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Behavioral and neuroplastic effects of low-frequency rTMS of the unaffected hemisphere in a chronic stroke patient: A concomitant TMS and fMRI study

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Repetitive Transcranial Magnetic Stimulation (rTMS) ameliorates motor and neuropsychological deficits following stroke, but little is known about the underlying neuroplasticity. We investigated neuroplastic changes following 5 days of low-frequency rTMS on the intact motor cortex to promote motor recovery in a chronic patient with subcortical stroke. The feasibility of administering multiple treatments was also assessed 6 months later by applying the same protocol over the patient's parietal cortex to improve visuospatial disorders. Behavioral improvements and no adverse events were observed. Neuroimaging findings indicated that motor symptoms amelioration was associated with downregulation and cortical reorganization of hyperactive contralesional hemisphere.

Keywords: Stroke; rTMS; Functional MRI; Motor recovery; Visual attention.

Repetitive Transcranial Magnetic Stimulation (rTMS) represents a potential tool for the rehabilitation of cognitive (Miniussi & Rossini, 2011; Miniussi et al., 2008) and motor disorders following stroke (Corti, Patten, & Triggs, 2012). However, little is known about rTMS-induced neuroplasticity.

The hemispheric rivalry account (Kinsbourne, 1977) proposes that symptoms following stroke may be explained not solely by inactivity of the damaged hemisphere but also by increased activity of the intact hemisphere, due to release of inhibition from the damaged hemisphere. Although no clear evidence has been collected about the neurophysiology of rTMS treatments in stroke, in accordance with the rivalry account, therapeutic effects are obtained either by downregulating the intact hemisphere through low-frequency rTMS (Avenanti, Coccia, Ladavas, Provinciali, & Ceravolo, 2012; Dafotakis et al., 2008; Emara et al., 2010; Fregni et al., 2006; Khedr, Abdel-Fadeil, Farghali, & Qaid, 2009; Mansur et al., 2005) or by

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upregulating the lesioned hemisphere through high-frequency rTMS (Ameli et al., 2009; Chang et al., 2010; Di Lazzaro et al., 2008; Emara et al., 2010). Between these protocols, inhibitory low-frequency rTMS (Chen et al., 1997) may be particularly safe in stroke patients who might be at highest risk of potential rTMS-induced seizures. Single sessions of low-frequency rTMS over the unaffected hemisphere enhance stroke patients' motor performance (Dafotakis et al., 2008; Mansur et al., 2005) and the application of this protocol for 5 (Fregni et al., 2006) or 10 (Avenanti et al., 2012; Emara et al., 2010) consecutive days induces significant motor improvement. Low-frequency rTMS over the unaffected hemisphere for several days also reduces visuospatial disorders following stroke (Brighina et al., 2003; Lim, Kang, & Paik, 2010; Song et al., 2009).

Recent neuroimaging studies have collected some evidence about brain activity changes following a single session of low- (Nowak et al., 2008) or high-frequency rTMS (Ameli et al., 2009), or 10 daily sessions of high-frequency rTMS coupled with motor training (Chang et al., 2012). However, to our knowledge, no previous studies have investigated neuroplastic changes following repeated sessions of low-frequency rTMS in chronic stroke. Repeated interventions, as employed in therapeutic trials, are likely to give rise to cumulative long-term after-effects that may differ from those following a single session of stimulation. With the present study, we explored neuroplasticity following 5 days of low-frequency rTMS over the intact motor cortex in a chronic stroke patient with contralesional motor and visuospatial disorders. Functional MRI was obtained before and after treatment during the performance of motor tasks and during the performance of a control visual task. Changes in motor cortex excitability (i.e., motor threshold) were also assessed using TMS. We hypothesized that several days of low-frequency rTMS targeting the unaffected upper limb motor area would have improved the patient's upper limb motor function without affecting visual field defects. The possibility that the intervention could modulate lower limb motor disorder was not excluded, given the anatomical contiguity of upper and lower limbs representations. In agreement with the rivalry account, we expected to observe reduced contralesional overactivity in motor cortex and no changes in visual cortex after treatment. Diffusion tensor imaging (DTI) was also performed to evaluate the fiber tracts which were damaged by the subcortical lesion and potential changes following treatment.

To assess the feasibility of administering multiple rTMS treatments in the same patient, 6 months later, the same protocol was applied to the patient's posterior parietal cortex to improve visuospatial disorders. To our knowledge, no previous studies have tested the feasibility of multiple subsequent rTMS treatments in the same patient, selectively targeting motor and cognitive symptoms which often coexist after stroke.

METHOD

Patient

LC was a 62-year-old right-handed woman who suffered from right subcortical ischaemic stroke 2 years before. The lesion involved the posterior limb and the retrolenticular part of the internal capsule, comprising corticospinal fibers and fibers coming from the lateral geniculate nucleus of the thalamus that posteriorly become the optic radiation (Figure 1).

