Low-frequency rTMS promotes use-dependent motor plasticity in chronic stroke

A randomized trial

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ABSTRACT

Objective: To investigate the long-term behavioral and neurophysiologic effects of combined time-locked repetitive transcranial magnetic stimulation (rTMS) and physical therapy (PT) intervention in chronic stroke patients with mild motor disabilities.

Methods: Thirty patients were enrolled in a double-blind, randomized, single-center clinical trial. Patients received 10 daily sessions of 1 Hz rTMS over the intact motor cortex. In different groups, stimulation was either real ($rTMS_R$) or sham ($rTMS_S$) and was administered either immediately before or after PT. Outcome measures included dexterity, force, interhemispheric inhibition, and corticospinal excitability and were assessed for 3 months after the end of treatment.

Results: Treatment induced cumulative rebalance of excitability in the 2 hemispheres and a reduction of interhemispheric inhibition in the $rTMS_R$ groups. Use-dependent improvements were detected in all groups. Improvements in trained abilities were small and transitory in $rTMS_S$ patients. Greater behavioral and neurophysiologic outcomes were found after $rTMS_R$, with the group receiving $rTMS_R$ before PT ($rTMS_R$-PT) showing robust and stable improvements and the other group (PT-$rTMS_R$) showing a slight improvement decline over time.

Conclusion: Our findings indicate that priming PT with inhibitory rTMS is optimal to boost use-dependent plasticity and rebalance motor excitability and suggest that time-locked rTMS is a valid and promising approach for chronic stroke patients with mild motor impairment.

Classification of evidence: This interventional study provides Class I evidence that time-locked rTMS before or after physical therapy improves measures of dexterity and force in the affected limb in patients with chronic deficits more than 6 months poststroke. *Neurology*® 2012;78:256–264

GLOSSARY

ANOVA = analysis of variance; **B&B** = Box and Block test; **FDI** = first dorsal interosseous; **iSP** = ipsilateral silent period; **JHFT** = Jebsen-Taylor Hand Function Test; **NHPT** = Nine-Hole Peg Test; **PT** = physical therapy; **rMT** = resting motor threshold; **rTMS** = repetitive transcranial magnetic stimulation; **$rTMS_R$** = real repetitive transcranial magnetic stimulation; **$rTMS_S$** = sham repetitive transcranial magnetic stimulation.

Physical therapy (PT) plays a critical role in promoting motor recovery after stroke; however, the functional outcomes are often of limited practical significance, particularly for chronic patients.[1–3] Recently, noninvasive brain stimulation[4–10] and, in particular, low-frequency repetitive transcranial magnetic stimulation[11–14] (rTMS), has been used to promote functional recovery of stroke patients by suppressing the contralesional intact motor cortex ($_{int}$M1) and thus reducing interhemispheric inhibition.

Although rTMS may represent an ideal tool to promote neural plasticity, especially when applied in multiple sessions,[6–10,13] information on the possible long-term effects (i.e., beyond 2 weeks) of multiple sessions of combined inhibitory rTMS and PT in chronic stroke patients is meager.

Brain stimulation protocols are thought to induce a temporary state in which learning is optimized[6–8]; this would suggest that a close temporal relation between rTMS and PT (time-

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Supplemental Data

CME

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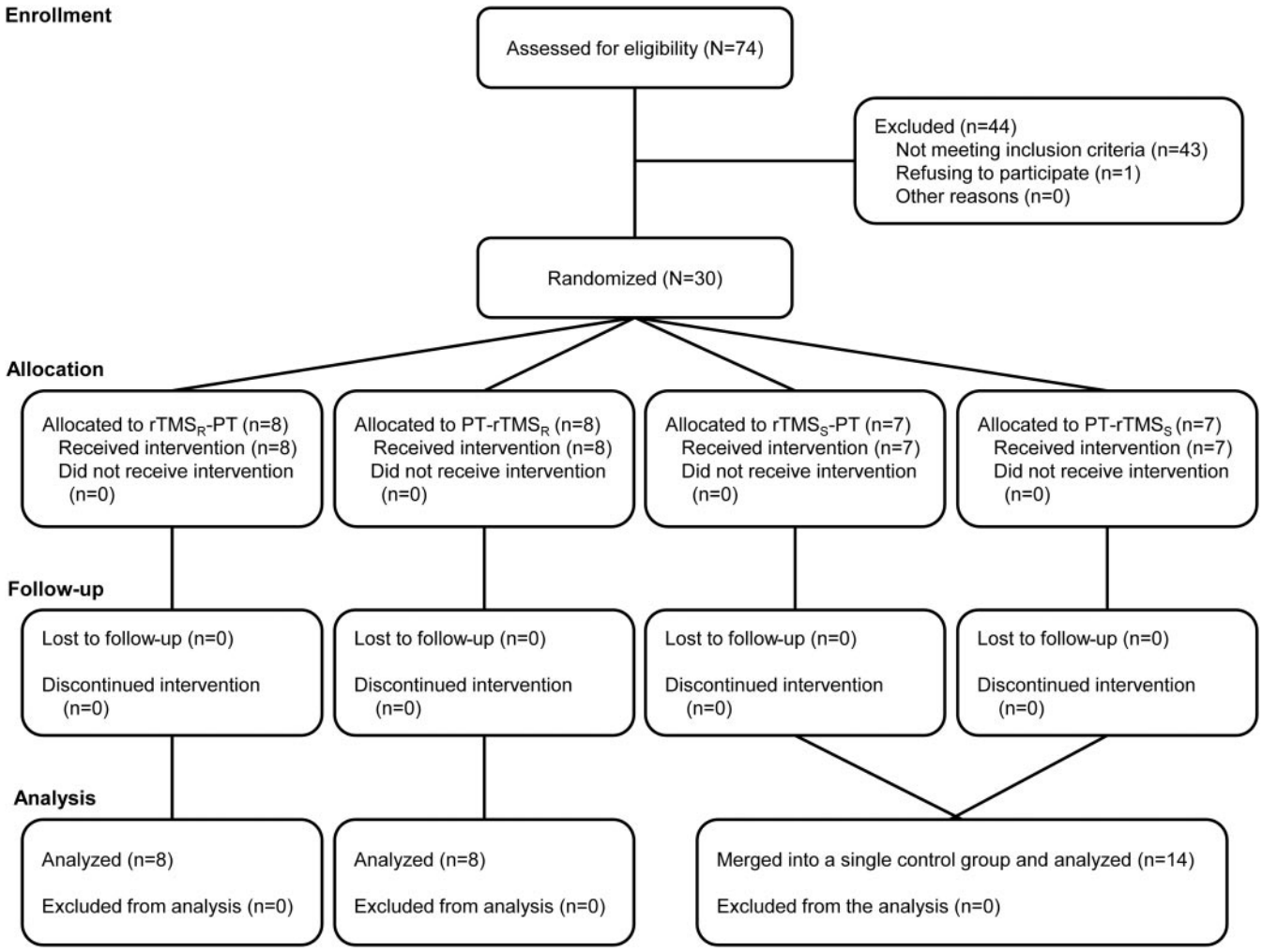
Figure 1 Participant flow through the trial



