Article

Low-frequency rTMS promotes use-dependent motor plasticity in chronic stroke: A randomized trial

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Data Supplement

Table e-1

		Baseline	Pre	Post	fu1	fu2	fu3	fu4	comparison
JHFT: execution time. Mean raw values in sec ± SD (change from Baseline ± SD)	$rTMS_S$-PT	108 ± 95	107 ± 95	102 ± 93	105 ± 96	107 ± 97	107 ± 96	108 ± 96	all p>0.4 (all p>0.2)
		-	(1% ± 3)	(5% ± 8)	(2% ± 6)	(1% ± 6)	(-1% ± 3)	(-2% ± 2)	
	PT- $rTMS_S$	79 ± 79	80 ± 79	74 ± 75	75 ± 78	76 ± 77	81 ± 84	81 ± 83	
		-	(-1% ± 2)	(2% ± 11)	(1% ± 7)	(0% ± 8)	(-1% ± 6)	(-3% ± 5)	
NHPT: execution time. Mean raw values in sec ± SD (change from Baseline ± SD)	$rTMS_S$-PT	196 ± 158	196 ± 158	187 ± 157	189 ± 162	192 ± 161	194 ± 159	197 ± 157	all p>0.7 (all p>0.2)
		-	(-1% ± 1)	(7% ± 4)	(7% ± 5)	(4% ± 6)	(1% ± 4)	(-1% ± 3)	
	PT- $rTMS_S$	151 ± 141	153 ± 145	142 ± 135	146 ± 146	147 ± 145	148 ± 142	149 ± 141	
		-	(0% ± 2)	(8% ± 2)	(8% ± 8)	(6% ± 6)	(4% ± 3)	(2% ± 4)	
B&B: number of lifted cubes. Mean raw values ± SD (change from Baseline ± SD)	$rTMS_S$-PT	16 ± 14	16 ± 13	17 ± 15	17 ± 15	16 ± 15	16 ± 15	16 ± 14	all p>0.7 (all p>0.1)
		-	(0% ± 1)	(12% ± 11)	(13% ± 15)	(1% ± 7)	(2% ± 7)	(0% ± 5)	
	PT- $rTMS_S$	23 ± 13	22 ± 13	24 ± 13	24 ± 14	24 ± 14	24 ± 14	23 ± 14	
		-	(-2% ± 7)	(8% ± 7)	(7% ± 8)	(7% ± 5)	(2% ± 9)	(-2% ± 4)	
Key-grip: max contraction. Mean raw values in Kg ± SD (change from Baseline ± SD)	$rTMS_S$-PT	4.1 ± 1.1	4.1 ± 1.1	4.4 ± 1.0	4.2 ± 1.1	4.2 ± 1.2	4.1 ± 1.2	4.0 ± 1.2	all p>0.4 (all p>0.1)
		-	(0% ± 2)	(6% ± 6)	(5% ± 7)	(1% ± 3)	(-1% ± 3)	(-1% ± 3)	
	PT- $rTMS_S$	5.1 ± 2.1	5.1 ± 2.1	5.5 ± 2.2	5.5 ± 2.3	5.2 ± 2.1	5.1 ± 2.2	5.1 ± 2.2	
		-	(0% ± 2)	(10% ± 5)	(10% ± 7)	(6% ± 5)	(0% ± 6)	(0% ± 2)	
Tip-pinch: max contraction. Mean raw values in Kg ± SD (change from Baseline ± SD)	$rTMS_S$-PT	3.1 ± 2.0	3.1 ± 2.0	3.3 ± 2.2	3.3 ± 2.2	3.2 ± 2.1	3.2 ± 2.1	3.2 ± 2.2	all p>0.3 (all p>0.1)
		-	(0% ± 2)	(6% ± 6)	(5% ± 7)	(1% ± 3)	(-1% ± 3)	(-1% ± 3)	
	PT- $rTMS_S$	3.6 ± 1.6	3.6 ± 1.7	4.0 ± 1.9	4.0 ± 1.9	3.8 ± 1.7	3.6 ± 1.7	3.6 ± 1.7	
		-	(0% ± 2)	(10% ± 5)	(10% ± 7)	(6% ± 5)	(0% ± 6)	(0% ± 2)	
Power-grip: **max contraction.** Mean raw values in Kg ± SD (change from Baseline ± SD)	$rTMS_S$-PT	14.5 ± 10.1	14.5 ± 10.0	14.6 ± 10.1	14.7 ± 10.1	14.3 ± 10.0	14.4 ± 10.0	14.5 ± 10.1	all p>0.2 (all p>0.1)
		-	(0% ± 2)	(2% ± 4)	(2% ± 4)	(-1% ± 6)	(0% ± 5)	(0% ± 3)	
	PT- $rTMS_S$	12.7 ± 8.7	12.8 ± 8.7	13.0 ± 8.7	13.1 ± 8.8	13.0 ± 8.7	13.0 ± 8.8	12.9 ± 9.1	
		-	(0% ± 3)	(3% ± 6)	(3% ± 6)	(3% ± 5)	(2% ± 5)	(-1% ± 5)	
rMT: max stimulator ouput. Mean raw values ± SD (change from Baseline ± SD)	$rTMS_S$-PT	65 ± 28	65 ± 28	65 ± 28	65 ± 27	65 ± 28	67 ± 27	67 ± 27	all p>0.6 (all p>0.2)
		-	(0% ± 2)	(2% ± 4)	(2% ± 4)	(-1% ± 6)	(0% ± 5)	(0% ± 3)	
	PT- $rTMS_S$	58 ± 12	59 ± 13	59 ± 13	59 ± 13	59 ± 12	59 ± 12	59 ± 12	
		-	(0% ± 3)	(3% ± 6)	(3% ± 6)	(3% ± 5)	(2% ± 5)	(-1% ± 5)	
iSP: duration. Mean raw values in ms ± SD (change from Baseline ± SD)	$rTMS_S$-PT	-	70 ± 14	72 ± 15	-	-	-	-	all p>0.7 (all p>0.3)
			-	(3% ± 6)					
	PT- $rTMS_S$	-	69 ± 16	70 ± 16	-	-	-	-	
			-	(3% ± 6)					