LC showed left spastic hemiparesis of contralesional limbs with complete plegia of the hand and the foot. She also showed contralesional visual field defects. In the acute phase, she manifested left unilateral neglect. No other concomitant cognitive impairments were found in the acute phase. LC signed a written informed consent to participate in the study, which was previously approved by the Local Ethical Committee.

EXPERIMENT 1

One Hz rTMS was applied over LC's contralesional primary motor (M1) cortex for 5 consecutive days. Neuropsychological, clinical, and neuroimaging assessments were performed before and after treatment.

Stimuli and procedure

Neuropsychological evaluation

Visual neglect was assessed through the bisection of five 180 mm long lines (Heilman & Valenstein, 1979) and the OTA's cancellation task (Ota, Fujii, Suzuki, Fukatsu, & Yamadori, 2001). Visual and tactile extinctions were assessed using conventional finger confrontation tests. Neglect dyslexia (Costello & Warrington, 1987) was evaluated by asking the patient to read 20 words and 20 legal non-words (each stimulus could be composed of

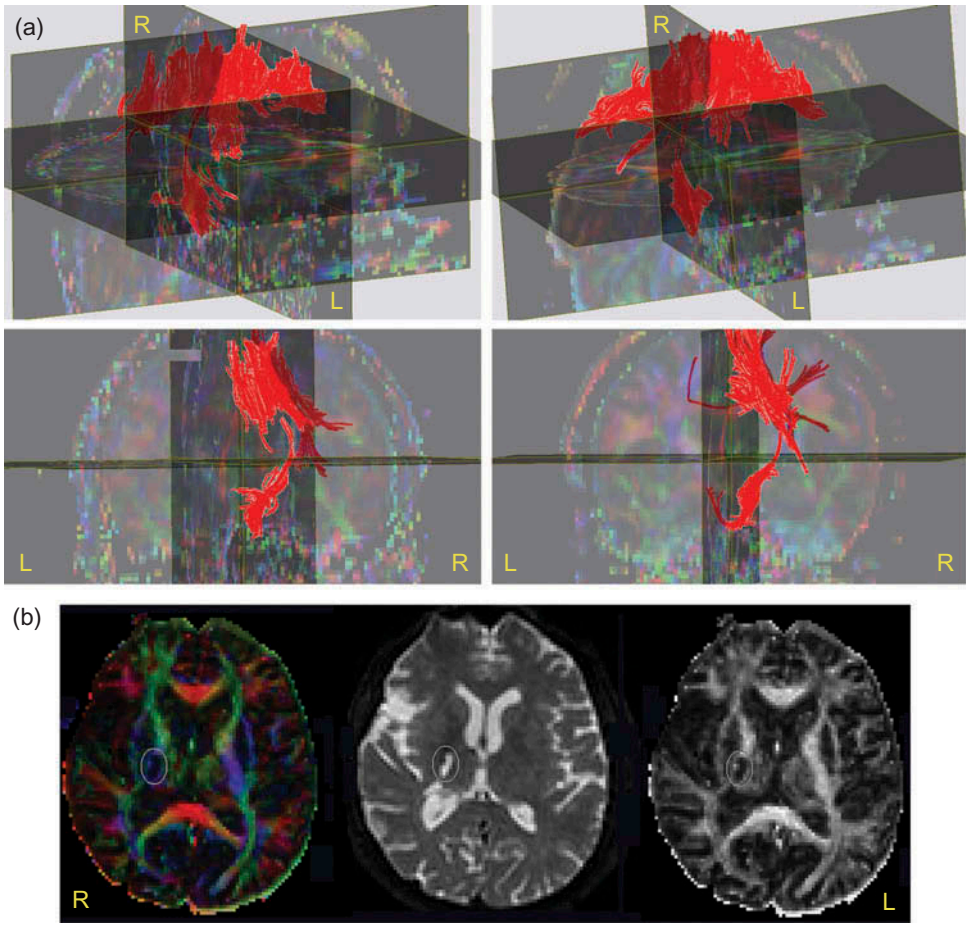

Figure 1. Comparison of DTI metrics and tractography for the Right CST pre- and post-treatment. (a) The fiber tracking before (on the left) and after (on the right) TMS. The Right CST appeared reduced in both conditions. (b) From left to right, the color coded FA, the Apparent Diffusion Coefficient (ADC) and the FA of the 31st slice. The white circle underlines the lesion. L = left, R = right, red = left–right direction, blue = inferior–superior direction, green = anteroposterior direction. [To view this figure in color, please see the online version of this Journal].

6, 7, or 8 letters and each letter was 1.1° of visual angle high and 0.7° wide) presented on the screen of the computer monitor. Two different series of words and non-words, balanced for linguistic features (Colombo, 1992; Miceli & Caramazza, 1993), were used for pre- and post-rTMS assessments.

