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Neurofunctional changes after a single mirror therapy intervention in chronic ischemic stroke

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Abstract

Background: Mirror therapy (MT) is becoming an alternative rehabilitation strategy for various conditions, including stroke. Although recent studies suggest the positive benefit of MT in chronic stroke motor recovery, little is known about its neural mechanisms. **Purpose:** To identify functional brain changes induced by

a single MT intervention in ischemic stroke survivors, assessed by both Transcranial Magnetic Stimulation (TMS) and functional Magnetic Resonance Imaging (fMRI). **Materials and Methods:** TMS and fMRI were used to investigate fifteen stroke survivors immediately before and after a single thirty minutes MT session. **Results:** We found statistically significant increase in post-MT MEP amplitude (increased excitability) from the affected primary motor cortex (M1), when compared to pre-MT MEP. Post-MT fMRI maps were associated with a more organized and constrained pattern, with a more focal M1 activity within the affected hemisphere after MT, limited to the cortical area of hand representation. Furthermore, we find a change in the balance of M1 activity toward the affected hemisphere. In addition, significant correlation was found between decreased fMRI β -values and increased Motor Evoked Potential (MEP) amplitude post-MT, in the affected hemisphere. **Conclusion:** Our study suggests that a single MT intervention in stroke survivors is related to increased MEP of the affected limb, and a more constrained activity of the affected M1, as if activity had become more constrained and limited to the affected hemisphere.

Keywords: Mirror therapy, ischemic stroke, stroke rehabilitation, Transcranial magnetic stimulation, functional Magnetic Resonance Imaging.

Introduction

Ischemic stroke is frequently accompanied by motor functions deficits, which evolves with limited spontaneous recovery particularly at the chronic stage of stroke (6 months after the insult).^{1,2} During this stage, patients usually undergo motor rehabilitation programs³⁻⁶

A promising rehabilitation approach is the use of mirror therapy (MT), which was originally designed and used to help alleviating phantom limb pain.⁷ More recently, it has been tested as an adjuvant therapy in stroke motor rehabilitation.⁸⁻¹¹ During an MT session for stroke, patients place their hands in a mirror box, composed of two compartments separated by a vertical mirror (sup. fig. 1). The affected hand is positioned in one side of the box, behind the mirror, and the unaffected hand in the other side, so the reflection of the unaffected hand is seen in the place of the affected one (supp.

fig. 1). Patients are oriented to look at the mirror and are instructed to move their unaffected hand, creating the illusion that the affected hand is moving.

Most studies that tested MT in stroke rehabilitation have observed improved motor performance after 6-8 weeks of MT-based programs.^{9,10,12} For instance, a recent randomized controlled trial suggests that 8 weeks of MT (40 sessions, 5/week) promotes significant improvement in motor function, when compared to standard motor rehabilitation.⁹ Nevertheless, the underlying neural mechanisms related to the motor recovery induced by MT are still unclear.

Functional Magnetic Resonance Imaging (fMRI) studies suggest that brain reorganization is not limited to the motor system, but also involves changes in the parieto-occipital lobe, and dorsal frontal areas.¹³ The most consistent finding, however, is that improved motor performance after MT-based programs are related to increased activity of the affected primary motor cortex (M1), as if MT changes the balance of M1 activity between hemispheres, toward the affected hemisphere.^{10,14,15}

There are also a few Transcranial Magnetic Stimulation (TMS) studies designed to understand the neural basis of MT interventions, and most of them have been conducted in healthy subjects. A consistent observation is an increased excitability of ipsilateral M1 (to the moving hand), both during the MT session,¹⁶⁻¹⁸ and immediately after the session.¹⁹ There is only one study in stroke survivors which used TMS stimuli during the MT execution.²⁰ The results suggest increased excitability of the affected M1, with respect to the unaffected M1.²⁰

Studies conducted in stroke so far have assessed MT-induced changes by TMS or fMRI, separately, and after the completion of many MT sessions (fMRI studies) or during MT execution (TMS studies). However, it is still not clear if MT neural changes already occur at the first hours after a single MT training. Therefore, this study sought to investigate MT sub-acute changes in the motor cortex of chronic stroke patients by both TMS and fMRI, and to explore the correspondences between them. Our initial hypothesis is that a single MT session would induce the following sub-acute changes: (i) increase TMS-MEP amplitude when the affected M1 is stimulated; (ii) increase fMRI activity in

the affected M1; **(iii)** changes in fMRI M1 activity correlates with TMS-MEP changes observed in the affected hemisphere.