Table e-1 Performance and motor excitability in the two groups receiving sham stimulation ($rTMS_S$). For $rTMS_S$, intervention order was not expected to influence outcomes. Mann-Whythney U tests on raw (and percentage change from Baseline) values were used to test this assumption: the $rTMS_S$-PT and PT-$rTMS_S$ groups were statistically comparable at all time points and for all the measures. Thus, the two groups were merged into a single control group

($rTMS_S$, N = 14). Mann-Whitney U was also used to compare $rTMS_S$-PT and PT-$rTMS_S$ groups for stroke duration (mean value ± SD: 39.1 m ± 32.8 vs 29.1 m ± 25.6; p=0.8), age (61.3 y ± 8.7 vs 66.7 y ± 15.0; p=0.5) and education (8.1 y ± 3.6 vs 8.1 y ± 3.6; p>0.9). Freeman-Halton extension of the Fisher exact probability test was used to compare $rTMS_S$-PT and PT-$rTMS_S$ groups for type of ictus (ischemic/hemorragic: 5/2 vs 4/3; p>0.9), lesion location (subcortical /cortical/cortico-subcortical: 6/2/0 vs 8/0/0; p>0.5), affected hand laterality (left/right: 4/3 vs 3/4; p>0.9) and sex distribution (females/males: 2/5 vs 4/3; p>0.6).