PT = physical therapy; rTMS$_R$ = real repetitive transcranial magnetic stimulation; rTMS$_S$ = sham repetitive transcranial magnetic stimulation.

locked rTMS) is optimal to potentiate the effect of PT. However, to date it is unclear whether time-locked rTMS should precede or follow the PT.[8] In the present research, we sought to investigate whether multiple sessions of time-locked inhibitory rTMS (1 Hz rTMS) applied as an add-on to PT may induce long-term neurophysiologic and behavioral improvements. To test the effect of treatment order, half of the patients received PT immediately after rTMS (rTMS-PT) and the other half received PT before rTMS (PT-rTMS). Moreover, to test the effect of treatment on use-dependent plasticity, both trained and untrained motor functions were monitored for 3 months following the end of the treatment. We expected that patients receiving real rTMS (rTMS$_R$) would show greater functional improvements than patients receiving sham rTMS (rTMS$_S$). Moreover, we hypothesized that rTMS$_R$ preceding PT could potentially prime functional networks for the physical intervention and would be most effective in promoting use-dependent plasticity. Thus, we expected to observe superior outcomes and training-specific effects in rTMS$_R$-PT than in the PT-rTMS$_R$ group.

METHODS **Patients.** We enrolled 30 chronic stroke hemiparetic patients at the neurorehabilitation clinic of the Hospital Riuniti of Ancona in 2007–2011 (figure 1). The diagnosis was made by clinical features and confirmed by CT and MRI. Inclusion criteria were 1) unilateral stroke sparing M1[15]; 2) >6 months after the first-ever stroke; and 3) mild upper-limb motor deficit (Motricity index range 72–76). We excluded patients with moderate to severe motor deficits or any other clinically significant medical comorbidity. Patients underwent prolonged EEG monitoring to exclude presence of epileptic activity.

Standard protocol approvals and patient consents. Institutional review boards approved the study, and written informed consent was obtained prior to enrollment.

Design. This study was a prospective, randomized, parallel- and factorial-design, sham-controlled, phase II trial conducted at a single center that had 4 phases: 1) randomization, 2) baseline evaluations, 3) treatment, and 4) follow-up evaluations. Thirty patients were randomly assigned, using a computer random-number generator, to 1 of 4 groups receiving either $rTMS_R$ or $rTMS_S$ that were administered either immediately before or after PT, following a 2 × 2 factorial design ($rTMS_R$-PT, PT-$rTMS_R$, $rTMS_S$-PT, PT-$rTMS_S$). Power analysis conducted in previous noninvasive brain stimulation studies[16] suggest a sample size of n = 8 for each group to be adequate. Eight patients were randomly assigned to each experimental group ($rTMS_R$-PT, PT-$rTMS_R$) and a total of 14 patients to the sham groups ($rTMS_S$-PT, PT-$rTMS_S$). Since for sham stimulation, intervention order is not expected to influence performance (statistical test of this assumption in table e-1 on the *Neurology*® Web site at www.neurology.org), the 2 $rTMS_S$ groups were merged into a single control group.

Intervention. Treatment lasted 10 days with 2 time-locked daily interventions: 1) 25 minutes of real/sham 1 Hz rTMS[17]; and 2) 45 minutes of standard task-oriented upper-limb exercises.[1–3] Low-frequency $rTMS_R$ was performed using a 70-mm focal coil connected to a Magstim Rapid2 stimulator (Magstim, UK). A single train of 1,500 pulses at 90% of resting motor threshold (rMT) was administered over the motor representation of the first dorsal interosseous (FDI) in the $_{int}$M1; $rTMS_S$ with the same parameters was applied by positioning a 90-mm circular coil perpendicularly to the scalp so that no current was induced in the brain. All participants were blinded to the rTMS conditions and none of them had any experience with rTMS before the study. To minimize the risk of unblinding, different coil types and stimulators were used for single-pulse TMS (administered for neurophysiologic assessment; see below) and $rTMS_S$, to prevent the patients' expectation that $rTMS_S$ should produce scalp sensations as single-pulse TMS.