Visual field defects evaluation

The patient's visual field defects (VFD) evaluation was performed 3 days before and 1 day after treatment, using a computer-based task. The target was a white circle (diameter: 0.8° of visual angle) that appeared for 150 ms on the left or right of a white central fixation cross (1.1° × 1.1° of visual angle), in one of eighteen possible locations (see Table 1 for details). For each location, the target was presented five times, for a total of 90 trials. Stimulus presentation followed a random order. For five times, no stimuli appeared. There were 20 practice trials.

LC fixated the central cross (exposure time 500 ms) before each trial and verbally reported whether the stimulus appeared to the left or right of the central cross. Trials in which LC moved her eyes were excluded from the analysis and rerun.

Motor impairment evaluation

LC's upper and lower limbs residual movements and muscle strength were evaluated by a licensed neurologist (who was blind to the specific site that was targeted by the intervention) using the "Medical Research Council Scale for muscle strength" (MRC, Her Majesty's Stationery Office, London, 1981). On the MRC scale the patient's effort is graded on a scale of 0–6: 0 = no contraction, 1 = flicker or trace contraction, 2 = active

TABLE 1
Visual field defects (VFD) evaluation: proportion of correct detections for the treatment over the motor cortex

Pre-rTMS							*Post-rTMS*						
Left				*Right*			*Left*				*Right*		
0/5	0/5	2/5		5/5	4/5	5/5	0/5	0/5	0/5		5/5	5/5	5/5
1/5	2/5	4/5	+	5/5	5/5	5/5	1/5	4/5	4/5	+	5/5	5/5	5/5
4/5	4/5	4/5		5/5	5/5	4/5	5/5	5/5	5/5		5/5	5/5	5/5

There were 18 possible locations on which the visual stimulus could appear, 9 to the left and 9 to the right of the fixation cross. On the horizontal axis, the stimulus could appear 6.4°, 10.2°, or 14.1° to the left or the right of the center, and on the vertical axis on the same vertical coordinate of the fixation cross or 14.1° above or below. Results show severe visual field defects for the left upper quadrant.

movement, with gravity eliminated, 3 = active movement against gravity, 4 = active movement against gravity and resistance, and 5 = normal power. To further discriminate the different degrees of muscle strength in movement against resistance, the neurologist used the following values: 4 = movement against resistance is barely possible, 4+ = movement against resistance is possible with a moderate reduction of muscle strength, and 4 $^1/_2$ = movement against resistance is possible with a minimal reduction of muscle strength. For the upper limb, the evaluated parameters were as follows: fingers flexion and extension, wrist flexion and extension, forearm flexion and extension, and arm abduction. For the lower limb, the evaluated parameters were as follows: leg flexion, foot flexion and extension. Evaluations were performed 1 hour before and 1 day, 1 month, and 6 months after treatment.

Transcranial magnetic stimulation

Magnetic stimulation was performed with a Magstim Rapid^2 Stimulator (Magstim Co., Whitland, Dyfed, UK). A 70-mm figure-of-eight coil was positioned over the patient's unaffected M1 at the optimum scalp position to elicit motor evoked potentials (MEPs) in the contralateral abductor pollicis brevis muscle (APB). Resting motor threshold (rMT) was defined as the minimum stimulus intensity that produced MEPs > 50 μV (peak-to-peak amplitude) in at least 5 out of 10 responses (Rossini et al., 1994). Electromyographic activity was recorded using surface electrodes (Xcalibur EMG system XLTEK). One Hz rTMS (900 pulses) was applied to the contralesional APB hotspot at 100% of rMT, on 5 consecutive days. rTMS was administered every day at the same hour. The rMT of the unaffected hemisphere was measured 1 day before and 1 day, 1 month, 6 months after treatment to assess the changes in motor cortex excitability. The patient was not informed that the treatment was specifically targeting the upper limb motor function. Therefore, she had no expectations about the specific limb that could be mainly affected by the intervention. Since her vision was also assessed, she had some expectations about the possibility that the treatment could affect visual disorders. Since visual assessment served as control condition, she was kept blind about this issue until the end of the study.

fMRI

The patient underwent the first fMRI session 2 days before and the second session the day after treatment.

Tasks and stimuli

Inside the scanner, LC performed motor tasks using her ipsi or contralesional limbs. LC was asked to try to perform the tasks with the plegic limbs in the same way she would do with the healthy limbs. For upper limbs motor task, the patient was asked to "open and close" her right and left hand alternately and relax in the rest condition. For lower limbs motor task, she had to "move up and down" her right and left foot alternately and relax in the rest condition. For the visual control condition, LC viewed colored patterns and a gray screen (rest condition) while keeping her gaze on a fixation cross.

fMRI analysis

Imaging data were analyzed using Brain Voyager QX 2.3 (Brain Innovation, Maastricht, Holland). The patient's functional data underwent the following preprocessing steps: mean intensity adjustment, head motion correction, slice scan time correction, spatial data smoothing

(FWHM = 4 mm), temporal filtering, temporal smoothing (FWHM = 2.8 sec). After preprocessing, the patient's slice-based functional scans were coregistered to her 3D high-resolution structural scan. The volume time course was created using the anatomical-functional coregistration matrix.