Materials and Methods

Participant

The Ethics and Research Committee of the Ribeirão Preto School of Medicine, University of São Paulo approved the study (#860004294-11). All subjects provided written informed consent prior to their participation in the study.

Ischemic stroke survivors were selected from the Ribeirão Preto Stroke Registry, according to the following inclusion criteria: **(i)** single ischemic stroke episode confirmed by computed tomography (CT) and/or magnetic resonance imaging (MRI), **(ii)** chronic stroke (more than 6 months after insult). **(iii)** be able to perform the fMRI task of opening and closing the hand. Participants were excluded if they had a general contraindication for TMS or fMRI assessments, such as: cardiac pacemaker, aneurysm clips, carotid or cerebral stents, metal implants, pregnancy.

Demographic and clinical data are presented in tables 1 and 2. Most participants were right-handed (93%, N=14), male (67%, N=10), adults (58 ± 10 y.o). All were stable in terms of neurological deficits. On average, the ischemic insult had occurred 25 months before the study, and in most cases (53%) compromised the left middle cerebral artery (MCA).

Clinical instruments

Clinical evaluation was used to characterize the upper limb motor function. The following tests were used, only at baseline (before MT): **(i) Box and block test** to assess gross manual dexterity.²¹ This test evaluates the number of blocks moved in 60 seconds from one compartment of the box to the other; **(ii) Purdue Pegboard test** to assess fine manual dexterity.²² This test evaluates the number of pins placed into small holes in 30 seconds; **(iii) Action Research Arm Test (ARAT)** to evaluate

compression activities, handgrip, clamping and outreach activities;²³ (iv) **Fugl-Meyer Assessment (FMA)** for the upper limbs to assess sensitivity, motor function, speed and coordination of the upper limbs.²⁴

Mirror Therapy intervention

During the MT session, we instructed participants to position their affected hand in one side of the mirror box, behind the mirror, and the unaffected hand on the other side, in front of the mirror (suppl. fig. 1). Participants were instructed to look at the reflected image of the unaffected hand while performing the following series of movements with the unaffected hand (suppl. fig. 1): abduction-adduction, flexion-extension, opponency of fingers with the thumb, and pronation-supination of the forearm. The intervention lasted 30 minutes. Participants were monitored to ascertain correct task execution, and to evaluate synkinesis of the affected hand, which was not supposed to move.

Experimental protocol

This manuscript is a descriptive study in which fifteen patients were included and evaluated before and after Mirror Therapy by scales, TMS and fMRI. Figure 1 shows a diagram of the experimental protocol. Assessments occurred on three separate days. At the first encounter, patients were submitted to a clinical neurological evaluation, and were submitted to the box of block test, the Purdue test, the ARAT, and the FMA assessments. At this same day, participants were randomized with respect to the order of the assessments: fMRI or TMS first.

TMS and fMRI sessions occurred on two separate days. In each day, TMS or fMRI was conducted immediately before and immediately after a 30 minutes MT intervention. In each of these sessions, patients enrolled in a baseline assessment (TMS or fMRI), followed by the MT intervention, and by a post-MT assessment (TMS or fMRI) (Fig. 1). Both evaluations (TMS and fMRI) were performed within a week.

TMS session

TMS stimulation was delivered to the affected hemisphere only, and Motor Evoked Potentials (MEP) were acquired on the affected hand only. We used a NeuroSoft TMS stimulator (Neuro-MS, Russia), with a butterfly coil (larger/smaller radius = 20 cm/10 cm) positioned over the primary motor cortex (M1) at an angle of $\sim 45^\circ$ with respect to the longitudinal fissure. MEPs were detected with two silver-surface electrodes, one positioned over the abductor muscle of the thumb, referenced against the one positioned over the prominence of the seventh cervical vertebra. Signals were amplified and conditioned by a Myosystem (12-bit A/D Converter) electromyography system, with a sampling rate of 4000 Hz, and a band pass filter set at 20-1000 Hz.