The PT was carried out by a therapist blinded to group allocation. PT was aimed at training hand dexterity by presenting patients with a number of daily routine tasks[2,3] (e.g., grasping and manipulating objects with different affordances, size, and weight). Finger force was also trained daily (for about 5–10 minutes) using task-oriented exercises (e.g., grasping and lifting objects with different weights, squeezing soft objects) focusing on key grip (i.e., involving the adduction of thumb and index finger), which may be particularly functional in hemiparetic patients.[18,19] Patients did not receive any other upper-limb PT intervention over the duration of the study.

Assessment. Primary outcomes were hand dexterity and force and were performed by a clinician blinded to group allocation. Trained (manual dexterity, key grip force) and untrained motor functions (tip-pinch and power-grip force) of both the affected and the unaffected hands were assessed. The Jebsen-Taylor Hand Function Test[20] (JHFT), the Nine-Hole Peg Test[21] (NHPT), and the Box and Block test[22] (B&B) were used to assess hand dexterity. Maximal force of key grip and tip-pinch was evaluated by means of a pinch-meter; a dynamometer was used for assessing power-grip force.[23] See data supplement for details on tests and assessment procedures. To check patients' stability, 2 pretreatment evaluations were performed 2 weeks (baseline) and 1 day before starting the treatment (pre). Post-treatment evaluations were performed 1 day (post) and 7 (follow-up 1), 14 (follow-up 2), 30 (follow-up 3), and 90 days (follow-up 4) after treatment. Before baseline, patients participated in 2 daily sessions in which they familiarized themselves with all the tests.

Secondary outcomes included measures of cortical excitability that were recorded by an experimenter unblinded to group allocation. Corticospinal excitability of both hemispheres was assessed by recording the rMT[24] using a Biopac MP-150 (Biopac Corp, CA) electromyograph and a 70-mm polyurethane-coated focal coil connected to a Magstim 200 stimulator (Magstim, UK). Evaluations of rMT were performed at baseline, pre, at the start of the sixth session on day 6 (midtreatment evaluation, mid), post, and follow-up 1–4. Evaluation of interhemispheric inhibition from $_{int}$M1 to $_{aff}$M1 was performed at pre and post by recording the ipsilateral silent period[25,26] (iSP) in the contracted FDI muscle of the affected hand by stimulation of $_{int}$M1 (e-Methods).

Analysis. Preliminary analyses assured that the different groups were entirely comparable before treatment (table e-2, table e-3). The effect of treatment was evaluated as follows: for each measure, evaluation at pre, post, and follow-up 1–4 was expressed as percentage from the baseline. The 2 $rTMS_S$ groups showed entirely comparable effects at all time points (table e-1); thus, to simplify the analysis, they were merged into a single group. Changes in dexterity, force, and rMT in the 3 groups ($rTMS_R$-PT, PT-$rTMS_R$, $rTMS_S$) were analyzed by means of Friedman nonparametric analysis of variance (ANOVA) for repeated measures and comparisons between pre and post-treatment conditions were evaluated with Bonferroni correction (0.05/5 = 0.01). Friedman nonparametric ANOVA was also used to evaluate the duration of the iSP at pre and post in each group. Between-groups differences were analyzed using Mann-Whitney *U* test with Bonferroni correction (0.05/2 = 0.025). To test whether post-treatment changes in neurophysiologic measures (iSP, $_{int}$M1, and $_{aff}$M1 rMT at post) predicted improvements in trained (mean changes in JHFT, NHPT, B&B, key grip performance) and untrained motor functions (tip-pinch, power-grip), a correlation analysis was performed using Spearman test.

RESULTS

The different groups did not differ in clinical features or demographic variables (table 1). Before treatment (baseline and pre evaluations), groups were comparable in all dexterity and force tests, showed pathologically lower performance in the affected hand, and presented a stable performance in the 2 pretreatment assessments (table e-2). Moreover, before treatment, groups showed comparable and stable motor excitability and presented higher rMT (lower excitability) in $_{aff}$M1 relative to $_{int}$M1.[27]

Treatment-related changes in motor excitability. During treatment there was a daily cumulative increase of $_{int}$M1 rMT in the 2 $rTMS_R$ groups only, indicating that $rTMS_R$ was effective in suppressing motor excitability[13,17,28]; this suppression was comparable in the 2 $rTMS_R$ groups and lasted only few days after treatment (figure e-1).