For each task, the following procedure was performed. A multi-study design matrix was specified and each defined boxcar was convolved with a predefined hemodynamic response function (HRF) (Boynton, Engel, Glover, & Heeger, 1996) to account for the hemodynamic delay. A statistical fixed effect analysis using the General Linear Model (GLM) with separate study predictors was performed to yield functional activation maps during the pre- and post-treatment separately. All voxels activated in the pre-treatment and those activated in the post-treatment were combined to create a mask ($3 \times 3 \times 3$ mm resolution; threshold $p < .05$, Bonferroni corrected for multiple comparisons; minimum cluster size 8 voxels in the native resolution) excluding the rest of the cerebrum and cerebellum; we used this mask to compute the GLM comparing post-treatment activations with pre-treatment activations [post-treatment (movement versus rest) minus pre-treatment (movement versus rest)]. Post- versus pre-treatment statistical comparisons were computed at a statistical threshold of $p < .05$, corrected for multiple comparisons using the false discovery rate (FDR) (Genovese, Lazar, & Nichols, 2002).

DTI

Diffusion tensor imaging (DTI) was performed 2 days before and the day after treatment.

The DTI scheme included the collection of 32 images with noncollinear diffusion gradients ($b = 800$ s/mm^2) and one nondiffusion-weighted image ($b = 0$ s/mm^2), employing a single shot echo planar imaging sequence. The patient was scanned twice, before and after treatment (TE = 72 ms, TR = 6700 ms, FoV 256×256 mm, acquisition matrix 128×128, 60 axial slices of 2 mm thickness, voxel $2 \times 2 \times 2$ mm).

Tractography

We used a 12 degrees of freedom affine transformation to coregister the pre- and post-treatment images.

Fractional anisotropy (FA) of each voxel was derived based on the three eigenvalues. The FA was used as a measure of the degree of diffusion anisotropy and varied between 0 (in the case of isotropy) and 1 (in the case of the diffusion taking place entirely in one direction).

The tractography for the Left and Right Cortico-Spinal Tract (CST) was implemented using DTI-Studio 2.4 free software (www.mristudio.org). Fiber assignment by continuous tracking, sampling every voxel, was used. To reconstruct tracts of interest, we used a multiple-region-of-interest (ROI) approach based on the existing anatomic knowledge of tract trajectories (Catani & Thiebaut de Schotten, 2008). The seed ROI for tracing the CST was placed in the cerebral peduncle. Then we used three filter ROIs to exclude non-CST fibers. They were placed in the pyramid of the medulla oblongata, the posterior limb of the internal capsule, and the pre- and post-central gyri, respectively. All the ROIs were drawn on the FA maps. Fibers passing through all the four ROIs were taken as the entire CST.

Once tracked, statistics for the bundles of interest were computed (two sample t tests), in particular for the FA, the first eigenvalue (axial diffusivity, AD) and the mean of second and third eigenvalues (radial diffusivity, RD) to assess qualitatively the changes between pre- and post-treatment conditions. In particular, we compared the mean values of the Left and Right CST separately (for the whole bundle and slice by slice) before and after treatment and between the Left and Right CST before and after the treatment (for the whole bundles and slice by slice).

Results

No adverse events occurred during or after treatment.

Neuropsychological evaluation

The patient did not show any neglect, tactile, or visual extinction on standard confrontation tests before treatment. To control for potential unexpected rTMS effects on patient visuospatial performance, the same tasks were repeated after treatment. No changes were detected.

Visual field defects evaluation

LC showed contralesional visual field defects mainly localized in the upper portion of the hemifield (see Table 1). Binomial two-tail test (with Bonferroni correction) showed that LC's stimulus detection within the left hemifield did not differ significantly from chance level in both pre- (47%) and post-treatment (53%) conditions.

Movement and strength evaluation

Scores for the patient's upper and lower limb motor evaluation are reported in Table 2. For the upper limb, the treatment did not affect LC's contralesional hand plegia. However, the first clinical evaluation after treatment revealed amelioration in forearm extension and arm abduction and a slight improvement in the forearm flexion. Improvements were maintained at 1 month and 6 months after treatment. The intervention did not affect lower limb motor function.