Table e-2

Affected hand	$rTMS_R$-PT		PT-$rTMS_R$		$rTMS_S$		Groups comparison at Baseline
	Baseline	Pre	Baseline	Pre	Baseline	Pre	
JHFT: execution time. Mean raw values in sec ± SD	101 ± 58	101 ± 58	102 ± 51	102 ± 50	93 ± 85	94 ± 85	all p>0.4
NHPT: execution time. Mean raw values in sec ± SD	197 ± 145	193 ± 139	169 ± 93	169 ± 93	156 ± 145	157 ± 147	all p>0.3
B&B: number of lifted cubes. Mean raw values ± SD	19 ± 12	19 ± 11	19 ± 11	19 ± 10	19 ± 13	19 ± 13	all p>0.7
Key-grip: max contraction. Mean raw values in Kg ± SD	4.8 ± 1.4	4.8 ± 1.5	5.5 ± 1.5	5.5 ± 1.6	4.6 ± 1.7	4.6 ± 1.7	all p>0.3
Tip-pinch: max contraction. Mean raw values in Kg ± SD	3.9 ± 1.7	3.9 ± 1.7	3.6 ± 1.0	3.6 ± 1.0	3.4 ± 1.8	3.4 ± 1.8	all p>0.4
Power-grip: **max contraction.** Mean raw values in Kg ± SD	12.4 ± 4.1	12.4 ± 4.3	13.7 ± 5.1	13.7 ± 5.2	13.6 ± 9.1	13.6 ± 9.1	all p>0.4
rMT: max stimulator ouput. Mean raw values ± SD	61 ± 12	62 ± 12	60 ± 19	61 ± 19	62 ± 21	62 ± 21	all p>0.6
iSP: duration. Mean raw values in ms ± SD	-	67 ± 21	-	69 ± 24	-	69 ± 14	all p>0.6

Unaffected hand	$rTMS_R$-PT		PT-$rTMS_R$		$rTMS_S$		Groups comparison at Baseline
	Baseline	Pre	Baseline	Pre	Baseline	Pre	
JHFT: execution time. Mean raw values in sec ± SD	11 ± 3	11 ± 3	10 ± 2	10 ± 2	11 ± 3	10 ± 2	all p>0.6
NHPT: execution time. Mean raw values in sec ± SD	25 ± 5	25 ± 5	23 ± 4	23 ± 4	25 ± 5	22 ± 4	all p>0.2
B&B: number of lifted cubes. Mean raw values ± SD	49 ± 7	50 ± 7	50 ± 13	50 ± 13	50 ± 7	50 ± 13	all p>0.5
Key-grip: max contraction. Mean raw values in Kg ± SD	8.3 ± 1.8	8.2 ± 1.9	8.1 ± 2.0	8.1 ± 2.0	8.2 ± 1.9	8.2 ± 2.0	all p>0.5
Tip-pinch: max contraction. Mean raw values in Kg ± SD	6.9 ± 1.8	6.9 ± 1.7	6.5 ± 1.7	6.5 ± 1.7	6.9 ± 1.7	6.5 ± 1.6	all p>0.8
Power-grip: **max contraction.** Mean raw values in Kg ± SD	29.2 ± 10.5	29.1 ± 10.6	30.3 ± 12.6	30.3 ± 12.6	29.1 ± 10.6	30.4 ± 12.6	all p>0.7
rMT: max stimulator ouput. Mean raw values ± SD	45 ± 10	44 ± 10	45 ± 10	45 ± 10	44 ± 10	45 ± 10	all p>0.5

Table e-2 Behavioral performance and corticospinal excitability at Baseline and Pre. Mean raw values ± SD. Upper and lower table show data from affected and unaffected hand. Mann-Whythney U tests confirmed that the three groups were entirely comparable at Baseline in all the measures (p-level reported in the table). Additional analyses were carried out by comparing performance and corticospinal excitability at Baseline and at Pre. Friedman ANOVA revealed that performance and corticospinal excitability was greater for the healthy than for the affected side (all $p<0.0001$). No difference between Baseline and Pre evaluation was found for both sides (all $p>0.2$).