In contrast, long-lasting changes in excitability were obtained in the $_{aff}$M1. Friedman ANOVA per-

Table 1 Sample characteristics[a]

Treatment allocation	Months after stroke	Lesion site	Type of ictus	Affected hand laterality
Real rTMS before PT (n = 8)				
$rTMS_R$-PT	28	Internal capsule	Ischemic	L
$rTMS_R$-PT	22	Internal capsule	Ischemic	R
$rTMS_R$-PT	54	Thalamus	Hemorrhagic	L
$rTMS_R$-PT	32	Internal capsule, basal ganglia	Hemorrhagic	R
$rTMS_R$-PT	66	Temporo-parietal cortex, basal ganglia	Ischemic	R
$rTMS_R$-PT	7	Basal ganglia, internal capsule	Ischemic	L
$rTMS_R$-PT	11	Internal capsule	Ischemic	L
$rTMS_R$-PT	26	Thalamus	Ischemic	R
Real rTMS after PT (n = 8)				
PT-$rTMS_R$	46	Internal capsule, thalamus	Hemorrhagic	L
PT-$rTMS_R$	38	Internal capsule, thalamus	Hemorrhagic	L
PT-$rTMS_R$	24	Internal capsule	Ischemic	R
PT-$rTMS_R$	26	Internal capsule	Hemorrhagic	R
PT-$rTMS_R$	16	Thalamus	Ischemic	L
PT-$rTMS_R$	7	Basal ganglia	Ischemic	L
PT-$rTMS_R$	37	Internal capsule	Ischemic	R
PT-$rTMS_R$	26	Fronto-insular cortex	Ischemic	L
Sham rTMS (n = 14)				
$rTMS_S$-PT	88	Temporo-parietal cortex	Ischemic	R
$rTMS_S$-PT	62	Thalamus	Ischemic	L
$rTMS_S$-PT	63	Thalamus	Ischemic	L
$rTMS_S$-PT	7	Fronto-insular cortex	Ischemic	R
$rTMS_S$-PT	6	Basal ganglia, internal capsule	Hemorrhagic	R
$rTMS_S$-PT	38	Internal capsule	Ischemic	L
$rTMS_S$-PT	10	Internal capsule	Hemorrhagic	L
PT-$rTMS_S$	14	Basal ganglia, thalamus	Ischemic	R
PT-$rTMS_S$	8	Internal capsule	Hemorrhagic	L
PT-$rTMS_S$	21	Basal ganglia, internal capsule	Ischemic	R
PT-$rTMS_S$	14	Basal ganglia, internal capsule	Hemorrhagic	L
PT-$rTMS_S$	28	Internal capsule	Hemorrhagic	L
PT-$rTMS_S$	83	Basal ganglia	Ischemic	R
PT-$rTMS_S$	36	Internal capsule	Ischemic	R

Abbreviations: PT = physical therapy; rTMS = repetitive transcranial magnetic stimulation; $rTMS_R$ = real repetitive transcranial magnetic stimulation; $rTMS_S$ = sham repetitive transcranial magnetic stimulation.

[a] In the $rTMS_R$-PT group, there were 4 women and 4 men (mean age ± SD: 60.9 ± 8.8 years; mean education ± SD: 8.4 ±4.0 years); in the PT-$rTMS_R$ group, there were 4 women and 4 men (age: 64.0 ± 7.7 years; education: 8.6 ± 4.3 years); in the $rTMS_S$ group, there were 6 women and 8 men (age: 64.0 ± 12.1 years; education: 8.1 ± 3.8 years). Mann-Whitney *U* was used to compare duration after stroke ($p > 0.6$), age ($p > 0.4$), and education ($p > 0.8$) in the different groups. Freeman-Halton extension of the Fisher exact probability test was used to compare the type of ictus (ischemic/hemorrhagic: $p > 0.9$), lesion location (subcortical/cortical/cortico-subcortical: $p > 0.9$), affected hand laterality (left/right: $p = 0.9$), and sex distribution (male/female: $p > 0.9$) across groups.

