recorded for each hemisphere and each group.

3.1.1. Resting motor threshold

Figure 2 shows the relative changes, induced by rTMS in corticospinal excitability measured using the RMT for each hemisphere and each group. The ANOVA demonstrated a significant main effect of HEMISPHERE ($F = 5.020$, $p = 0.047$). RMT in the stimulated hemisphere was increased following rTMS, whereas it decreased in the non-stimulated hemisphere. However, the ANOVA did not reveal any significant effect of the GROUP factor, and the interaction between the HEMISPHERE and GROUP was not statistically significant (all $p > 0.05$).

3.1.2. Motor evoked potentials

Figure 3 presents the relative changes, induced by rTMS in corticospinal excitability (in this case MEP amplitude) for each hemisphere and each group. The ANOVA shows a main effect of HEMISPHERE. ($F = 35.20$, $p < 0.001$). The amplitude of the MEPs evoked by single-pulse over the stimulated hemisphere was decreased after rTMS, whereas the amplitude of the MEPs evoked by single-pulse over the non-stimulated hemisphere was increased. The ANOVA did not show any effect of the GROUP factor ($p > 0.05$). The interaction between the HEMISPHERE and GROUP was significant ($F = 6.58$, $p = 0.026$). *Post-hoc* testing revealed that the increase in MEP size over the non-stimulated hemisphere following rTMS was larger for patients than for healthy controls ($p < 0.05$). It should be noted, that on average the baseline (pre) MEP amplitude was slightly greater in the controls than in patients. This
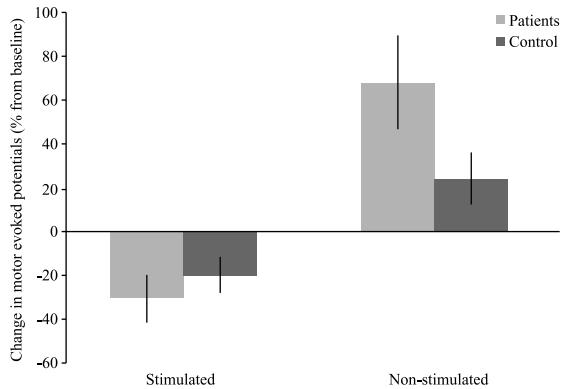
Fig. 3. Relative changes in the amplitudes of MEPs induced by rTMS ($\Delta = $[post-pre]/pre) for each hemisphere and each group. Error bar: standard error.

Fig. 4. Relative rTMS-induced changes in short interval intra-cortical inhibition (SICI) at interstimulus intervals of 3 ms and intra-cortical facilitation (ICF) at 12 ms ($\Delta = $[post-pre]/pre) for each hemisphere and each group. Error bar: standard error.

difference was significant for the non-stimulated hemisphere.

The MEP latencies elicited by TMS at 120% of the RMT in the relaxed muscle were also measured. The ANOVA showed a significant effect of HEMISPHERE ($F = 6.769$, $p = 0.025$). The latency of the MEPs evoked by single-pulse over the stimulated hemisphere was slightly increased after rTMS, whereas the latency of the MEPs evoked by single-pulse over the non-stimulated hemisphere was decreased. The ANOVA did not detect any significant effect of the GROUP factor, and the interaction between the HEMISPHERE and GROUP was not statistically significant (all the $p > 0.05$).

3.1.3. Cortical silent period

A stimulation intensity of 120% of the RMT was applied while sustaining 20% of MVC before and after TMS. The ANOVA showed that the HEMISPHERE and GROUP had no significant main effect on the relative changes observed in the cortical SP. There was no significant interaction between the HEMISPHERE and GROUP ($p > 0.05$).

3.1.4. Intracortical inhibition/facilitation

Figure 4 displays the relative changes, induced by rTMS in SICI and ICF for each hemisphere and each group.

