Differential Effect of Conditioning Sequences in Coupling Inhibitory/Facilitatory Repetitive Transcranial Magnetic Stimulation for PostStroke Motor Recovery

Chih-Pin Wang,¹ Po-Yi Tsai,^{2,3} Tsui Fen Yang,^{2,4} Kuang-Yao Yang^{3,5} & Chien-Chih Wang²

¹ Department of Emergency, Mackay Memorial Hospital, Taipei, Taiwan, China

² Department of Physical Medicine and Rehabilitation, Taipei Veterans General Hospital, Taipei, Taiwan, China

³ School of Medicine, National Yang-Ming University, Taipei, Taiwan, China

⁴ Department of Physical Medicine and Rehabilitation, National Yang-Ming University, Taipei, Taiwan, China

⁵ Department of Chest Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, China

Keywords

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Correspondence

Dr. Po-Yi Tsai, Department of Physical Medicine and Rehabilitation, Taipei Veterans General Hospital, Taipei, Taiwan, China. and

School of Medicine, National Yang-Ming University, No. 201, Shih-Pai Rd, Sec. 2, Taipei, 11217 Taiwan, China.

Tel.: +886 28757293;

Fax: +886 2 28757359;

E-mail: pytsai@vghtpe.gov.tw

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SUMMARY

Introduction: While neuromodulation through unihemispheric repetitive transcranial magnetic stimulation (rTMS) has shown promise for the motor recovery of stroke patients, the effectiveness of the coupling of different rTMS protocols remains unclear. **Aims:** We aimed to test the long-term efficacy of this strategy with different applying sequences and to identify the electrophysiological correlates of motor improvements to the paretic hand.

Results: In our sham-controlled, double-blinded parallel study, 48 stroke patients (2–6 months poststroke) were randomly allocated to three groups. Group A underwent 20-session rTMS conditioning initiated with 10-session 1 Hz rTMS over the contralesional primary motor cortex (M1), followed by 10-session intermittent theta burst stimulation (iTBS) consequently over the ipsilesional M1; Group B underwent the same two paradigms but in reverse; and Group C received sham stimulation that was identical to Group A. We tested cortical excitability and motor assessments at the baseline, postpriming rTMS, postconsequent rTMS, and at 3-months follow-up. Group A manifested greater improvement than Group B in Fugl-Meyer Assessment (FMA), Wolf Motor Function testing (WMFT) score, and muscle strength ($P = 0.001$ – 0.02) post the priming rTMS. After the consequent rTMS, Group A continued to present a superior outcome than Group B in FMA ($P = 0.015$) and WMFT score ($P = 0.008$) with significant behavior-electrophysiological correlation. **Conclusions:** Conditioning the contralesional M1 prior to ipsilesional iTBS was found to be optimal for enhancing hand function, and this effect persisted for at least 3 months. Early modulation within 6 months poststroke rebalances interhemispheric competition and appears to be a feasible time window for rTMS intervention.

Introduction

For the majority of people who have experienced a hemiplegic stroke, even when they have undergone intensive rehabilitation, their motor function recovery is usually incomplete and disappointing [1]. Even 3–6 months after stroke, 55–75% of survivors experience major functional limitation in the affected limbs which compromises their quality of life [2]. Brain plasticity associated with spontaneous recovery usually comprises many forms of reorganization that occur in parallel, including structural, functional, and connectional remodeling in both the perilesional and contralesional motor systems; these may be accompanied by a shift in motor cortex laterality toward to contralesional hemisphere (interhemispheric

imbalance) that may hamper any consequential adaptive neuroplasticity [3–5].

Brain plasticity associated with treatment-induced recovery helps neuromodulation process return to a normal degree of brain laterality that has been demonstrated to have a positive impact on functional recovery [6,7]. There is now converging evidence to suggest that repetitive transcranial magnetic stimulation (rTMS) in this field of neuromodulation is an effective approach [8]. This may be achieved either by directly enhancing the synaptic strength of the affected motor area with high-frequency rTMS under the mechanism known as “long-term potentiation” (LTP) [9–11] or by reducing transcallosal inhibition from the contralesional homologous motor areas using low-frequency rTMS under the mechanism known as “long-

term depression" (LTD) [12,13]. These lead to effects on behavior and learning that could be harnessed for clinical application [14]. However, the conventional protocols using inhibitory or facilitatory rTMS have shown only short-term or modest beneficial effects on motor recovery [12,15–17]. Patterned or complex coupled stimulation protocols (with priming stimulation or repeated sessions) have potential to be used to enhance the efficacy of stimulation [14].