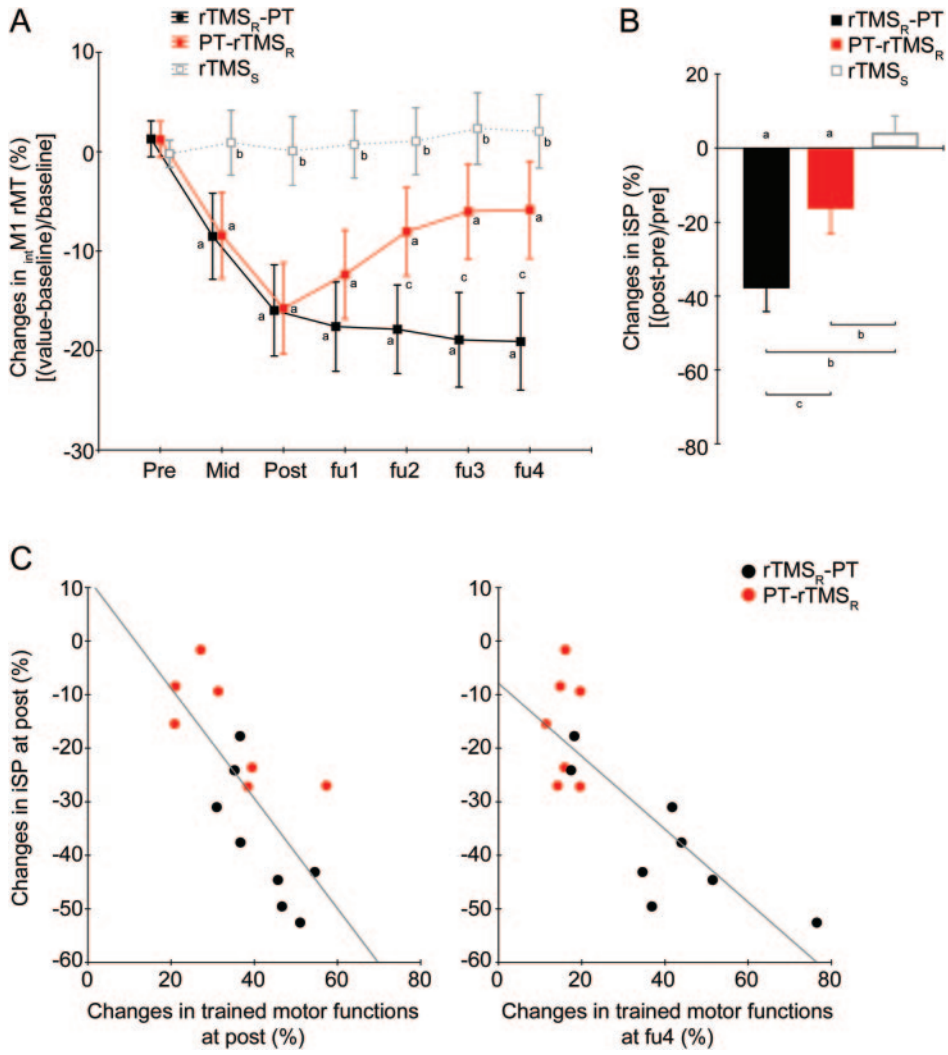
formed on $_{aff}$M1 rMT (figure 2A) was significant in the 2 $rTMS_R$ groups ($p < 0.01$) but not in the $rTMS_S$ group ($p = 0.6$), indicating that treatment selectively affected the $_{aff}$M1 of patients receiving active rTMS. At mid, post, and follow-up 1–4, the $_{aff}$M1 rMT in the 2 $rTMS_R$ groups was significantly lower than at pre ($p < 0.01$), indicating an increase of $_{aff}$M1 excitability. The 2 $rTMS_R$ groups were comparable to the $rTMS_S$ group at pre ($p > 0.2$), however they showed lower $_{aff}$M1 rMT at mid, post, and follow-up 1–4 ($p < 0.01$). Notably, while the $rTMS_R$-PT group presented a stable change in excitability, the PT-$rTMS_R$ group presented a slight decline at the last follow-ups: the 2 $rTMS_R$ groups resulted comparable at pre, mid, post, and follow-up 1 ($p > 0.1$), however at follow-up 2–4 the $rTMS_R$-PT group presented lower rMT (greater $_{aff}$M1 corticospinal excitability) than the PT- $rTMS_R$ ($p < 0.025$).

In the 2 $rTMS_R$ groups, assessment of iSP revealed a significant reduction of transcallosal inhibition at post relative to pre ($p < 0.01$; figure e-1); no similar reduction was found in the $rTMS_S$ group ($p = 0.6$). Moreover, the $rTMS_R$-PT group showed greater iSP reduction relative to PT-$rTMS_R$ group and $rTMS_S$ group ($p < 0.01$; figure 2B) and the PT-$rTMS_R$ group showed greater iSP reduction than $rTMS_S$ group ($p < 0.01$). Thus, for the iSP, the superior outcome of the $rTMS_R$-PT group was already detectable at post.

Trained motor functions. In tests tapping trained motor functions, all the groups showed an increase in performance after treatment. This increase was present in the affected but not in the unaffected hand (figure e-2) and varied in the different groups (figure 3). While improvements in the $rTMS_S$ group were modest and transitory, long-lasting increases in performance were detected in the 2 $rTMS_R$ groups: the $rTMS_R$-PT group showed a strong improvement that was maintained until the last follow-up; in contrast, the PT-$rTMS_R$ group showed a slight improvement decline over time. This indicates that $rTMS_R$-PT was particularly effective in promoting use-dependent plasticity.

Friedman ANOVAs performed on JHFT (figure 3A) and NHPT (figure 3B) resulted significant in all the groups (all $p < 0.01$), indicating that treatment affected fine manual dexterity. At post and all follow-ups, dexterity performance in the 2 $rTMS_R$ groups was significantly greater than prelevels ($p < 0.01$). The $rTMS_S$ group showed a modest but significant improvement at post and follow-up 1–2 ($p < 0.01$) that however returned to pretreatment level at follow-up 3–4 ($p > 0.03$). The 2 $rTMS_R$ groups were comparable to the $rTMS_S$ group at pre ($p > 0.3$); however, they showed greater JHFT and NHPT performance at all post-treatment time points ($p < 0.025$). Performance in the 2 $rTMS_R$