With respect to SICI, the ANOVA did not reveal any significant effect of the HEMISPHERE and of GROUP and the interaction between the HEMISPHERE and GROUP was not statistically significant ($p > 0.05$). Repetitive TMS increased SICI (experimental values became more negative) regardless of the HEMISPHERE and GROUP.
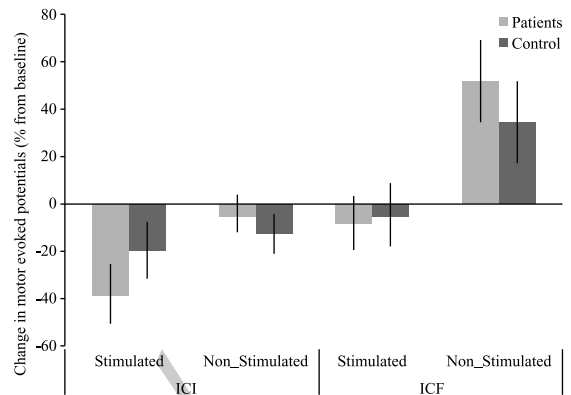
For ICF, the ANOVA showed a significant main effect of the HEMISPHERE ($F = 7.084$, $p = 0.022$). ICF decreased in the stimulated hemisphere after rTMS was applied whereas it increased in the non-stimulated hemisphere. The ANOVA did not reveal any effect of the GROUP factor and the interaction between the HEMISPHERE and GROUP was not statistically significant ($p > 0.05$).

3.2. Behavioral outcome measures

3.2.1. Motor function

For the 9-hole peg task, the ANOVA showed a significant effect of the SIDE ($F = 6.16$ $p = 0.03$, Fig. 5, Table 3). Performance improved (i.e., the time taken to complete the task decreased) in the hand ipsilateral to the stimulation (the affected hand in patients) but not for the hand contralateral to the stimulation. The ANOVA did not show any significant effect of the GROUP factor, and the interaction between the SIDE and GROUP was not statistically significant (all $p > 0.05$, Table 3).

For the pinch grip strength task, the ANOVA showed a significant effect of the SIDE ($F = 38.48$, $p < 0.000$). Muscle strength increased for the hand ipsilateral to the stimulation (the affected hand in patients), but not for the hand contralateral to the stimulated side. The effect of the GROUP was not statistically significant ($p > 0.05$). The interaction between the SIDE and GROUP was significant ($F = 17.26$, $p < 0.02$). *Post-hoc* testing showed that the effect of stimulation was stronger for patients than for controls ($p = 0.002$, Fig. 6, Table 3).

Table 3

Effects of 1 Hz rTMS on the hand performing behavioral tasks, including the 9-hole peg test (9-HPT), pinch grip strength task (PGS), finger tapping task (FTT) and simple reaction time task (RT) in the stimulated (contralateral to the rTMS intervention side) and non-stimulated (ipsilateral to the rTMS stimulation side) M1 for patients and controls. $\Delta = $ [post-pre]/pre

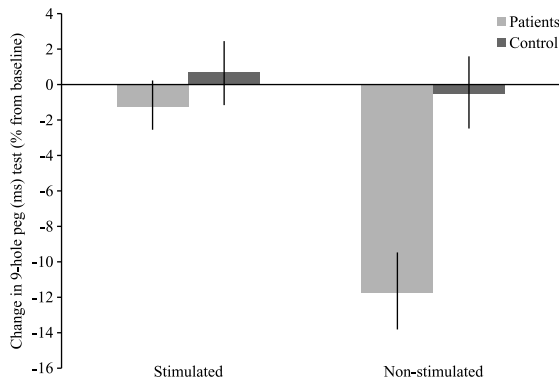|	Patients		Controls	
Hemisphere	Non-Stimulated	Stimulated	Non-Stimulated	Stimulated
9-HPT (sec)	$\Delta = -11.80 \pm 2.18$	$\Delta = -1.24 \pm 1.41$	$\Delta = -0.48 \pm 2.07$	$\Delta = 0.69 \pm 1.82$
PGS (kg)	$\Delta = 23.86 \pm 6.16$	$\Delta = -1.12 \pm 3.33$	$\Delta = 6.61 \pm 3.28$	$\Delta = 2.51 \pm 2.49$
FTT (no)	$\Delta = 45.44 \pm 4.20$	$\Delta = -0.45 \pm 3.21$	$\Delta = 1.50 \pm .1.65$	$\Delta = -2.40 \pm 1.58$
RT (ms)	$\Delta = -2.21 \pm 1.10$	$\Delta = 2.04 \pm 2.26$	$\Delta = -1.55 \pm 2.42$	$\Delta = 5.25 \pm 2.02$

Fig. 5. Relative changes of the 9-hole peg (ms) performance induced by rTMS ($\Delta = $ [post-pre]/pre) in each hemisphere and each group. Error bar: standard error.