Motor cortex excitability

Before treatment, LC's resting Motor Threshold (rMT) of the unaffected hemisphere was 40% of maximum machine output. At the end of treatment, rMT was 44%, showing an increase of 10% with respect to pre-treatment value. This increment was maintained at 1 month (45%) and 6 months (48%) from the treatment. In healthy participants, a difference between rMT of about 10% has been found to be statistically significant (Fitzgerald, Brown, Daskalakis, Chen, & Kulkarni, 2002). However, given that the present data were collected on a single stroke patient no clear conclusions can be drawn from this finding.

fMRI

Pre- rTMS: Motor tasks

In the pre-treatment condition, during movements of the unaffected hand and foot, activations were found in the primary sensorimotor areas (hand and foot somatotopic projections), premotor, supplementary, and mesial motor areas as well as cerebellum, for both effectors. When the patient tried to move the left plegic hand more widespread, groups of activations were found in the same areas as in the contralateral side but they also included parietal territory. A similar result was found during movement of the foot.

Pre- versus post- rTMS: Motor tasks

The statistical comparison of imaging data acquired before and after treatment revealed areas of increased hemodynamic response and areas of reduced hemodynamic response in the post-rTMS condition. During right hand movement, increased activation was found in the left primary motor cortex (BA 4) corresponding to the hand area and reduced activation in the foot area (Figure 2a). On the other hand, during right foot movement, increased BOLD signal was found in the left hemisphere primary motor cortex (BA 4) corresponding to the foot area and reduced BOLD signal was found in the hand area (Figure 2c). When the patient tried to move the left hand, increased activations were found in the premotor and sensorimotor cortex as well as in the superior parietal lobule (BA 6, 4, 7) (Figure 2b). Reduced activations were found in the primary motor cortex, in the foot area. When the patient tried to perform the motor task with the left foot, increased activations were found in the foot primary motor area, premotor cortex and superior parietal lobule (BA 4, 6, 7) (Figure 2d). Reduced activations were found in the sensorimotor cortex, hand area.

Pre- versus post- rTMS: Visual task

The comparison of imaging data acquired before and after treatment did not reveal any significant change in the activations of visual areas.

TABLE 2
Upper and lower limb movements and strength evaluation (MRC scale)

Upper limb	*Pre-rTMS*	*Post-rTMS 1*	*Post-rTMS 2*	*Post-rTMS 3*
Fingers flexion/extension	0/5	0/5	0/5	0/5
Wrist flexion/extension	0/5	0/5	0/5	0/5
Forearm flexion	4/5	4 +/5	4½/5	4 +/5
Forearm extension	3/5	4/5	4½/5	4½/5
Arm abduction	3/5	4/5	4 +/5	4 +/5
Lower limb				
Leg flexion	4 +/5	4 +/5	4 +/5	4 +/5
Foot flexion	4/5	4/5	4/5	2/5
Foot extension	3/5	3/5	3/5	2/5

pre-rTMS = 1 hour before treatment; post-rTMS 1 = 1 hour after treatment; post-rTMS 2 = 1 month after treatment; post-rTMS 3 = 6 months after treatment.