Our previous study investigating the coupling protocol with inhibitory and facilitatory rTMS montage in chronic stroke patients had successfully demonstrated the superior aftereffects of this strategy compared with those obtained from a single course (10-session) of rTMS intervention alone [18]. However, the long-term efficacy of this regimen for motor enhancement remains an area of uncertainty, and it is unclear, for instance, whether facilitatory rTMS should precede or follow inhibitory rTMS. Under the framework of LTP/LTD model and building upon this recent work, we hypothesized that this protocol with inhibitory rTMS applied over the unaffected hemisphere (UH) and additional sessions of facilitatory rTMS applied over the affected hemisphere (AH) in the primary motor cortex (M1) would improve poststroke motor recovery of the paretic hand. Our randomized, sham-controlled study aimed (1) to assess both the immediate and long-term effects of this protocol with a 3-month follow-up and (2) to compare the efficacy of different conditioning sequences for achieving motor recovery, as measured by upper extremity motor function tests and corticomotor excitability.

Materials and methods

Subjects

Ninety-five patients admitted to the stroke unit were evaluated consecutively for participation in the study. Two patients declined to participate and 48 patients met the inclusion criteria: (1) diagnosis of unilateral hemiplegia secondary to a ischemic stroke, confirmed by magnetic resonance imaging; (2) 2–6 months after stroke; (3) the muscle strength of the finger flexors (distal Medical Research Council Scale) and anterior deltoid of the upper extremity (proximal MRC) ≤ 3 grade; (4) no history of dementia, cognitive impairment, or other neurodegenerative diseases; and (5) absence of aphasia, spatial neglect, visual field deficit, or emotional problems. Neurological assessments included (1) the severity of the stroke, using the National Institute of Health Stroke Scale (NIHSS) [19]; (2) sequential motor recovery following stroke using the Brunnstrom Approach [20]; and (3) cognitive and physical disability, using the Functional Independence Measure [21].

Forty-eight patients (10 women and 38 men aged 32–87 years) participated the study and gave their written informed consent prior to the intervention, in accordance with the 2008 Declaration of Helsinki and with the approval of the local Institutional Review Board (Figure 1).

Subject characteristics are given in Table 1. In summary, the mean age was 65 years (range, 32–87). All three groups shared the same overall characteristics in terms of age range, time post-stroke, NIHSS, Brunnstrom stage, MRC and functional indepen-

Table 1 Demographic data and clinical characteristics of all patients in three groups

	Group A (n = 17)	Group B (n = 15)	Group C (n = 16)	P
Male/female	14/3	13/2	11/5	
Age	62.2 ± 12	63.1 ± 12.1	62.5 ± 13.4	0.60
Cortical/subcortical	7/10	7/6	9/6	0.44
Months poststroke	4.6 ± 3.9	4.5 ± 3.4	4.4 ± 3.1	0.67
NIHSS	13.5 ± 4.6	12.8 ± 3.3	13.1 ± 5.1	0.75
Br stage, proximal	2.9 ± 1.3	2.8 ± 1.4	2.8 ± 1.3	0.63
Br stage, distal	3.2 ± 1.4	3.1 ± 1.6	3.1 ± 1.5	0.71
DM history (%)	29.4	23.0	20	0.79
Hyperlipidemia (%)	23.5	23.0	26.6	0.84
Hypertension (%)	94	92.3	93.3	0.76

NIHSS, National Institute of Health Stroke Scale; Br, Brunnstrom; DM, diabetic mellitus.

dence measure scores (including cognition and psychosocial domains), with *P*-values in the range of 0.4–0.83.

Experimental Design

We conducted a sham-controlled, double-blinded parallel study design (Figure 2). The randomization order was computer generated and concealed in sequentially numbered envelopes by an independent statistician. All subjects were allocated to one of the three groups by a researcher blinded to the assessment. The Group A and Group B experimental groups comprised 17 and 15 subjects, respectively, each of whom underwent real rTMS, which proceeded with either 10-session 1 Hz rTMS over the UH followed by 10-session intermittent theta burst stimulation (iTBS) over the AH (Group A) or 10-session iTBS over the AH followed by 10-session 1 Hz rTMS over the UH (Group B). Group C (n = 16) received sham stimulation at the same sites in the same order as Group A. One Hertz rTMS trains consisting of 600 pulses were applied at 90% of resting motor threshold (rMT) over the UH M1 "hot spot" for 10 min. iTBS was performed at 80% of active MT over the AH M1 hot spot, consisting of bursts containing three pulses at 50 Hz each, repeated at 200 millisecond intervals for 2 seconds. A 2-second train of iTBS was repeated every 10 second to give a total time of 190 second and 600 pulses [22–24]. The "hot spot" for the first dorsal interosseous (FDI) served as the target for the rTMS modulation. Each subject received 20 daily rTMS sessions for 20 consecutive weekdays, over a 4-week period. The daily rTMS session comprised either 10 min of 1 Hz rTMS or 190 seconds of iTBS protocol.