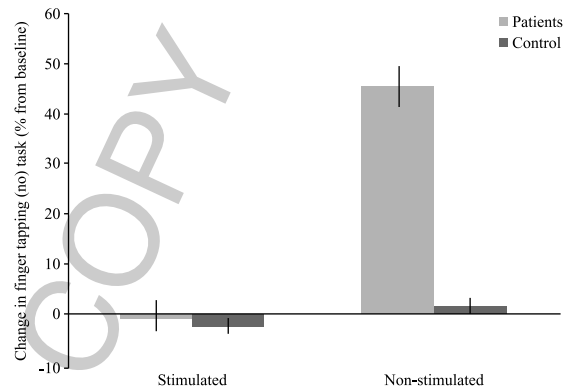Fig. 6. Relative changes of the pinch grip strength (Kg) performance induced by rTMS ($\Delta = $ [post-pre]/pre) in each hemisphere and each group. Error bar: standard error.

Fig. 7. Relative changes of the finger tapping (no) performance induced by rTMS ($\Delta = $ [post-pre]/pre) in each hemisphere and each group. Error bar: standard error.

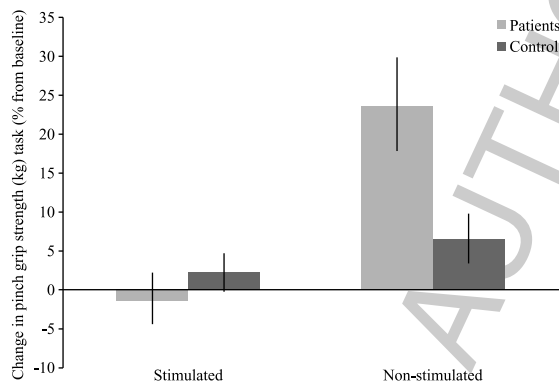For the finger-taping task, the ANOVA showed a significant effect of the SIDE ($F = 56.72$, $p < 0.000$). Finger tapping speed increased in the hand ipsilateral to the stimulation (the affected hand in patients), but not in the hand contralateral to the stimulation. The effect of the GROUP was not statistically significant ($p > 0.05$). The interaction between the SIDE and GROUP was significant ($F = 39.88$ $p = 0.000$)

Post-hoc testing showed that these effects were greater for patients than for controls ($p < 0.03$, Fig. 7, Table 3).

For the simple reaction time task, the ANOVA showed a significant effect of the SIDE ($F = 7.363$ $p = 0.02$). Reaction time performance improved (i.e., reaction time decreased) for the hand ipsilateral to the stimulation (the affected hand in patients), whereas performance decreased (reaction time increased) for the hand contralateral to the stimulation. The ANOVA did not show any significant effect of the GROUP factor, and the interaction between the SIDE and GROUP was not statistically significant (all $p > 0.05$, Fig. 8, Table 3).

4. Discussion

In this study, we found that navigated rTMS at 1 Hz over the contralesional motor cortex (M1) reduced the corticospinal excitability of this region, while increasing in the ipsilesional hemisphere, corresponding with improvement of the motor function of the affected hand in stroke patients compared to healthy controls. Of note, significant ICF increment
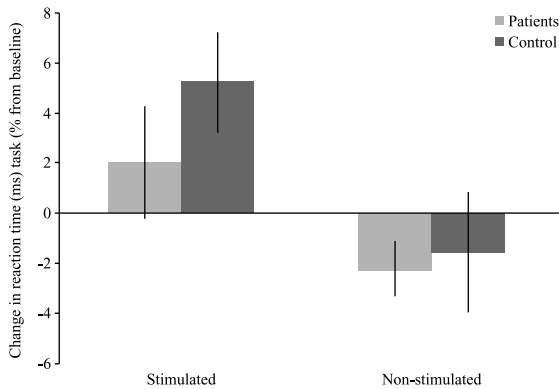
Fig. 8. Relative changes of the reaction time (msec) performance induced by rTMS (Δ = [post-pre]/pre) in each hemisphere and each group. Error bar: standard error.

was observed in the ipsilesional motor cortex following rTMS in both stroke and control groups.