Table e-3

Affected hand		Pre	Post	fu1	fu2	fu3	fu4	NNT - ARR at Post	NNT - ARR at fu4
JHFT: execution time. Change from Baseline ± SD	$rTMS_S$-PT	-1% ± 3	40% ± 10*	39 % ± 10*	40% ± 9*	41% ± 6*	36% ± 13*	1.08 - 0.93	1.14 - 0.88
	PT $rTMS_S$	0% ± 2	32% ± 10*	25 % ± 13*	21% ± 13*	21% ± 14*	17% ± 8*	1.24 - 0.80	4.00 - 0.25
	$rTMS_S$	0% ± 1	11% ± 5*	11 % ± 8*	8% ± 5	4% ± 5	2% ± 4		
NHPT: execution time. Change from Baseline ± SD	$rTMS_S$-PT	0% ± 3	38% ± 15*	37 % ± 14*	41% ± 14*	42% ± 17*	38% ± 20*	1.00 - 1.00	1.33 - 0.75
	PT $rTMS_S$	0% ± 3	26 % ± 15*	24 % ± 17*	16% ± 10*	17% ± 7*	13% ± 8*	1.60 - 0.65	4.00 - 0.25
	$rTMS_S$	0% ± 2	8% ± 4*	8 % ± 6*	5% ± 5	2% ± 3	0% ± 4		
B&B: number of lifted cubes Mean raw values ± SD	$rTMS_S$-PT	2% ± 4	58% ± 33*	54 % ± 30*	53% ± 28*	52% ± 20*	54% ± 35*	1.37 - 0.73	1.14 - 0.88
	PT $rTMS_S$	-1% ± 3	44% ± 39*	44 % ± 40*	24% ± 18*	20% ± 15*	17% ± 10*	2.80 - 0.36	4.00 - 0.25
	$rTMS_S$	-1% ± 1	10% ± 2*	10 % ± 3*	4% ± 2	2% ± 2	-1% ± 1		
Key-grip: max contraction. Change from Baseline ± SD	$rTMS_S$-PT	0% ± 1	33% ± 12*	34 % ± 17*	33% ± 17*	35% ± 16*	33% ± 18*	1.14 - 0.88	1.14 - 0.88
	PT $rTMS_S$	-1% ± 4	23% ± 12*	17 % ± 10*	16% ± 11*	14% ± 6*	13% ± 5*	1.60 - 0.63	8.00 - 0.13
	$rTMS_S$	-1% ± 2	8% ± 4*	5 % ± 5*	1% ± 5	-1% ± 4	-2% ± 5		
Tip-pinch: max contraction. Change from Baseline ± SD	$rTMS_S$-PT	0% ± 1	27% ± 20*	22 % ± 16*	21% ± 19*	17% ± 14*	13% ± 11*	2.00 - 0.50	4.00 - 0.25
	PT $rTMS_S$	0% ± 2	32% ± 27*	24 % ± 17*	19% ± 14*	20% ± 10*	14% ± 11*	1.60 - 0.63	2.67 - 0.38
	$rTMS_S$	0% ± 2	8% ± 5*	7 % ± 7	3% ± 5	0% ± 5	-1% ± 3		
Power-grip: **max contraction.** Change from Baseline ± SD	$rTMS_S$-PT	0% ± 3	22% ± 11*	15 % ± 7*	15% ± 10*	14% ± 10*	11% ± 11*	2.00 - 0.50	8.00 - 0.13
	PT $rTMS_S$	0% ± 2	20% ± 23*	16 % ± 18*	14% ± 15*	13% ± 14*	10% ± 11*	2.00 - 0.50	4.00 - 0.25
	$rTMS_S$	0% ± 2	2% ± 5	2 % ± 5	1% ± 6	1% ± 5	-1% ± 4		
rMT: max stimulator ouput. Change from Baseline ± SD	$rTMS_S$-PT	1% ± 3	-16% ± 7*	-18 % ± 7*	-18% ± 8*	-19% ±11*	-19% ±12*		
	PT $rTMS_S$	1% ± 2	-16% ± 9*	-12 % ± 8*	-8% ± 8*	-6% ± 4*	-6% ± 4*		
	$rTMS_S$	0% ± 2	0% ± 3	1 % ± 4	1% ± 2	2% ± 3	2% ± 4		
iSP: duration. Change from Pre ± SD	$rTMS_S$-PT	-	-38% ±12*	-	-	-	-		
	PT $rTMS_S$	-	-16% ±10*	-	-	-	-		
	$rTMS_S$	-	3% ± 11	-	-	-	-		