Figure 2 Changes in motor excitability



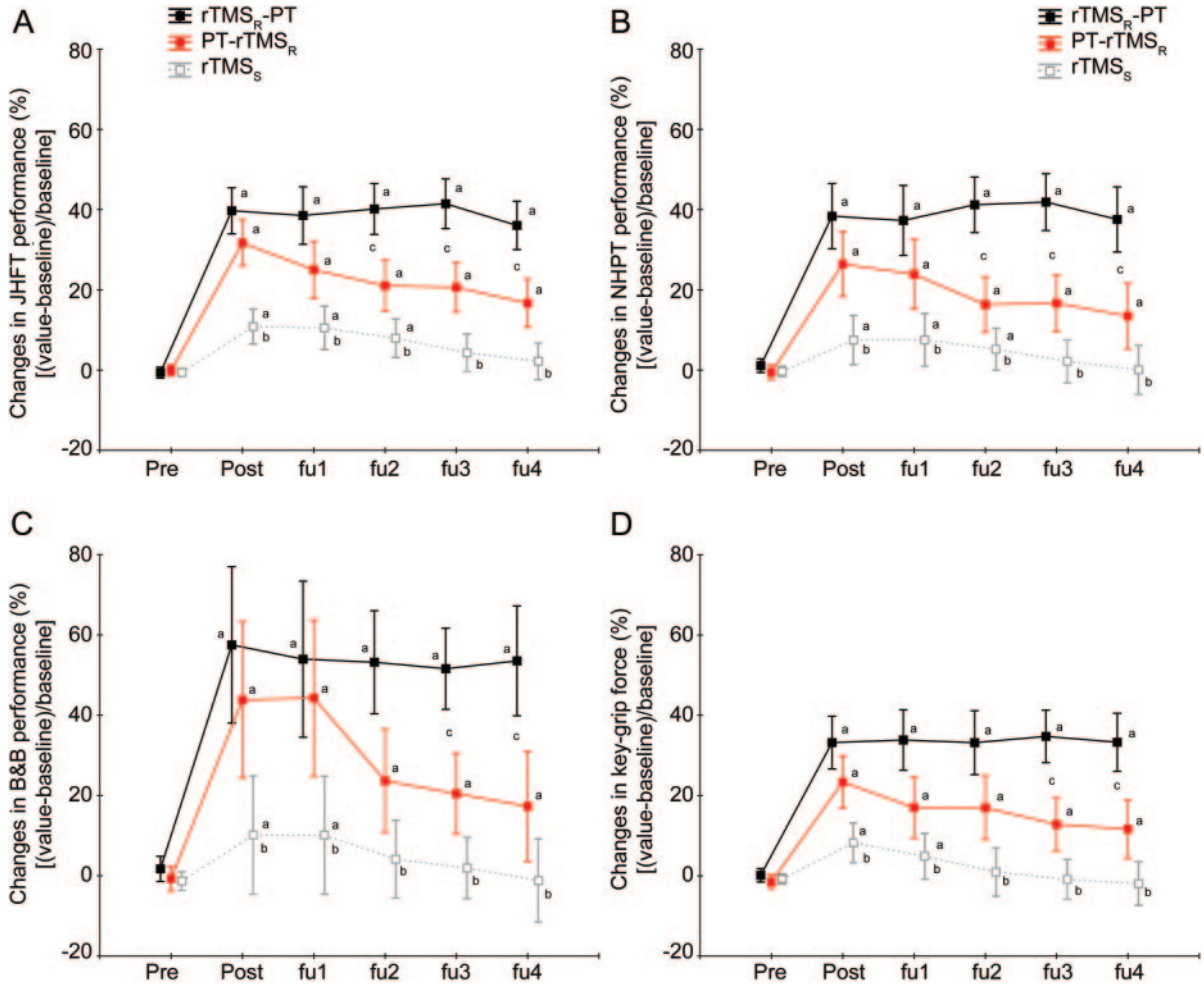
(A) Changes in $_{aff}$M1 resting motor threshold (rMT) over time. (B) Changes in ipsilateral silent period (iSP) at post. (C) Relation between changes in iSP at post and trained motor function (average of Jebsen-Taylor Hand Function Test, Nine-Hole Peg Test, Box and Block test, key-grip) at post and follow-up 4. Bars denote 95% confidence interval. Symbols indicate significant comparisons. a = Significant difference with respect to pre ($p < 0.01$); b = significant between-group difference: sham repetitive transcranial magnetic stimulation (rTMS$_S$) group vs both real repetitive transcranial magnetic stimulation (rTMS$_R$) groups ($p < 0.025$); c = significant between-group difference: rTMS$_R$-physical therapy (PT) group vs PT-rTMS$_R$ group ($p < 0.025$).

groups resulted comparable at pre, post, and follow-up 1 ($p > 0.1$); however, at follow-up 2–4 dexterity in the rTMS$_R$-PT group was significantly greater than in the PT-rTMS$_R$ group ($p < 0.025$).

Comparable results were obtained in tests requiring less fine motor control. Friedman ANOVAs performed on B&B (figure 3C) and on key-grip force (figure 3D) resulted significant in all the groups ($p < 0.01$). At post and all follow-ups, dexterity and force performance in the 2 rTMS$_R$ groups was significantly greater than at pre ($p < 0.01$). The rTMS$_S$ group showed a modest but significant improvement at post and follow-up 1 ($p < 0.01$) that however returned to pretreatment level at follow-up 2–4 ($p > 0.03$). The 2 rTMS$_R$ groups were comparable to the rTMS$_S$ group at pre ($p > 0.3$); however, they showed greater performance at all post-treatment time points ($p < 0.01$). B&B and key-grip performance in the 2 rTMS$_R$ groups resulted comparable at pre, post, and follow-up 1–2 ($p > 0.03$); however, at follow-up 3–4 dexterity in the rTMS$_R$-PT group was significantly greater than in the PT-rTMS$_R$ group ($p < 0.025$).

Untrained motor functions. A general improvement in untrained motor functions was found in the rTMS$_R$ groups with no differential effects for rTMS$_R$-PT and PT-rTMS$_R$ (figure 4). The rTMS$_R$ groups showed a similar trend with a peak at post and a slight performance decline over time without returning to pretreatment levels.