Individual hot spots were determined as the site of stimulation that evokes the largest MEP amplitude. Individual motor threshold was determined as the minimal stimulation intensity that evokes MEP responses of at least 50 μV in five out of 10 consecutive TMS pulses.²⁵ MEP response was composed by the average of ten consecutive TMS pulses applied to the individual hot spot at 110% of the individual motor threshold, randomly distributed across a 60 seconds interval. The TMS protocol was applied when the subjects were at rest.

fMRI session

Magnetic resonance images (MRI) were collected in a 3T scanner (Philips, Achieva, The Netherlands). fMRI statistical maps were based on an Echo Planar Image (EPI) sequence with the following acquisition parameters: TR/TE = 2000/60 ms, flip angle = 90° , FOV = 230 mm, slice thickness = 3 mm, 30 slices, 118 volumes. A set of high-resolution 3D T1-weighted anatomical images (1 mm³) were also obtained, with the following parameters: TR = 5.7 ms; TE = 2.6 ms; flip angle = 8° , matrix = 256 x 256, FOV = 256 mm, slice thickness = 1 mm.

The fMRI motor task consisted of opening and closing the affected hand at self-pace. The chosen protocol used an event-related design to avoid habituation effects, commonly observed in stroke.²⁶ The protocol encompassed alternating periods of movement (6s) with periods of rest (16s).

Auditory cues were delivered to signal a change between conditions. In order to assure task compliance, participants performed a dry run before fMRI acquisition.

Data Analysis

Scales

Statistical analysis of the scales was performed with a two-tailed Wilcoxon test (data is not normally distributed), using the GraphPad Prism software (GraphPad Software v5.0, San Diego, USA), comparing scores from the affected against the unaffected hand at baseline. We expressed values as median, Z-value and statistical significance was set at $p < 0.05$.

TMS

MEP analysis was conducted in MATLAB 7.10. Group analysis compared the grand median MEP values (pre-MT vs post-MT), obtained from the 10 individual MEPs detected before and after the MT intervention. A Wilcoxon test (data is not normally distributed) was used to compare MEPs from the two endpoints (before x after MT). Statistical threshold was set at $p < 0.05$ (two-tailed). We expressed values as median, Z value and statistical significance was set at $p < 0.05$.

For individual subject inspection, the 10 detected MEPs for each subject were compared (pre-MT vs post-MT) using the Mann-Whitney test, since the data is not normally distributed and the samples are independent. We expressed values as median, U-value and statistical significance was set at $p < 0.05$.

fMRI

fMRI preprocessing and statistical analyses were performed in SPM12 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, UK). Preprocessing included slice-time correction, head motion correction, spatial smoothing (Gaussian kernel, FWHM=5 mm), and a high-pass filter set at 0.02 Hz. Images were normalized to the MNI152 template. To have all lesions falling

onto the same side, participants with right-sided lesions had their images flipped about the mid-sagittal plane.

First-level individual analysis used the general linear model (GLM) with a single regressor of interest modeling task periods. A single regressor was obtained by convolving a boxcar function of the task periods with a double-gamma hemodynamic response function. The six motion parameters (three rotations and three translations) were included in the model as regressors of no interest. Contrast images were obtained for each subject for the task > rest condition using a $p < 0.05$, uncorrected. These contrast images were used in a subsequent second-level group analysis to examine differences between pre- and post-MT. Whole brain analysis was conducted with a small volume correction at two regions of interest (ROI): the bilateral primary motor cortex (M1) and Supplementary Motor Area (SMA). These two ROI were extracted from the sensorimotor network atlas.²⁷ Contrast maps were calculated with respect to: (i) pre-MT; (ii) post-MT; (iii) pre-MT > post-MT and; (iv) post-MT > pre-MT. Statistical threshold was set at $p < 0.05$, and clusters with $p_{\text{fwe}} < 0.01$ at peak level.

fMRI x TMS correlation

To evaluate the correspondence between TMS and fMRI changes elicited by MT, Pearson's correlation coefficient was calculated between individual M1 fMRI β -values changes $\left(\frac{\text{post\_MT} - \text{pre\_MT}}{\text{pre\_MT}} \right)$, and the mean MEP amplitude changes $\left(\frac{\text{post\_MT} - \text{pre\_MT}}{\text{pre\_MT}} \right)$ from the affected hemisphere. Individual fMRI β -values were extracted from a mask created by the intersection between the individual first-level statistical maps, before the MT intervention, and the M1 ROI defined in the sensorimotor network atlas.²⁷

Results

Fifteen patients were included in the study. Six participants performed the fMRI assessments first, and in nine TMS was applied first (table 2). Two participants had to be excluded from fMRI analysis: one due to significant hearing impairment, and the other due to claustrophobia. Five subjects had to be

excluded from TMS analysis: in two, no MEP was detected, in the other two, EMG signal was too noisy, and one patient did not complete the post-MT session. fMRI was successfully conducted in 13 subjects, TMS in 10 subjects, and 8 patients were successfully submitted to both fMRI and TMS analysis.