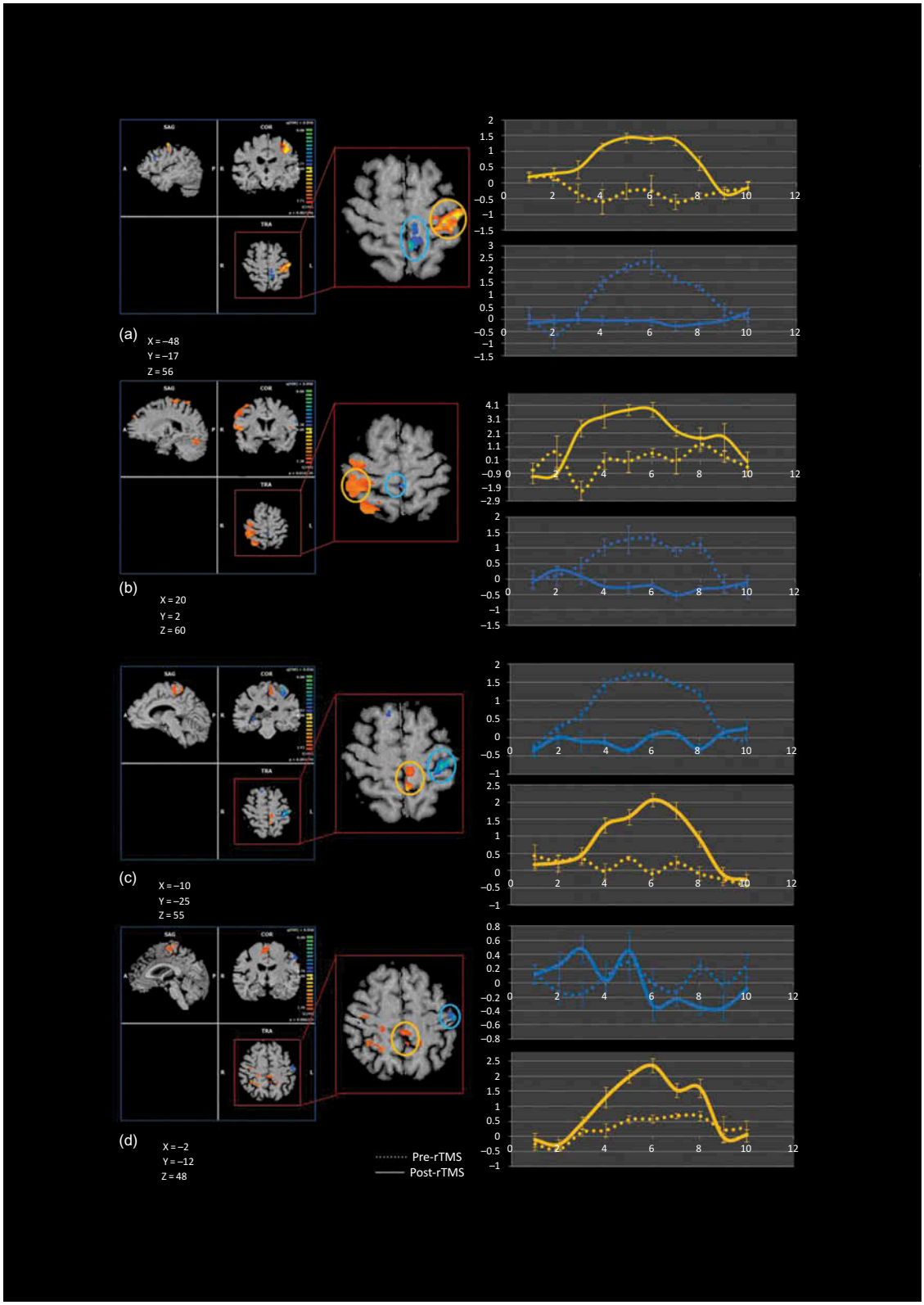

Figure 2. Comparison between pre- and post-treatment fMRI motor activations. Left panels show the statistical comparison ($p < .05$ FDR corrected for multiple comparison) for: (a) right hand, (b) left hand, (c) right foot, and (d) left foot. Right panels show the event related averages of pre- and post-treatment conditions of the ROIs indicated in the correspondent figure in the left panel. [To view this figure in colour, please see the online version of this Journal].

DTI

The Left CST pre- and post-treatment did not show any difference ($p > .05$) in FA, AD, and RD, for the whole bundle (mean ± standard deviation; FA pre = 0.48 ± 0.13, FA post = 0.48 ± 0.13; AD pre = 1.16 ± 0.21 $mm^2/sec \times 10^{-3}$, AD post = 1.20 ± 0.24 $mm^2/sec \times 10^{-3}$; RD pre = 0.53 ± 0.13 $mm^2/sec \times 10^{-3}$ RD post = 0.56 ± 0.18 $mm^2/sec \times 10^{-3}$) or slice by slice (data not shown).

The Right CST pre- and post-treatment did not show any difference ($p > .05$, see also fiber tracking in Figure 1a) in FA, AD, and RD, for the whole bundle (mean ± standard deviation; FA pre = 0.42 ± 0.11, FA post = 0.42 ± 0.11; AD pre = 1.17 ± 0.17 $mm^2/sec \times 10^{-3}$, AD post = 1.22 ± 0.22 $mm^2/sec \times 10^{-3}$; RD pre = 0.63 ± 0.12 $mm^2/sec \times 10^{-3}$ RD post = 0.66 ± 0.16 $mm^2/sec \times 10^{-3}$) or slice by slice (data not shown).

The Right and Left CST were significantly different (see Figure 1b) both before and after treatment for the mean values of FA, AD, RD and in particular between the 23rd and the 32nd slices.

EXPERIMENT 2

Six months later the same rTMS protocol used in experiment 1 was applied to LC's intact posterior parietal cortex (PPC) to ameliorate contralesional visuospatial deficits. Although the patient did not show any neglect on paper and pencil tests or extinction on fingers confrontation tests, she manifested subtle visual extinction when assessed using a more demanding PC-based task (Ricci & Chatterjee, 2004; Ricci, Genero, Colombatti, Zampieri, & Chatterjee, 2005). In this task, contralesional stimuli were presented in a portion of space that, according to VFD evaluation of experiment 1, was partially spared by the scotoma. For this treatment, LC was not willing to undergo additional fMRI sessions.

Cortical excitability

The rMT of the patient's unaffected hemisphere before the second rTMS treatment was 48% of the TMS output.

Stimuli and procedure

Visual field defects evaluation

VDF evaluation was performed 1 hour before the beginning of treatment and 2 hours after the end of treatment using the same procedure described for experiment 1.