Interventions

Intermittent theta burst stimulation and 1 Hz rTMS were conducted using Magstim Rapid² (Magstim Company, Withland, Dyfed, UK) connected with a 70-mm figure-of-eight coil. We used a placebo coil (Magstim Company) for the sham stimulation, which delivered <5% of the magnetic output with audible click-on discharge. As none of the patients had ever undergone TMS, they would not have known whether the stimulation was real or sham.

Figure 1 The flowchart illustrates recruitment, group allocation, allocation treatment, follow-up and analysis.

Each subject received the same amount of conventional rehabilitation program immediately following the daily rTMS or iTBS protocol during the 4-week period. The conventional physiotherapy (1 h daily, five times per week) included task-oriented training (e.g., squeezing and releasing soft objects, pushing and lifting objects and key gripping); these emphasized the active participation of the affected limb and individualized motor task training. After the rTMS protocols, the subjects continued the physiotherapy programs twice a week during the 3-month follow-up period. The physiotherapy was conducted by a therapist blinded to group allocation.

Assessments

An independent assessor, blinded to treatment allocation, performed the baseline measurements shortly before the first session of rTMS intervention (baseline), on the day after the 10th rTMS session (post 1), on the day after the 20th rTMS session (post 2), and at 3 month after the last rTMS session (post 3). The motor assessments included the Fugl-Meyer Assessment—upper extremity (FMA) [25] and the Wolf Motor Function test (WMFT)—functional ability scale [26].

Corticomotor excitability of both hemispheres was measured with monophasic Magstim 200 via a 70-mm figure-of-eight coil. Procedures of corticomotor assessment of rMT, amplitude and latency of the maximal motor evoked potential (MEP), as well as motor map area were conducted using standard techniques that have been described previously [18,27]. Subjects were instructed to sit in a comfortable chair with both hands relaxed and remain conscious. An elastic cap with a 7 × 7 cm grid was applied to the subject's head with reference to two anatomical landmarks, namely the nasion-inion line and the interaural line. We moved the coil along each grid position and kept the coil tangentially to the scalp and 45° from mid-sagittal line. MEPs evoked by ipsilesional or contralesional cortical magnetic stimulation were

recorded bilaterally from the FDI muscles using surface Ag/AgCl electrodes. A Dantec Keypoint electromyograph (EMG; Dantec, Skovlunde, Denmark) was connected to the stimulator to record the MEP signals. The amplified (100 μ V–1 mV/div) and bandpass-filtered (20–2000 Hz) signal was digitized at a 20 kHz sampling rate.

As a first step, the “hot spot” for FDI was determined to be the optimal scalp location from which the greatest amplitude of MEP was elicited with the lowest intensity. The rMT for MEP was defined as the minimal intensity at which MEPs of at least 50 μ V amplitudes could be elicited in half of 10 consecutive stimuli. We then determined the maximal MEP amplitude and shortest latency using 100% of maximal output among five consecutive stimuli separated with 5-second interval. The motor map area was assessed over bilateral frontal motor area with a grid of 49 positions, spaced at 1 cm relative to the interaural and sagittal lines. The motor map area was determined as the sum of the excitable sites where at least two MEPs were elicited of three stimuli at 110% of the rMT intensity [28].

Statistics

At the baseline assessments prior to the rTMS intervention, the mean values of the motor assessments in terms of FMA, WMFT, MRC scoring and electrophysiological findings were compared between the groups, using one-way analysis of variance (ANOVA) for independent samples for continuous data or the chi-squared test for categorical data.

For intergroup comparisons across all time-points, we used repeated-measure ANOVA with Bonferroni correction. The time-points at baseline, post 1, post 2, and post 3 were employed as the within-patient factors, and the Group A, B, and C were used as the between-patient factors.