Individual results for the ARAT, Fugl-Meyer, box of blocks and Purdue assessments are presented in table 2. Motor deficits observed at initial clinical evaluation involved mainly subtle aspects of movements, such as fine manual dexterity (Purdue test, $p=0.02$, $Z=-2.29$, $\Delta\text{median}=3$), sensitivity and coordination (Fugl-Meyer, $p=0.002$, $Z=-3.06$, $\Delta\text{median}=2$) (Table 1). We did not observe statistically significant differences in the ARAT, nor in the box of blocks tests from the affected hand, when compared to unaffected hand (Table 1).

Figure 2a shows the median MEP detected in the affected hand before and after MT. We found significantly increased MEP amplitude (increased excitability) from the affected M1 post-MT, when compared to pre-MT (Fig. 2a, $p=0.03$, $Z=-2.19$, median difference=0.25). Median values changed from 0.88mV to 1.41mV. Figure 2b shows the MEP changes (ΔMEP) observed at the individual subject level. MEP amplitude measured in the affected hand of four participants (marked in black) increased significantly after therapy (Fig. 2b, $p<0.05$). Analytical individual MEP results from all participants are presented in table 3.

Figure 3 shows group level fMRI results, pre- and post-MT. The motor task engaged the bilateral M1 and SMA, both before and after MT (Fig. 3). Figure 4 shows the between session comparison (post-MT vs pre-MT). MT led to changes of the primary motor cortex (M1). Post-MT fMRI maps were associated with a more organized and constrained pattern, with a more focal M1 activity within the affected hemisphere after MT, limited to the area of hand representation (Fig. 4). Furthermore, we find a change in the balance of M1 activity toward the affected hemisphere. At the individual level, the same patterns of reorganization were observed in 10 participants (77%) (suppl. fig. 2).

Figure 5 shows a significant Pearson's correlation between individual fMRI β -values changes with respect to individual amplitude MEP change in the affected M1.

Discussion

In this study we used both TMS and fMRI to investigate sub-acute neural changes induced by a single MT intervention in ischemic stroke survivors. At the group level, TMS revealed increased excitability of the affected M1, observed as increased MEP amplitude post-MT, with respect to pre-MT. fMRI reveals a change in the balance of M1 activity toward the affected hemisphere, with increased fMRI focal activity post-MT in the affected M1, and a decreased activity in the unaffected M1. We also found a significant correlation between increased MEP amplitude and decreased fMRI β -values in the affected M1.

Changes in MEP status are frequently observed in stroke, with decreased MEP amplitude (decreased excitability) of the affected M1 with respect to the unaffected M1.^{28–31} In fact, the TMS excitability of the affected M1 is an important marker of motor recovery after stroke.^{30,32,33} For instance, evidence suggest that the MEP amplitude from the affected M1 measured one day after an ischemic stroke is predictive of the motor recovery observed 14 days after the insult.³⁰ Presumably, the excess of transcallosal inhibitory activity from the unaffected M1 to the affected hemisphere compromises the motor recovery of these patients.^{34–37}

In line with these findings, an increasing number of studies have suggested that increased fMRI activity of the unaffected M1, with respect to the affected M1, is associated with poorer motor recovery.^{6,10,38–42} On the contrary, we found significant increased focal fMRI activity in the affected M1, and decreased activity in the unaffected M1, after a single MT intervention, with a more restricted M1 activity.^{43–45} Furthermore, increasing evidence points to a widespread recruitment of brain regions early after the stroke insult, followed by a progressive reduction in this task-related recruitment over the course of rehabilitation sessions.^{6,39,46} For instance, an fMRI study in stroke observed a linear decrease in the size of unaffected M1 activity, along the course of a 2 weeks rehabilitation program

with constraint-induced movement therapy, which correlated with the observed motor improvement.³⁹ Also, rehabilitation programs with functional and/or nonfunctional exercises observed decreased sparseness in fMRI activity maps, reflecting a decreased activity of the unaffected M1, decreased perilesional M1 activity.⁶