Visual extinction evaluation

LC's visual extinction was assessed using a computer-based task. The patient was presented with a red T (1.7° × 2.1° of visual angle) which could appear on the left, right, both or neither sides of a white fixation cross always present on the center of the computer monitor (1.9° × 1.9° of visual angle). The distance between stimuli and the central cross was 9.7° of visual angle. Stimuli were flashed for 50 ms on a black background. LC was asked to point with her ipsilesional limb toward the location(s) in which the stimulus (i) appeared. No response was required when no stimuli were presented. There were 224 trials, 56 for each condition, presented in a random order and 20 practice trials. The trials during which an eye movement was detected were excluded from the analysis and rerun.

Visual extinction was assessed twice before treatment (T-1 = two weeks before treatment; T0 = the first day of treatment) and twice after treatment (T1 = immediately after treatment; T2 = 7 weeks after treatment).

Transcranial magnetic stimulation

The same TMS device and procedure of experiment 1 were used. The rTMS was applied to LC's contralesional PPC over P5 (according to the 10/20 EEG system).

Results

No adverse events occurred during or after stimulation.

Visual field defects

As for experiment1, LC showed severe VFD for the left upper quadrant (see Table 3). Binomial two-tail test (with Bonferroni correction) showed that LC's stimulus detection within the left hemifield did not differ significantly from chance level in both pre- (58%) and post-treatment (49%) conditions.

TABLE 3
Visual field defects (VFD) evaluation: proportion of correct detections for the treatment over the parietal cortex

Pre-rTMS							*Post-rTMS*						
Left				*Right*			*Left*				*Right*		
1/5	0/5	0/5		5/5	4/5	5/5	0/5	0/5	1/5		5/5	5/5	5/5
2/5	3/5	5/5	+	5/5	5/5	5/5	1/5	1/5	4/5	+	5/5	5/5	5/5
5/5	5/5	5/5		5/5	5/5	5/5	5/5	5/5	5/5		5/5	5/5	5/5

There were 18 possible locations on which the visual stimulus could appear, 9 to the left and 9 to the right of the fixation cross. On the horizontal axis the stimulus could appear 6.4°, 10.2°, or 14.1° to the left or the right of the center, and on the vertical axis on the same vertical coordinate of the fixation cross or 14.1° above or below. Results show severe visual field defects for the left upper quadrant.

Visual extinction

LC showed some degree of contralesional visual deficits during double simultaneous stimulation and contralesional single stimulation. She was always correct in detecting ipsilesional single stimuli or no stimuli in all assessments. On double simultaneous stimulation, LC extinguished 39% of contralateral stimuli at T-1, 36% at T0, 21% at T1, and 56% at T2.

The binomial two-tail test with Bonferroni correction for multiple comparisons ($p < .05$) indicates that the rate of contralesional stimulus detection during double stimulation significantly differed ($p < .0001$) from chance level at T1, while no significant differences were observed at T-1, T0, and T2. On single contralesional stimulus presentation the patient omitted 59% of stimuli at T-1, 39% at T0, 36% at T1, and 42% at T2. None of these performances significantly differed from chance level according to a binomial two-tail test with Bonferroni correction.

To summarize, LC's visual extinction seemed to improve after treatment and to return to baseline value at follow-up evaluation. It is important to mention that the independence assumption of the binomial test may not be strictly satisfied here.

DISCUSSION

To our knowledge, this is the first study investigating behavioral and neuroplastic after-effects induced by 5 days of low-frequency rTMS over the intact motor cortex in chronic stroke. The feasibility of applying multiple rTMS treatments in the same patient was also assessed.

The application of rTMS over the hand motor area of the intact hemisphere improved LC's contralesional upper limb proximal movements. These effects were maintained at 1 and 6 months after treatment. The intervention did not affect LC's lower limb motor function nor visual field defects. Thus, it specifically affected the motor function targeted by the coil, suggesting that the observed outcome could not be explained by unspecific rTMS effects over the healthy hemisphere. BOLD signal changes following treatment revealed reduced overactivity of the intact hemisphere and activity re-focalization to the specific motor areas of representation, during movement of the unaffected limbs. Increased activity focalization around the areas of limbs motor representation was also found when LC was trying to move the affected limbs. However, in these conditions a more widespread group of activations was observed, comprising premotor and superior parietal areas. Activation of these additional regions was likely due to the attentional effort made by the patient to accomplish the task.

In line with behavioral data, no changes were observed after treatment in the pattern of brain activation within visual areas, in the control visual condition.

The treatment did not significantly change the DTI metrics, probably because the induced functional changes were not sufficient to modify the anatomy or the structure of the bundles. However, it is also possible that a less damaged bundle or a more intensive or prolonged treatment would have modified the DTI metrics.