Correlation analysis for the baseline levels and changes in motor function as well as cortex excitability was performed using the Spearman test. This study achieved a statistical power of 0.97 at 95% (two-tailed $\alpha = 0.05$) for the WMFT score. The gain scores (outcome minus baseline) were calculated and expressed as a percentage improvement from the baseline of MEP parameters and motor assessments.

Results

This study addressed two questions, which were, firstly, whether a differential effect exists between the two experimental groups and, secondly, whether the motor improvement that occurs following the current coupling protocols parallels the electrophysiological change. The results indicated that Group A showed greater motor improvement than Group B or sham group C for all motor scores. The close relationship between FMA improvement and motor map area change was found only for Group A, but not for Group B and Group C.

Treatment-Related Changes in Motor Performance

Mean group data for the motor assessments of the affected upper extremity are shown in Table 2. In summary, no significant differ-

ence was observed at the baseline between the three groups in their MRC, FMA, or WMFT scores for the affected upper extremity.

For Group A, all assessments, namely MRC (muscle strength), FMA, and WMFT showed significant improvements over the sham group at post 1 ($P = 0.001$, $F_{2,45} = 12.28$; $P = 0.004$, $F_{2,45} = 9.11$; $P = 0.016$, $F_{2,45} = 7.82$, respectively), at post 2 ($P = 0.002$, $F_{2,45} = 9.32$; $P = 0.003$, $F_{2,45} = 7.76$; $P = 0.014$, $F_{2,45} = 8.43$, respectively), and at post 3 ($P = 0.012$, $F_{2,45} = 6.75$; $P = 0.001$, $F_{2,45} = 9.81$; $P < 0.001$, $F_{2,45} = 9.9$, respectively) after rTMS intervention.

For Group B, only WMFT and proximal MRC scores showed significant improvements over the sham group at post 1 ($P = 0.027$, $F_{2,45} = 7.82$; $P = 0.018$, $F_{2,45} = 7.45$, respectively), at post 2 ($P = 0.011$, $F_{2,45} = 8.43$; $P = 0.003$, $F_{2,45} = 9.71$, respectively), and at post 3 ($P = 0.043$, $F_{2,45} = 9.9$; $P < 0.001$, $F_{2,45} = 12.63$, respectively). No significant changes in the distal MRC and FMA scores were detected for Group B.

There were also significant changes between post 1 and post 2 for the FMA, WMFT, and proximal MRC scores in Group A, indicating that there was further behavioral gain after a second course of conditioning (iTBS). A less profound result was found for Group B, whereby only the WMFT and distal MRC scores displayed a statistical significant improvement after the second course of conditioning (1 Hz rTMS).

Table 3 depicted the motor performance comparisons between the two experimental groups using *post hoc* analysis. Group A manifested greater improvements in FMA, WMFT, and distal MRC scores ($P = 0.017$ – 0.001) than Group B at post 1, and in FMA and WMFT scores at post 2 ($P = 0.015$, $F_{2,45} = 8.76$, and $P = 0.008$, $F_{2,45} = 8.43$, respectively).

Overall, Group A protocol produced a 50% behavioral gain in terms of FMA and WMFT scores at post 2 as well as a 60–70% improvement at post 3 (Figure 3A,B). In contrast, Group B gave a 20–30% improvement, whereas the sham-controlled Group C showed no significant improvement even for the best outcome, which was at 3-month follow-up.

Treatment-Related Changes in Cortical Excitability

The mean group data for cortical excitability measurements are shown in Table 4. No significant difference was observed between the three groups in their MEP parameters and motor map areas at baseline.

Both the experimental groups showed significant changes in corticomotor excitability compared with the sham groups. For Group A, the treatment resulted in a facilitation of ipsilesional excitability that was reflected in an AH map area increment (post 1: $P = 0.008$, $F_{2,22} = 9.0$; post 2: $P = 0.012$, $F_{2,22} = 6.82$) and in a suppression of contralesional excitability as demonstrated in an UH map area decrement (post 1: $P = 0.001$, $F_{2,45} = 11.2$; post 2: $P = 0.001$, $F_{2,45} = 10.21$). Significant influences on the bihemispheric map area, on the contralesional rMT, on MEP amplitude, and on MEP latency were found, and these were maintained up to the 3-month follow-up ($P = 0.0001$ – 0.0016 , Table 4).