These sub-acute MT modifications are consistent with previous fMRI studies that found changes in the motor cortex after many MT session.^{10,14} For example, previous studies in stroke survivors suggests that the improved motor performance after 30-40 MT sessions is accompanied by increased activity of the affected M1.^{10,14} Overall, these results suggest that a single MT intervention may already reduce excessive transcallosal inhibitory activity from the unaffected hemisphere over the affected one.³⁷

Individual subject variability is generally high for both TMS and fMRI evaluations and, therefore, most studies restrict their analysis to the group level. To draw reader's attention to the current clinical relevance of our study, the results are also presented at the individual subject level. Significant increased MEP amplitudes were observed post-MT in 40% of the participants. MEP did not decrease for any subject. Congruent focal increased activity of the affected M1, and decreased activity of the unaffected M1 was identified in 54% of the participants. It is possible that these changes become more consistent with increasing number of interventions. Furthermore, such variability may be an important early marker of the clinical benefit specific patients might expect after an MT-based rehabilitation program.

We also found a significant correlation between increased MEP amplitudes and decreased β -values extracted from the affected M1 ROI. Increased MEP amplitude was significantly correlated with decreased β -values after MT. Although the correspondences between fMRI and TMS variables are difficult to trace, it has been observed that patients with more pronounced fMRI activity in the affected hemisphere also had a more balanced transcallosal inhibition.³⁷

Our results bring preliminary evidence of the beneficial effect of a single MT session.

However, it is important to point out some limitations and caveats of our study: **(i)** the absence of a control group (without intervention), which limits our ability to associate the observed changes to the MT intervention; **(ii)** the number of patients is small; **(iii)** we did not evaluate patient's clinical improvement; **(iv)** the cohort was restricted to well-recovered patients, with mild motor impairment, as study participants should be able to move their hand during the fMRI protocol; **(v)** the use of fixed-effects models, which limits the conclusion of our results to the population studied; **(vi)** it is known that within-session fMRI reliability is higher than between-session, particularly in stroke survivors who show altered cerebrovascular coupling, which may interfere with fMRI analysis and interpretation^{26,47,48},

Overall, our study suggests that a single MT intervention in stroke survivors is related to increased MEP of the affected limb, a more constrained activity of the affected M1, a correspondence between the observed changes due to MT upon fMRI and TMS parameters, as if activity had become more constrained and limited to the affected hemisphere. This work contributes to better understand the neural mechanisms of MT in stroke survivors. Besides, exposing individual data may allow other researchers to find similarities with our data and contribute to understanding nuances of MT intervention in stroke survivors.

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Disclosure of interest

The authors report no conflicts of interest.

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Fig. 1. Experimental design. Clinical, fMRI and TMS evaluations were performed on three different days. At day one, patients were submitted to a clinical evaluation (Box of blocks, Purdue, ARAT and Fugl-Meyer tests) and were randomly assigned to the order of the assessments (fMRI or TMS). Each assessment (fMRI and TMS) was composed of: i) the (TMS or fMRI) evaluation immediately before the MT session, ii) the MT session (30 minutes), and iii) the (TMS or fMRI) evaluation immediately after the MT session.

Fig. 2. MT-induced MEP changes. Motors Evoked Potentials (MEP) amplitude of the affected hand on the group of patients before and after therapy. (a) MEP median values of all participants before and after MT (* $p < 0.05$; paired two tailed Wilcoxon test; $N = 10$). (b) Individual subject MEP changes (MEP after - MEP before) of all individuals. The solid horizontal line represents the mean. MEP amplitude in the affected hand of four participants (marked in black) increased significantly after therapy ($p < 0.05$, Mann Whitney test)

Fig. 3. Group response to the fMRI motor task. The proposed motor task elicited significant activity in bilateral M1 and SMA with asymmetrical ($p_{\text{voxel}} < 0.001$, FWE corrected).

Fig. 4. Between session comparison (Post-MT vs Pre-MT). Cluster within affected M1 showed increased activity (in red), and cluster within bilateral M1 showed decreased activity (in blue). $p_{\text{voxel}} < 0.05$, uncorrected, and clusters with $p < 0.01$ at peak level. $N=13$.