Figure 3 Changes in performance in tests tapping trained motor functions



(A) Jebsen-Taylor Hand Function Test (JHFT). (B) Nine-Hole Peg Test (NHPT). (C) Box and Block (B&B). (D) Key-grip force. Bars denote 95% confidence interval. Symbols indicate significant comparisons. a = Significant difference with respect to pre ($p < 0.01$); b = significant between-group difference: sham repetitive transcranial magnetic stimulation (rTMS$_S$) group vs both real repetitive transcranial magnetic stimulation (rTMS$_R$) groups ($p < 0.025$); c = significant between-group difference: rTMS$_R$–physical therapy (PT) group vs PT-rTMS$_R$ group ($p < 0.025$).

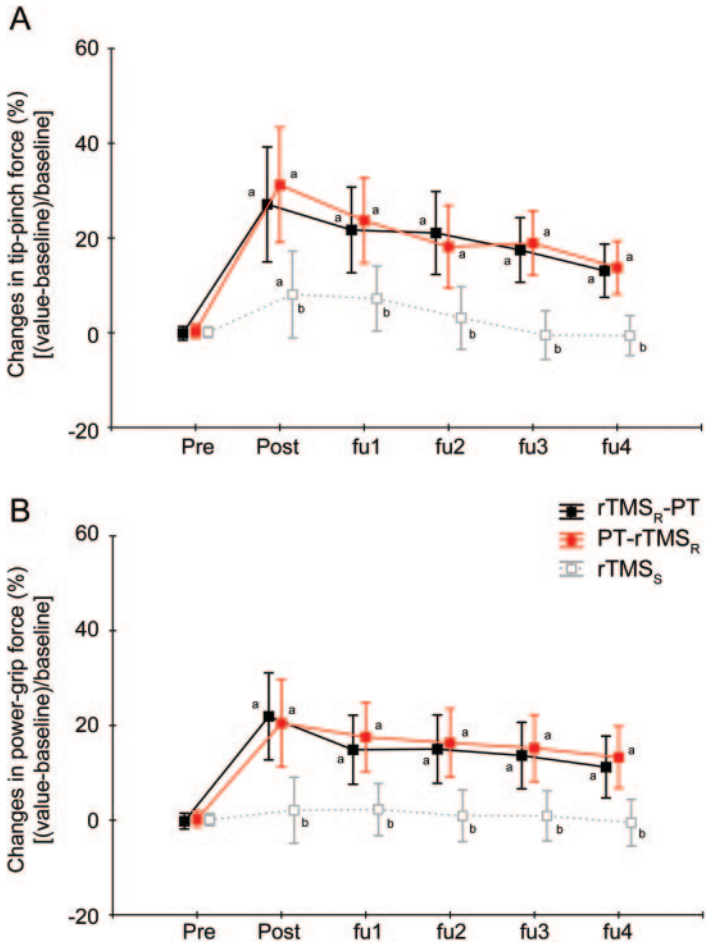
Friedman ANOVAs performed on changes in pinch-grip force were significant in all groups ($p < 0.01$; figure 4A). The 2 rTMS$_R$ groups showed a significant increase in force at post and follow-up 1–4 relative to pretreatment levels ($p < 0.01$). The rTMS$_S$ group showed a significant increase in force at post ($p < 0.01$) but not at follow-up 1–4 ($p > 0.03$). At pre the 3 groups were comparable ($p > 0.3$); however, the 2 rTMS$_R$ groups outperformed the rTMS$_S$ group at all post-treatment time points ($p < 0.025$). No difference between the 2 rTMS$_R$ groups was found at any post-treatment time point ($p > 0.7$).

Friedman ANOVAs performed on changes in power-grip force resulted significant in the 2 rTMS$_R$ groups ($p < 0.01$; figure 4B) that showed a slight but significant increase in force at post and all follow-ups relative to pre levels ($p < 0.01$). No significant change in force was detected in the rTMS$_S$ group ($p = 0.1$). At pre the 3 groups were comparable ($p > 0.5$); however, force improvements in the 2 rTMS$_R$ groups resulted greater than in the rTMS$_S$ group at all post-treatment time points ($p < 0.01$). No difference between the 2 rTMS$_R$ groups was found at any post-treatment time point ($p > 0.5$).

Correlation analyses. In the rTMS$_R$ groups, changes in iSP predicted improvements in tests tapping trained motor functions both at post and follow-up 4 ($r = 0.74$ and $r = 0.77$, respectively, $p < 0.01$;figure 2C). No similar relations were found for untrained motor functions ($p > 0.1$). Moreover, no relation was found between changes in $_{aff}$M1 or $_{int}$M1 rMT and behavior ($p > 0.2$). Correlation analyses carried out in the rTMS$_S$ group revealed no significant correlation ($p > 0.2$).