Results of the first study are in line with previous findings showing long-lasting motor disorders amelioration and reduced contralesional motor cortex excitability following low-frequency rTMS treatment over the healthy hemisphere in chronic stroke patients (Avenanti et al., 2012; Fregni et al., 2006; Khedr et al., 2009). Moreover, neuroimaging results are consistent with fMRI evidence in acute patients with motor disorders showing overactivity within ipsilesional and contralesional motor-related brain

regions during movements of the affected hand and the observation that motor recovery negatively correlates with overactivity (Marshall et al., 2000; Ward, Brown, Thompson, & Frackowiak, 2003).

Neuroimaging findings for the condition in which LC tried to move the affected limbs are difficult to interpret because of the lack of real movement. However, during movements of the unaffected limbs, reduction of contralesional motor cortex overactivity was observed after treatment. Thus, in accordance with the assumptions of the rivalry account, low-frequency rTMS over the healthy hemisphere may have reestablished inter-hemispheric balance and enhanced contralesional motor function by inhibiting maladaptive widespread activations in the intact hemisphere. These findings are consistent with those of previous neuroimaging studies showing improved dexterity of the affected hand and reduced overactivity in contralesional motor areas in patients with subcortical stroke soon after a single session of low-frequency rTMS over the intact M1 (Nowak et al., 2008) or high-frequency rTMS over the affected M1 (Ameli et al., 2009). However, in our study neuroimaging changes followed repeated sessions of inhibitory rTMS and were measured 1 day after treatment. Therefore, the observed changes reflect long-lasting cumulative after-effects rather than short-term effects. Long-lasting after-effects are likely to underlie behavioral improvement following repeated sessions of rTMS, as employed in therapeutic trials.

Recently, Chang et al. (2012) have shown that 10 daily sessions of high-frequency rTMS coupled with motor training enhance activity of the affected hemisphere, besides improving motor performance. Our findings, taken together with those of Chang et al. (2012), support the hypothesis that the neural mechanisms underlying enhancement of motor performance by several sessions of low- or high-frequency rTMS in stroke are likely due to reduction of maladaptive contralesional overactivity or enhancement of ipsilesional hypoactivity, respectively.

In stroke patients, neuroplastic changes due to limb disuse may also interfere with motor recovery (Roberts et al., 2007, 2010). Thus, brain stimulation in these patients may not only restore inter- and intra-hemispheric imbalance following the brain lesion but also contrast neuroplastic changes due to limb disuse (Ricci et al., 2008).

In experiment 2, treatment over parietal cortex seemed to ameliorate the patient's residual visual extinction. However, the amelioration was not maintained at 2 months after treatment.

Visual extinction is a deficit of visuospatial attention, characterized by the failure to detect contralesional stimuli when ipsilesional stimuli are presented simultaneously. This symptom follows unilateral lesions of PPC, temporoparietal junction (Becker & Karnath, 2007; di Pellegrino, Rafal, & Tipper, 2005) or subcortical tracts or structures which are richly connected to the above regions (Hillis, 2006). The results of the second study are in line with previous findings (Brighina et al., 2003; Lim, Kang, & Paik, 2010; Song et al., 2009) showing improvement of contralesional space awareness after low-frequency rTMS over the intact parietal cortex. Although the treatment on parietal cortex ameliorated LC's residual extinction, it did not affect the left upper quadrant scotoma nor contralesional single stimuli detection. Indeed, modulation of contralesional awareness during double stimulation occurred in a portion of space which was partially spared by the scotoma. It is likely that stimuli omissions in this portion of space were at least in part due to hemi-spatial neglect. Taken together, these findings suggest a specific effect of parietal rTMS on visual extinction and no effect on visual field defects and/or contralesional neglect. Low-frequency rTMS might have temporarily reduced the activity of contralesional parietal cortex without affecting the activity of ipsilesional parietal and visual cortices. Thus inhibitory rTMS may have improved contralesional extinction by suppressing the salience of ipsilesional competing stimuli. Future investigations are needed to further explore this hypothesis.

Findings of this study provide evidence that multiple low-frequency rTMS treatments are safe and well-tolerated interventions to promote cortical reorganization and symptoms amelioration in chronic stroke. They also suggest that the underlying neuroplastic changes are mediated by inhibition of the healthy hemisphere and neural activity re-focalization.

The main limitation of this study is the lack of a sham stimulation to control for unspecific rTMS effects, therefore a placebo effect cannot be completely ruled out. However, in experiment 1, the fact that the patient was blind to the specific function targeted by the coil and the lack of effects on lower limb and visual function make it unlikely. In addition, neuroimaging data showing motor cortex reorganization and no changes in visual cortex after the intervention seem even

harder to be explained by spontaneous recovery or placebo phenomena. Similarly, in experiment 2, placebo or practice effects can hardly account for the selectivity of parietal rTMS effects on extinction errors.

In future, double-blind placebo-controlled studies using multiple integrated techniques on large groups of patients are needed to better understand rTMS-induced neuroplasticity. This information will help identify optimal rehabilitation protocols and potential biomarkers of rTMS treatment response.

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