Table e-3 Changes in affected hand performance and corticospinal excitability over time. * = significant comparison with Pre ($p<0.01$). For behavioral tests, the Number Needed to Treat (NNT) and the Absolute Risk Reduction (ARR) were computed for outcome at Post and fu4 and reported in the last two columns. NNT and ARR were computed separately for the two $rTMS_R$-groups and were calculated with reference to the $rTMS_S$-group. An increase in performance greater than 20% of Baseline was considered as a positive outcome.

Figure e-1

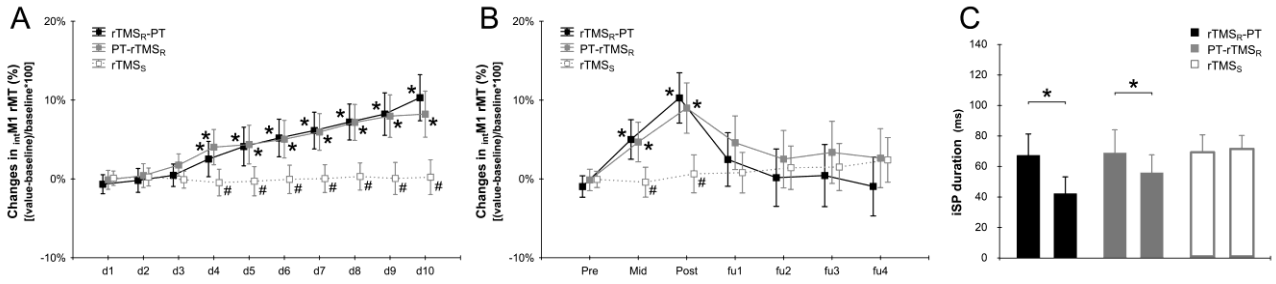

Figure e-1. Changes in motor excitability. (A) Changes in $_{int}$M1 rMT during treatment (% of change from the Baseline). A linear cumulative increase of $_{int}$M1 rMT (indexing a reduction of excitability of corticospinal motor neurons in the intact hemisphere) was detected in the two rTMS$_R$-groups. In both rTMS$_R$-groups the increase of rMT started to become significant after 3 days of treatment (on day4; ($p<0.005$). (B) Changes in $_{int}$M1 rMT over time (% of change from the Baseline). The two rTMS$_R$-groups showed an increase of $_{int}$M1 rMT at Mid and Post ($p<0.005$), however at fu1-4 their $_{int}$M1 rMT returned to pre-treatment levels ($p>0.16$). (C) Raw iSP duration at Pre and Post (in ms). In the two rTMS$_R$-groups, the duration of iSP at Post was lower than at Pre ($p<0.01$), indicating a decrease of transcallosal inhibition from the $_{int}$M1 to the $_{aff}$M1. Bars denote 95% confidence interval. Symbols indicate significant Bonferroni corrected comparisons: * = different with respect to Pre/d1; # = rTMS$_R$-groups different with respect to rTMS$_S$-group.