For Group B, a significant suppression of contralesional motor excitability was reflected in a UH map area decrement throughout

Table 2 Mean group data ($\pm$ SD) of motor function assessments in affected upper extremity at baseline and after rTMS interventions

	Group A			Group B			Group C					
	Baseline	Post 1	Post 2	Post 3	Baseline	Post 1	Post 2	Post 3	Baseline	Post 1	Post 2	Post 3
MRC (Proximal)	2.6 ± 1.5	3.2 ± 1.4*	3.4 ± 1.3** ^a	3.9 ± 1.0**	2.6 ± 1.3	3.1 ± 1.6*	3.5 ± 1.7*	3.8 ± 1.5**	2.3 ± 1.6	2.4 ± 1.6	2.5 ± 1.6	2.36 ± 1.5
MRC (Distal)	2.3 ± 1.6	3.1 ± 1.7**	3.3 ± 1.5**	3.4 ± 1.4*	2.4 ± 1.3	2.6 ± 1.6	3.2 ± 1.6** ^a	3.7 ± 1.3**	2.2 ± 1.5	2.3 ± 1.5	2.6 ± 1.6	2.1 ± 1.5
FMA	26.2 ± 21.6	29.6 ± 22.5*	33.6 ± 23.1** ^a	36.6 ± 24.0***	28.4 ± 24.1	30.4 ± 23.6	33.7 ± 23.6	34.7 ± 28.3	26.2 ± 19.0	32.3 ± 24.0	27.2 ± 19.6	27.4 ± 19.3
WMFT	30.4 ± 14.5	32.9 ± 15.0**	38.0 ± 16.1*** ^a	40.3 ± 29.1***	30.9 ± 15.7	32.5 ± 16.7*	34.7 ± 14.8** ^a	36.5 ± 23.5*	31.4 ± 13.9	31.3 ± 17.0	31.6 ± 14.8	31.4 ± 20

MRC, Medical Research Council Scale; FMA, Fugt-Meyer Assessment; WMFT, Wolf motor function test; rTMS, repetitive transcranial magnetic stimulation. Significance levels: **P* < 0.05; ***P* < 0.01; ****P* < 0.001, in comparison with baseline level; ^aSignificance between post 1 and post 2 results.

MRC, Medical Research Council Scale; FMA, Fugl-Meyer Assessment; WMFT, Wolf motor function test; rTMS, repetitive transcranial magnetic stimulation. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, in comparison with baseline level; ^aSignificance between post 1 and post 2 results.

Table 3 Comparison of improvement in motor assessments for Group A and Group B at Post 1 and Post 2 visits

Improvement	Group A		Group B		Group A versus Group B <i>P</i> -value	
	Post 1-baseline	Post 2-baseline	Post 1-baseline	Post 2-baseline	Post 1-baseline	Post 2-baseline
FMA	3.4 + 3.0	8.4 + 8.7	1.6 + 3.2	3.8 + 5.7	$P = 0.02^*$, $F_{2,45} = 6.23$	$P = 0.015^*$, $F_{2,45} = 7.76$
WMFT, score	2.4 + 2.5	8.3 + 9.5	1.5 + 2.9	2.7 + 4.7	$P < 0.001^*$, $F_{2,45} = 10.84$	$P = 0.008^*$, $F_{2,45} = 8.43$
MRC, distal	0.7 + 0.5	1.1 + 0.7	0.2 + 0.1	0.9 + 0.4	$P = 0.017^*$, $F_{2,45} = 7.47$	$P = 0.51$, $F_{2,45} = 1.95$

MRC, Medical Research Council Scale; FMA, Fugl-Meyer Assessment; WMFT, Wolf motor function test. Significance levels from *Post hoc* analyses: * $P < 0.05$.

follow-up (post 1, 2, 3: $P < 0.001$) and as a decrement in UH MEP amplitude (post 2: $P = 0.005$, $F_{2,45} = 8.3$; post 3: $P = 0.006$, $F_{2,45} = 9.2$). Ipsilesional cortical facilitation was also found and was reflected in the results as AH amplitude augmentation (post 1: $P = 0.018$, $F_{2,22} = 6.2$; post 2: $P = 0.01$, $F_{2,22} = 5.81$).

The *post hoc* analyses revealed that there was no significant difference between Group A and Group B in terms of MEP parameters and cortical excitability. These findings thus indicate that both the real rTMS protocols showed a profound ability to suppress contralesional corticomotor activity (40% depression) and that there is no significant difference between Groups A and B (Figure 3C). However, in contrast to the above, Group C demonstrated increasing activity with time in contralesional motor cortex.