Post-stroke motor recovery relies on cortical reorganization of the ipsilesional and contralesional hemispheres through disinhibition of the intact motor cortex via transcallosal fibers (Tombari et al., 2004). The speculative notion is that the more severely a unilateral hemisphere is damaged, the more distinct the trans hemispheric disinhibition is. Therefore, significant contralesional activation indicates more severe damage and poorer recovery. Although the roles played by ipsilesional and contralesional hemispheres in post-stroke recovery remains controversial, previous studies have shown that increased recruitment of the ipsilesional hemisphere and reduced recruitment of the contralesional hemisphere relate to improved motor recovery (Calauttite al., 2001; Cao et al., 1999 and Carey et al., 2005; Cinzia, et al., 2007; Cramer et al., 1997, 2004; Coupar et al., 2012; Johansen-Berg et al., 2002; Lioumis et al., 2012; Stinear, 2010; Zemke et al., 2003;).

Several studies have suggested that the down regulation of the contralesional motor cortex results in an improvement in the motor function of the affected hand in chronic stroke (Avenanti et al., 2012; Hummel et al., 2005; Kobayashi et al., 2004; Maeda et al., 2000, Mansur et al., 2005; Pal et al., 2005; Ziemann, 2005). Behaviorally, we also found that navigated rTMS led to improvements in all of the tasks performed with the hand ipsilateral to the stimulated side. In the grip strength and finger tapping tasks, these effects were greater in stroke patients than in controls. Finger tapping is reported as an assay of human voluntary motor control in healthy adults and in stroke recovery (Calautti et al., 2010; Shimoyama

et al., 1990 and Pineiro et al., 2011). Here we show an increase in this ability in the affected hand of stroke patients, and importantly, no reduction in performance of the unaffected hand; seldom assessed in protocols aiming to reduce corticospinal excitability in the unaffected hemisphere. These results are consistent with the neurophysiological finding of a greater interhemispheric impact in patients than in controls. Our results expand prior findings demonstrating that suppression of primary motor cortex cortical excitability using 1 Hz rTMS may result in faster serial button pressing with the ipsilateral hand in healthy humans (Bashir et al., 2011; Kobayashi et al., 2003). Furthermore, 1 Hz rTMS applied over the right primary motor cortex has been found to modify several kinematic parameters in a sequential finger opposition task performed with the ipsilateral hand (Avenanti et al., 2012).

Regarding changes in corticospinal excitability, previous studies showed that 1 Hz rTMS led to a reduction of MEP amplitude of stimulated primary motor cortex with increased MEP amplitude of the non-stimulated motor cortex in stroke (Fregni et al., 2005; Takeuchi et al., 2008) as well as in healthy controls (Gugino et al., 2001; Heide et al., 2006), which was similar to our study. As described above, this finding also follows the mechanism of reduced disinhibition of the ipsilesional hemisphere, plausibly by the disruption of transcallosal inhibition. When considered in stroke, disinhibition in the ipsilesional hemisphere is known to be prominent in early period of stroke (Cicinelli et al., 2003; Liepert et al., 2007; Manganotti et al., 2002) whereas ICF has been reported to be normal both during the first 2 weeks (Liepert et al., 2007; Manganotti et al., 2002; Shimizu et al., 2002) and in the chronic stage (Butefisch et al., 2003, 2005; Swayne et al., 2008). Our results regarding measurements of excitatory and inhibitory pathways showing increased ICF in the ipsilesional hemisphere would support this finding, which might have related to facilitation due to the activation of glutamatergic interneurons (Cicinelli et al., 2003). Although the results of studies investigating the clinical implications of the intracortical excitability of the contralesional hemisphere are controversial, increased intracortical excitatory activity has been demonstrated in the contralesional hemisphere of recovering stroke patients (Bütefisch et al., 2003; Lioumis et al., 2012; Talelli et al., 2006). Due to small sample size, it would be difficult to draw inference from this result, however it could be possible that increased ICF might be related to