Figure e-2

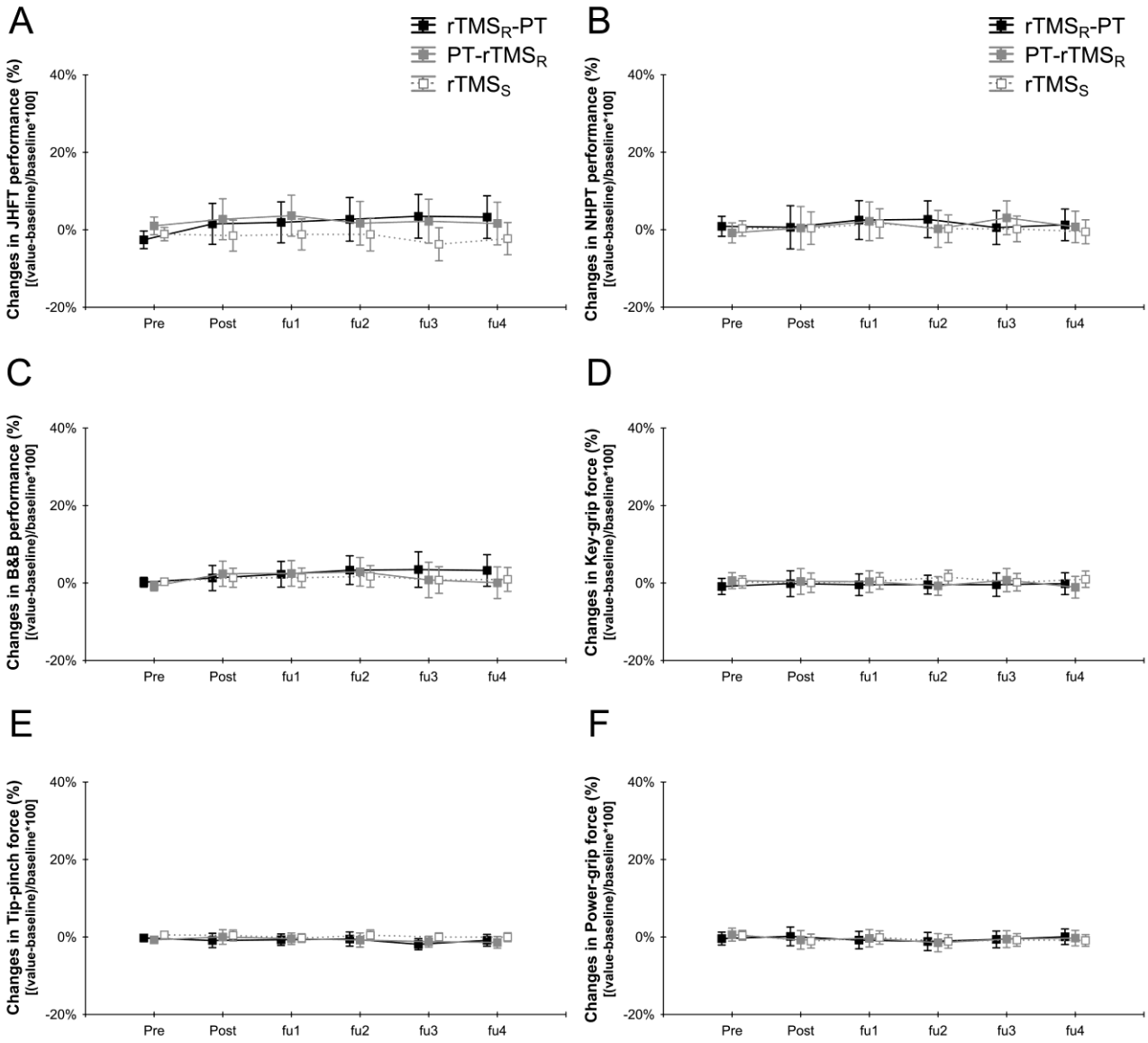

Figure e-2. Changes in performance for the unaffected hand. Friedman ANOVA conducted on each group and measure showed no significant change in dexterity and force over time (all $p>0.3$).

e-Methods

Behavioral assessment

Behavioral assessment was peformed at Baseline, Pre, Post and fu1-4 and included manual dexterity and force.

Manual dexterity was assessed by means of the Jebsen-Taylor Hand Function Test (JHFT), the Nine Hole Peg Test (NHPT) and the Box and Block test (B&B). The JHFT is a well-standardized functional assessment that consists of 7 subtests evaluating a broad range of everyday hand functions: copying a 24-letter sentence, turning cards over, picking up small objects and placing them in a can, simulated feeding (picking up beans with a spoon and placing them in a can), stacking checkers, and moving large light objects and large heavy objects from one location to another. The dependent measure is mean time to perform these tasks[e1]. The NHPT involves the subject placing nine dowels in nine holes. Patients are scored based on the amount of time it takes to place and remove all nine pegs[e2]. The B&B requires patients to transfer small cubes from a full box into an empty box by moving their arm across a barrier that is placed between the two boxes. The number of cubes transferred in 30 sec is recorded[e3].