Correlation Analyses

In the real rTMS groups, the motor improvement was found to correlate well with some MEP parameters and excitability measures. Improvement of FMA correlated positively with the increased rMT in the UH ($P = 0.001$, $r = 0.59$) at post 2. Similar relationship was also detected between MRC and UH rMT ($P = 0.021$, $r = 0.40$) and between WMFT and UH rMT ($P < 0.001$, $r = 0.66$) at post 2. Decrement in UH motor map area was paralleled with the increment in FMA ($P = 0.035$, $r = -0.34$) at post 2 analysis. In contrast, correlation analyses carried out in Group B and the sham group revealed no significant correlation ($P > 0.2$). Figure 4 illustrated the relationship between changes of FMA related to UH map area among each group. Better FMA outcomes were associated with smaller map area, with closer relation for Group A ($P = 0.035$, $r = -0.34$). The steeper regression line noted for Group A indicates that for a specific decrease in UH map area, the subjects in Group A reached greater improvement as measured by FMA than was the case for Group B and for the sham group.

Discussion

In this sham-controlled rTMS trial involving hemiplegic stroke patients, we found that a 20-session coupled rTMS protocol was safe and demonstrated significantly greater improvement in motor recovery compared with a control group. Priming the UH firstly with inhibitory rTMS and then facilitatory AH conditioning was found to be optimal for enhancing hand dexterity, and this effect persisted for at least 3 months until the last follow-up testing. The existence of significant behavior-electrophysiological correlations

substantiated our hypothesis that this novel approach leads to the amelioration of interhemispheric imbalance and the consolidation of LTD and LTP-like neuroplasticity.

The rationale for targeting the ipsilesional motor cortex with facilitatory rTMS and the contralesional motor cortex with inhibitory rTMS was based on functional MRI and TMS studies showing that recruitment of the ipsilesional M1, supplementary motor area, and cerebellum correlated with full motor recovery [4], whereas contralesional recruitment and the induced MEP from contralesional TMS correlated with poor motor recovery [28,29]. Hyperactivity in the contralesional M1 tends to be disinhibited from the affected motor cortex, and this reciprocally exerts transcallosal influence on the lesioned M1 as interhemispheric competition [1,4]. The enhanced transcallosal inhibition from the intact motor cortex will exaggerate motor recovery from stroke. Applying inhibitory rTMS over contralesional M1 has been shown to harmonize interhemispheric imbalance that is associated with hand function restoration [9,12]. Within the 1–3 months after the occurrence of a stroke, amelioration of the maladaptive neuroplasticity in the intact brain has been found to occur parallel with functional improvement [30]. This timeframe is compatible with our patient recruitment. However, for more chronic stroke patients who have developed mature motor patterns [31], the other strategy of facilitating contralesional premotor cortex is thought to be beneficial for motor recovery following injury to M1. The other rationale for our conducting multiple rTMS treatment sessions is that evidence suggests that the facilitation of motor improvement parallels the number of treatment sessions, which would consolidate long-lasting neuroplastic changes in the human brain [32]. Our study demonstrated that the extended conditioning timeframe with 20-daily rTMS (4-week protocol) in ischemic stroke patients results in robust hand function improvement during the early stage of motor recovery.

Moreover, the coupling approach produced greater improvement in motor task measures, along with cortical excitability change, after a second course of rTMS applied over the opposite M1. As shown in Tables 2 and 4 and Figure 2, we observed that the subsequent protocol undertaken had a significantly additive effect on motor function, as reflected in the FMA and WMFT results and in distal and proximal muscle strength, in comparison with that observed after the first course of conditioning. At the final follow-up, this effect had been maintained. This was paralleled by a continuous decrease in UH cortical excitability over time, including in UH MEP amplitude and motor map area. The additive effect after the second course of conditioning has been

Figure 2 Experimental design. The daily repetitive transcranial magnetic stimulation (rTMS) session comprised either 10 min of 1 Hz rTMS or 190 second of intermittent theta burst stimulation (iTBS) protocol. Group A received 10 sessions of 1 Hz rTMS initially then followed with 10 sessions of iTBS protocol, over a period of 4 weeks. Group B underwent the same paradigms but in reverse; and Group C received sham stimulation identical to Group A. Each subject received 1-h physiotherapy immediately following rTMS. Evaluations were arranged prior to the intervention, after 10 sessions of rTMS (post 1), after 20 sessions (post 2), and 3 months after the intervention (post 3).