the patient characteristics of this study, including good motor recovery. The integrity of SICI in contralesional hemispheres after rTMS was also found to predict good motor recovery, because it reflects the maintenance of transcallosal inhibition by ipsilesional hemispheres (Manganotti et al., 2002; Liepert et al., 2007), however, we couldn't find some difference when compared to controls, although it was relatively decreased in the patients group. The reason for this might be because of the difference of the time duration from onset of stroke, which was measured in chronic stage in this study.

We did not detect any effects of rTMS on the cortical silent period (CSP). However, our assessment of the CSP could be improved by more quantitative approaches, and in any case, the dissociation in the neurobiological substrates of the CSP and other measures of corticospinal excitability have been repeatedly supported.

Navigated rTMS allows stimulation of brain areas with greater spatial accuracy and reproducibility than conventional TMS method in healthy subjects (Bashir et al., 2010). Neuronavigation was shown to improve accuracy and reproducibility; however the superiority of this targeting method has not yet been tested in the rehabilitation setting for stroke.

The most likely interpretation of this finding is that rTMS over the lesioned M1 reduced the excessive inhibition over the contralateral motor cortex. This disinhibition of the lesioned motor cortex may partly contribute to the functional improvement in the affected hand by unmasking the latent networks (Kobayashi et al., 2003). However, we did not observe any change in the SICI of the ipsilesional hemisphere following rTMS. This hypothesis needs to be investigated using a larger number of stroke patients.

Several studies have suggested that the up regulation of the lesioned motor cortex results in an improvement in the motor function of the affected hand in chronic stroke (Avenanti et al., 2012; Hummel et al., 2005; Kobayashi et al., 2004; Maeda et al., 2000; Mansur et al., 2005; Pal et al., 2005; Ziemann, 2005). In the present study navigated rTMS at 1 Hz induced an increase in pinch force. Therefore, rTMS may be important in the rehabilitation of patients after stroke, and imparting additional motor training while the changes are being generated by rTMS at 1 Hz and conducting rTMS cumulatively would sustain the effect of rTMS and improve the function of the affected hand.

In conclusion, our results demonstrate that navigated rTMS over the contralesional hemisphere can lead to an improvement in the motor function in the affected hand of chronic stroke patients. These findings may be pertinent to the design and optimization of neurorehabilitation strategies for patients after stroke. However, the present study has several limitations that should be considered when interpreting the results. First, we compared the stroke condition with healthy subjects (and not with a sham condition) making it difficult to draw inference about rTMS effect directly.

Second, we performed this TMS study only once, although previous studies have shown that there is an evolution of brain activation over time. Therefore, a longitudinal study would have undoubtedly benefited our predictions of clinical outcomes. Third, the subject group was not homogeneous with respect to stroke type. Moreover, the time since the occurrence of stroke varied among subjects (Table 1). This type of subject heterogeneity may have obscured the interpretation of the results. Fourth, there is limited power in the current study because of its small sample size. Despite the above limitations, we suggest that the integrity of the corticospinal output and normal inhibitory and excitatory intracortical processes, as demonstrated by navigated TMS in healthy subjects, may be relevant to the reorganization process required for motor recovery following stroke. Future studies of NIBS in healthy subjects should take caution speculating on likely effects for patients, because patients may have heightened susceptibility for improvement as this study shows.

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Conflict of interest statement

Dr. Pascual- Leone serves on the scientific advisory boards for Nexstim, Neuronix, Starlab, Neuroelectrics, Axilum Robotics, Magstim, and Neosync; and is listed as an inventor on several issued and pending patents on the real-time integration of transcranial magnetic stimulation (TMS) with electroencephalography (EEG) and magnetic resonance imaging (MRI).

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