Fig. 5. Pearson's correlation between changes of β -values and MEP mean amplitude. Values are represented as individual β -values changes ((after – before)/before) and the mean amplitude MEP change ((after – before)/before) of the affected M1. Significant inverse correlation was found ($r = -0.74$, $p=0.03$, $N=8$).

Table 1. Demographic data and clinical characteristics (box and block test, ARAT, Fugl-Meyer and Purdue) of all participants.

NOMINAL VARIABLE	NUMBER OF PARTICIPANTS (%)		
Sex			
Male		10	(67%)
Female		5	(33 %)
Affected hemisphere			
Left		8	(53 %)
Right		7	(47 %)
Affected cerebral artery			
Middle cerebral artery		12	(80%)
Internal carotid artery		2	(13%)
Vertebral artery		1	(7%)
Manual dominance			
Left		1	(7 %)
Right		14	(93 %)
Cognition			
Deficit		2	(13 %)
No deficit		13	(87 %)
CONTINUOUS VARIABLE		MEAN $\pm$ SD	
Age (year)		58 $\pm$ 10	
Time after stroke (months)		25 $\pm$ 22	
MOTOR IMPAIRMENT	Dif. median	Z	P
Box and block test	10	-1.79	0.07
ARAT	0	-1.83	0.07
Fugl-Meyer	2	-3.06	0.002**
Purdue	3	-2.29	0.02*

Statistical significance were found in the Fugl-Meyer (**p<0.005) and Purdue (*p<0.05). Dif. Median= difference in median. Wilcoxon two-tailed, N=15.

Table 2. Personal and clinical characteristics of all participants.

Sub j.	Gender	Age	Time since stroke (months)	Affected Hem.	Manual Dominance	CT & MRI Affected artery	AR AT / A	FM / A	BB T / U	BB T / A	Purdue / U	Purdue / A	First assessment	Second assessment
1	M	60	24	R	R	MCA	57	76	49	38	9.33	7.67	TMS	fMRI
2	M	59	7	R	R	MCA and ACA	57	76	32	36	11.33	11.33	TMS	fMRI
3	M	47	41	L	R	MCA	57	70	43	41	10.67	5.67	fMRI	TMS
4	M	54	19	R	R	ICA	57	72	41	40	7.67	8.33	TMS	fMRI
5	M	65	24	L	R	MCA	57	69	54	38	10.67	7.67	fMRI	TMS
6	M	60	36	R	R	MCA	57	76	60	58	13	10.67	TMS	fMRI
7	F	76	11	L	R	MCA	57	74	27	31	9.67	8.67	fMRI	TMS
8	F	59	22	L	R	MCA	57	75	68	76	13	13.67	fMRI	TMS
9	M	68	32	L	R	MCA	57	74	47	53	8.67	10.67	TMS	fMRI*
10	F	34	96	L	R	MCA	57	74	65	62	11.67	15.33	TMS*	fMRI
11	M	58	13	L	R	Vertebral artery	3	43	60	0	12	0	fMRI	TMS*
12	F	56	12	L	R	MCA	57	75	48	43	12.67	8.33	TMS*	fMRI
13	M	64	6	R	R	ICA	56	72	47	39	10	6.33	fMRI	TMS*
14	M	62	14	R	L	MCA	45	60	57	16	12	1	TMS*	fMRI
15	F	54	14	R	R	MCA	48	64	62	27	11.33	5.33	TMS	fMRI*

M = male, F = female, R = right, L = left, MCA = Middle Cerebral Artery, ICA = Internal Carotid Artery, ACA = Anterior Cerebral Artery, FM=Fugl-Meyer, BBT=Box and block test. Participants with significant TMS changes are represented in **bold**. *data was not considered for statistical analysis.

Table 3. Individual between-session (post-MT – pre-MT) Motor Evoked Potentials (MEP).

Participants	Median difference	U-value	p-value
1	0.41	42	0,57
2	1.85	10	0,001
3	0.03	25	0.063
4	3.17	0	<0.0001
5	0.08	43	0.61
6	1.3	23	0.043
7	0.07	40	0.47
8	0.29	35	0.28
9	0.62	12	0.003
15	0.03	49	0.95

Participants with significant TMS changes are represented in **bold**. The 10 detected MEPs for each subject were compared (pre-MT vs post-MT) using the Mann-Whitney test. We expressed values as median, U-value and statistical significance was set at $p < 0.05$