DISCUSSION After a unilateral lesion, $_{int}$M1 is disinhibited by the reduction in the transcallosal inhibition

Figure 4 Changes in performance in tests tapping untrained motor functions



(A) Tip-pinch force. (B) Power-grip force. Bars denote 95% confidence interval. Symbols indicate significant comparisons. a = Significant difference with respect to pre ($p < 0.01$); b = significant between-group difference: sham repetitive transcranial magnetic stimulation (rTMS$_S$) group vs both real repetitive transcranial magnetic stimulation (rTMS$_R$) groups ($p < 0.025$). PT = physical therapy.

from $_{aff}$M1.[9,29,30] Subsequently, this phenomenon is thought to lead to an increased interhemispheric inhibition of the $_{aff}$M1 by the disinhibited $_{int}$M1. As a result, chronic hemiparetic patients, like those who took part in our study, typically show less excitability in the $_{aff}$M1as compared to $_{int}$M1.[27]

Studies suggest that abnormal interhemispheric inhibition may impede functional motor recovery in unilateral stroke[29] and single sessions of low-frequency rTMS over $_{int}$M1 have been proved to transiently improve affected hand motor functions[11,12,14] by downregulating transcallosal inhibition from the $_{int}$M1.[9,12]

Here we addressed the issue of how to combine low-frequency rTMS with PT interventions in stroke patients with mild motor deficits. We used a time-locked rTMS strategy and tested the effect of interventions order (rTMS$_R$-PT vs PT-rTMS$_R$) on use-dependent plasticity. We hypothesized that rTMS$_R$ preceding PT could potentially prime functional networks for the physical intervention, leading to superior outcomes.[6–8] However, an alternative hypothesis would predict that rTMS$_R$ after PT can provide a further modulation of cortical excitability that might selectively promote the stabilization of activity-dependent motor networks.[10,31] Our study provides evidence that time-locked rTMS$_R$ and PT induce 1) a reduction of interhemispheric inhibition from $_{int}$M1 to $_{aff}$M1, 2) a long-term potentiation-like increase of $_{aff}$M1 excitability, and 3) conspicuous use-dependent functional improvements, in particular when PT is preceded, not followed, by rTMS. The major functional benefit of priming PT with rTMS was particularly evident at the last follow-ups: 1–3 months after treatment the PT-rTMS$_R$ group started to show a decline in performance and $_{aff}$M1 excitability; in contrast, the outcomes of the rTMS$_R$-PT group remained stable over time. This suggests that rTMS$_R$-PT more than PT-rTMS$_R$ boosts use-dependent plasticity mainly by stabilizing consolidation processes.

Notably, our data suggest a link between optimized consolidation due to priming PT with rTMS$_R$ and inhibitory interactions between hemispheres. Indeed, at post, the superior outcome of the rTMS$_R$-PT group was already visible in the iSP. Moreover, changes in iSP at post correlated with activity-dependent behavioral gains at post as well as at follow-up 4, suggesting that measures of GABAergic-mediated interhemispheric inhibition were particularly sensitive to detect treatment-related neuroplastic changes and predicted functional improvements. This would be in keeping with the notions that 1) activity-dependent plasticity critically relies on the main inhibitory neurotransmitter GABA[32,33]; and 2) reduction of abnormal interhemispheric inhibition plays an important role in the functional recovery of stroke patients with motor deficits.[4–14]

To induce long-lasting effects, in the present research we applied low-frequency rTMS in daily multiple sessions.[8,9,13] Notably, during treatment, we found evidence of a daily cumulative increase of rMT in $_{int}$M1,[9,17,28,34] reflecting a decrease of membrane excitability of corticospinal neurons in the healthy hemisphere.[35,36] This was paralleled by a strong cumulative increase of $_{aff}$M1 excitability as evidenced by rMT assessment at pre, mid, and post. These findings provide direct neurophysiologic evidence that 10 days are more effective than 5 days of treatment. Moreover, they further indicate that treatment rebalanced motor excitability in the 2 hemispheres.

Our findings suggest that rTMS$_R$ boosts the effect of PT. It should be noted that rTMS$_S$ groups

showed a modest improvement lasting only few weeks and no significant change in motor excitability. This is not surprising since PT duration was relatively short, patients were all in a chronic stage, and all of them had already received cycles of rehabilitation. While it is well known that PT at this stage is less effective,[1–3] our data indicate that time-locked rTMS may overcome this limitation.

A potential limitation of rTMS studies is the sham method.[10–14] An ideal $rTMS_S$ condition should produce the same scalp sensation as the $rTMS_R$. Given that all our patients were naive to rTMS, it is unlikely that this might have unblinded the rTMS treatment. Moreover, the different groups showed a comparable and stable pattern of results in the healthy hand, suggesting that during the evaluation they were similarly engaged in the tests.

Our study indicates that $rTMS_R$-PT leads to superior outcomes in tests tapping trained motor functions, suggesting that priming motor networks with rTMS promotes use-dependant plasticity.[37] Additional factors may have contributed to the present findings. For example, it is possible that patients receiving PT after rTMS were more attentive to the PT. Were this the case, however, we should have detected greater behavioral improvement also at post. In contrast, after treatment performance in the 2 $rTMS_R$ groups was comparable and a clear effect of interventions order was observed only in the last follow-ups. This would speak against an interpretation of the data in terms of attention and motivation. Rather we suggest that priming PT with time-locked inhibitory rTMS can create a state in which consolidation processes are optimized[6–10] and GABAergic neuroplastic changes in the motor system are favored. Further studies are needed to evaluate the effect of intervention order of time-locked rTMS in the same patients. Moreover, future studies should assess whether the present findings can be extended to stroke patients with moderate to severe motor impairments.

AUTHOR CONTRIBUTIONS

A.A., E.L., L.P., and M.G.C. conceived and designed the study. A.A. (neurophysiological assessment), M.C. (behavioral testing), L.P., and M.G.C. (clinical screening) conducted the study. A.A. and M.C. analyzed the data. A.A. wrote the paper.

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DISCLOSURE

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