Hand force (key-grip, tip-pinch and power-grip) was assessed by a pinch meter and a dynamometer. Pinch meter was used to measure finger force: patients held the end of the pinch gauge between the pad of the thumb and the lateral surface of the index (key-grip) or between the tip of the thumb and index finger (tip-pinch). For the power-grip, patients held the handle of a dynamometer with a whole hand grip. The average of three consecutive maximal contractions was considered as a measure of pinch and grip force[e4].

In the main analysis reported in the main text each behavioral test was expressed with respect to the Baseline evaluation [(value-baseline)/baseline*100]. For the JHFT, an average of the seven subscales was computed. JHFT and NHPT scores were inverted so that a reduction in execution time relative to Baseline (better performance) was expressed as a positive value, as for B&B and force measures.

Neurophysiological assessment

Neurophysiological assessment included rMT and iSP. EMG signals were acquired by means Biopac MP-150 (Biopac Corp, Goletta, CA.) electromyograph, band-pass filtered (30 Hz-1.0 kHz, sampled at 5 kHz), digitized and stored on a computer for off-line analysis. A 7 cm-diameter focal coil connected to a Magstim 200 stimulator (Magstim, Whitland, Dyfed, U.K.) was placed over the motor cortex (with the handle pointing backward at 45˚ from the midline) contralateral to the

recorded muscles. Motor-evoked potentials (MEPs) were induced in the in the first dorsal interosseous (FDI) muscles. In both hemispheres the optimum scalp position (OSP) was chosen so as to produce maximum amplitude MEPs in the FDI muscles. Measures of excitability (rMT and iSP) were collected by stimulating the OSP with single-pulse TMS.

The rMT in $_{aff}$M1 and $_{int}$M1 was measured according to standard procedures[e5]. Evaluations were peformed at Baseline, Pre, Mid, Post and fu1-4. The rMT was defined as the minimal intensity of the stimulator output that produces MEPs with amplitudes of at least 50 µV with 50% probability (using about 20 pulses). Values of rMT were expressed with respect from Baseline.

Measurements of rMT in the $_{int}$M1were also performed on Day 1-10 using a 7cm focal-coil connected to a Magstim Rapid2 stimulator (because of the daily measurements to adjust rTMS intensity; see figure e-1, panel A).

The iSP was measured to assess transcallosal inhibition from $_{int}$M1to $_{aff}$M1[e6,e7]. Evaluations were performed at Pre and Post only. We asked participants to perform a maximal contraction of the FDI muscle in the affected hand while their $_{int}$M1was stimulated at 150% rMT. Participants were asked to perform the contraction with the requirement of maintaining the force constant after the magnetic stimulus until ordered to relax[e8]. Pulses were given 1-3 s after the target force was attained. A total of 20 trials were recorded. The duration of the iSP was measured as the period of relative EMG suppression after the TMS pulse, i.e. when the EMG activity dropped below the background activity[e6,e9,e10]. The mean amplitude of the rectified EMG before the stimulus for 100 ms was defined as the background activity. The iSP duration was measured from where the EMG activity clearly fell below the background activity to where the EMG activity again reached the background activity[e6]. Evaluation of iSP was performed by raters blinded to the rTMS conditions.

Data from 3 patients (1, 1and 1 from PT-rTMS$_R$, rTMS$_S$-PT and PT-rTMS$_S$ group, respectively) are not available due to technical failure. Additional neurophysiological testing (contralateral silent period, input-output-curve) was carried out at Pre and Post, however, these data are not critical for the main hypotheses and will be reported in a separate pubblication.

e-References

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