demonstrated in our previous study with a designation of a sham rTMS following or preceding a real course of rTMS program [18]. The significant behavior improvement in our subjects after an adjuvant session of rTMS accorded with previous research on healthy patients [33], in which reaction time, finger tapping, and pinch force performance continued to benefit from a second single session of rTMS applied to the opposite M1. Previous researchers have likewise demonstrated that bihemispheric cathodal and anodal stimulation of healthy individuals produces greater behavioral effects than unihemispheric transcranial direct-current stimulation (tDCS) [34,35]. The extent of the improvement in our study was larger (50% after intervention and 60–70% at 3-month follow-up, Figure 3) compared with the previous reports, which ranged from 10% to 30% following using unihemispheric conditioning [12,17,36]. Meanwhile, we provided strong electrophysiological evidence of a significant correlation between UH rMT, MEP latency, cortical map area, and motor recovery, reflecting a shift of activation toward the stroke-AH and matching more closely the greater neuromodulatory effect of a bihemispheric approach.

The significant correlation found in this study between the electrophysiological evidence and clinical improvement indicates that the harmonization of abnormal interhemispheric competition, consolidated by a combined modulation, plays an important role in the functional recovery of stroke patients. Decreasing membrane excitability of corticospinal neurons, as reflected in the cumulative increase of rMT in the UH, has already been associated with motor recovery [37,38]. Similarly, previous researchers have pointed out that enlargement of the cortical output centers [39] and the change in MEP latency [40] have been paralleled by better outcomes in motor performance. These earlier observations are comparable to our own findings in motor map area and MEP latency in relation to FMA improvement under compound rTMS

protocol. This significant behavior-electrophysiological correlation strengthens our contention that bilateral modulation leads to a re-balancing of motor excitability in the two hemispheres.

We found that the patients who firstly underwent priming of the UH with 1 Hz rTMS (Group A) experienced greater motor performance improvement (Table 4, Figure 3) than the patients who firstly underwent iTBS priming in the AH (Group B). It is plausible that the efficacy of LTD or LTP depends on the integrity of the corticospinal pathway, plus its nearby area or an area around the reorganized motor pathway, for there to be an effective intracortical connection [41,42]. Previous researchers have found that the induction of movement of the paretic hand could be achieved only by rTMS conditioning on an intact corticospinal pathway [41–43]. Therefore, for Group A, it was possible to achieve a superior motor outcome because it was possible to more effectively prime a hemisphere that was intact than, as with Group B, an affected motor cortex with damaged integrity. It is notable that the FMA and WMFT assessments differed from the MRC assessment, in revealing significant improvement in Group A as compared with Group B. The discrepancy between these tests could derive from their differing specialization as to the components they measured. WMFT incorporates measures of task accomplishment and hand dexterity and is therefore thought to more accurately distinguish between motor recovery and compensation [2,34]. FMA involves hand coordination and dysmetria measurement, which is a more integral motor function assessment than merely testing strength. These results may be attributable to the projection of different movement components of various activated motor areas in the brain, as concluded by a number of previous fMRI studies. These earlier studies revealed that increasing complexity of movement led to increased activity in the bilateral superior parietal areas and contralateral inferior parietal areas [44], a component of the putative human mirror neuron system [45]. In this way, modulating

Table 4 Mean group data ($\pm$ SD) of bilateral corticomotor excitability at baseline and after rTMS

	Group A			Group B			Group C		
	Baseline	Post 1	Post 2	Post 3	Baseline	Post 1	Post 2	Post 3	Post 3
UH									
rMT (% maximal)	71.9 $\pm$ 14.4	73.8 $\pm$ 13.5	73.7 $\pm$ 12.1	74.4 $\pm$ 11.4*	70.9 $\pm$ 9.2	74.0 $\pm$ 12.3	74.1 $\pm$ 12.6	76.0 $\pm$ 13.0*	72.4 $\pm$ 12.5
MEP amplitude, μ V	5.0 $\pm$ 4.3	4.4 $\pm$ 3.4	4.2 $\pm$ 2.7*	3.7 $\pm$ 2.0*	3.4 $\pm$ 1.8	3.5 $\pm$ 2.4	2.5 $\pm$ 1.8**	2.1 $\pm$ 1.4**	3.1 $\pm$ 2.4
MEP latency, millisecond	22.9 $\pm$ 1.5	23.1 $\pm$ 2.5	23.4 $\pm$ 2.0	24.1 $\pm$ 2.0***	23.2 $\pm$ 2.6	23.2 $\pm$ 2.1	23.5 $\pm$ 1.8	23.8 $\pm$ 2.0	21.4 $\pm$ 2.4
Motor map area	12.5 $\pm$ 3.0	10.3 $\pm$ 3.2***	9.3 $\pm$ 2.1**	8.6 $\pm$ 2.1***	15.3 $\pm$ 3.2	13.0 $\pm$ 3.3***	11.2 $\pm$ 3.5***	9.6 $\pm$ 2.5***	14.6 $\pm$ 3.8
AH									
rMT (% maximal)	85.1 $\pm$ 13	80.4 $\pm$ 14.6	78.4 $\pm$ 14*	79.5 $\pm$ 15.0	77.6 $\pm$ 14.9	74.6 $\pm$ 16.5	74.8 $\pm$ 14.3	75.5 $\pm$ 12.3	86.0 $\pm$ 17.9
MEP amplitude	1.1 $\pm$ 1.1	2.2 $\pm$ 3.2	1.3 $\pm$ 1.5	1.82 $\pm$ 2.5	0.9 $\pm$ 1.4	2.3 $\pm$ 2.7*	2.1 $\pm$ 2.1*	1.8 $\pm$ 2.0	0.7 $\pm$ 1.4
MEP latency	29.6 $\pm$ 7.4	26.8 $\pm$ 5.7	26.6 $\pm$ 4.8	26.9 $\pm$ 3.9	25.5 $\pm$ 4.9	26.1 $\pm$ 1.7	24.5 $\pm$ 1.9	24.7 $\pm$ 0.9	27.1 $\pm$ 2.8
Motor map area	8.7 $\pm$ 2.0	11.5 $\pm$ 3.9**	11.8 $\pm$ 3.8**	11.6 $\pm$ 2.7**	8.6 $\pm$ 2.0	10.3 $\pm$ 2.2*	10.3 $\pm$ 2.8	11.3 $\pm$ 3.3	6.0 $\pm$ 2.3

UH, unaffected hemisphere; AH, affected hemisphere; rMT, resting motor threshold; MEP, motor evoked potential; rTMS, repetitive transcranial magnetic stimulation. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, in comparison with baseline level; ^aSignificance between post 1 and post 2 results.

Figure 3 The effects of repetitive transcranial magnetic stimulation (rTMS). (A) Change in Fugl-Meyer Assessment (FMA) along the visits. (B) Change in Wolf Motor Function test-Functional Ability Scale (WMFT) along the visits. (C) Change of contralesional motor map area for each visit. Significance levels: ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$, in comparison with baseline level over sham Group C; ^dSignificance between Post 1 and Post 2 results; ^eSignificance between Group A (1 Hz rTMS-iTBS) and Group B (iTBS-1 Hz rTMS). Error bar = SD. iTBS, intermittent theta burst stimulation.

Figure 4 Linear relationships of Fugl-Meyer Assessment (FMA) and UH motor map area. A better FMA outcome is associated with a smaller volume of excitable motor area ($P = 0.035$, $r = -0.34$) with closer relationship in Group A. UH, unaffected hemisphere.

the UH motor system prior to modulating the AH would facilitate motor planning and hand dexterity, resulting in bilateral interaction in accordance with the mirror neuron system. A superior aftereffect as registered in the Group A was achieved through reducing abnormal interhemispheric inhibition and promoting a shift of corticomotor laterality back to the affected motor cortex.

Although our patients demonstrated good tolerance for the rTMS sessions, with no adverse side-effects observed during the

conditioning and assessments, further study including electroencephalogram recording to detect ictal activity, epileptiform discharges, or notable differences before, during or after rTMS will assure the safety of this compound protocol. Stricter stratification of stroke subtypes during patient recruitment would improve disease homogeneity and ensure the accuracy of the findings. The location of the lesion (cortical or subcortical stroke) and the extent of the lesion could also yield a variety of recovery mechanisms and should be evaluated in separate groups.

Overall, with patients' enhanced motor recovery in strength and dexterity, sustained at least 3 months beyond the intervention and accompanied by neurophysiological changes in corticomotor excitability, this study demonstrated an optimal rTMS strategy for promoting motor recovery in chronic stroke patients. Further investigation with functional imaging evidence would more fully clarify the role of different pathologies or lesion locations in underlying neuroplasticity processes.

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Conflict of Interest

The authors declare no conflict of interest.

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