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May Dual Transcranial Direct Current Stimulation Enhance the Efficacy of Robot-Assisted Therapy for Promoting Upper Limb Recovery in Chronic Stroke?

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Abstract

Objective. To assess whether dual transcranial direct current stimulation (tDCS) may enhance the efficacy of exoskeleton robotic training on upper limb motor functions in patients with chronic stroke. *Methods.* A prospective, bi-center, double-blind, randomized clinical trial study was performed. Patients with moderate-to-severe stroke (according to The National Institute of Health Stroke Scale) were randomly assigned to receive dual or sham tDCS immediately before robotic therapy (10 sessions, 2 weeks). The primary outcome was the Fugl–Meyer for Upper Extremity, assessed before, after, and at the 12-week follow-up. Neurophysiological evaluation of corticospinal projections to upper limb muscles was performed by recording motor evoked potentials (MEPs). ClinicalTrials.gov-NCT03026712. *Results.* Two hundred and sixty individuals were tested for eligibility, of which 80 were enrolled and agreed to participate. Excluding 14 dropouts, 66 patients were randomly assigned into the 2 groups. Results showed that chronic patients were stable before treatment and significantly improved after that. The records within subject improvements were not significantly different between the 2 groups. However, a post-hoc analysis subdividing patients in 2 subgroups based on the presence or absence of MEPs at the baseline showed a significantly higher effect of real tDCS in patients without MEPs when compared to patients with MEPs ($F = 4.6$, $P = .007$). *Conclusion*. The adjunction of dual tDCS to robotic arm training did not further enhance recovery in the treated sample of patients with chronic stroke. However, a significant improvement in the subgroup of patients with a severe corticospinal dysfunction (as suggested by the absence of MEPs) suggests that they could benefit from such a treatment combination.

Keywords

robotic therapy, exoskeleton, tDCS, non-invasive brain stimulation, interhemispheric balance, stroke, rehabilitation

Introduction

Several studies have demonstrated that recovery after stroke is slow and often incomplete. Indeed, in 30% to 66% of hemiplegic stroke patients, the paretic arm remains without function when measured 6 months after stroke.[1] Recovery of voluntary upper extremity (UE) movement after stroke varies widely.[2] This process is strongly linked to brain plasticity phenomena that include changes in the excitability of neuronal circuits in perilesional areas and the formation of new functional neuronal connections.[3] Unfortunately, stroke-related brain plasticity is short-lived, it is maximal in the subacute phase (3-6 months after stroke), also called the "critical period" and then gradually diminishes in the following chronic phase, in which there is limited or absent

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spontaneous recovery.[4] In an attempt to enhance recovery in chronic patients, technologically- and robotic-assisted treatments have recently been proposed.[5] The passive and active motion of upper limbs can now be performed using robots.[6] Scientific literature has shown that robotic-assisted rehabilitation is more effective in improving upper limb motor function recovery, especially in late subacute and chronic stroke patients and in moderate–severe subjects with limited potential for spontaneous recovery.[7,8] In this intriguing scenario of novel technological approaches in stroke rehabilitation, one of the most promising treatments tries to boost brain plasticity in chronic stroke patients by manipulating cortical excitability using non-invasive brain stimulation techniques (NIBS). The aim of this approach is to augment plasticity in the subacute stroke phase.[9] To this end, transcranial direct current stimulation (tDCS) has been used in combination with several rehabilitation approaches such as conventional neurorehabilitation, robot-assisted therapy, and virtual reality.[10,11] Data from a recent meta-analysis conducted by Reis et al[12] show that, at present, there is not enough evidence about the benefits of tDCS as an add-on intervention to robot-assisted therapy on upper-limb motor function or activity in individuals with stroke. Nevertheless, while the efficacy of mono-hemispheric tDCS (anodal or cathodal independently) is limited when compared to sham conditions or other NIBS like the transcranial magnetic stimulation (TMS), the effect of dual tDCS, which could led to more consistent results, is still poorly investigated. tDCS offers different advantages that make this technique a possible choice for future studies: (a) the devices are cheap and easily available; (b) tDCS can be delivered simultaneously with robotic rehabilitation; (c) tDCS can be used as "at-home" treatment; and (d) tDCS does not share several TMS contraindications (eg, epilepsy and implantable devices). All of these advantages provide the basis for a new trial based on the association of tDCS and robotic rehabilitation despite the negative results of other already published trials. According to our previous studies on robotic therapies and given the high variability among patients, it is important not only to evaluate the general efficacy of a specific intervention but also to identify the discriminants that could allow the selection of patients who could benefit from the combination of robot-assisted therapy and NIBS interventions for a more tailored therapy.[13,14]

One of the proposed neurophysiological tools that can predict the recovery and efficacy of neurorehabilitation is the evaluation of Motor Evoked Potentials (MEP).[15] MEP can be recorded by TMS of the brain over the primary motor cortex (M1). MEPs reflect cortical excitability and are helpful in assessing inter-hemispheric imbalance between affected and unaffected hemispheres caused by cerebrovascular events.[9] In particular, according to the bimodal balance–recovery model, the main discriminant between the 2 main models of functional recovery—that is, interhemispheric imbalance model and vicariation model—is the structural reserve. If the patient has an elevated structural reserve, the interhemispheric competition model can predict recovery better than the vicariation model and vice versa.[9,15] Accordingly, the more reliable parameter to evaluate the amount of structural reserve is the presence or absence of MEP, the former being associated with more structural reserve and the latter linked to less reserve.[9,16] On the other hand, it has been demonstrated that tDCS can produce prolonged changes in motor cortical excitability both in healthy subjects[17] and in stroke patients,[18] resulting in MEP suppression when tDCS is delivered with cathodal montage and MEP increase when delivered with anodal montage.[19,20] Accordingly, tDCS has the potentiality of amending inter-hemispheric imbalance in subacute stroke. We hypothesized that dual-tDCS could enhance the benefits of robotic therapy, by favoring the restoration of interhemispheric balance and by boosting plasticity-dependent recovery in patients with stroke in chronic phase (by an enhancement of the excitability of the affected motor cortex and a concomitant suppression of the excitability of contralesional motor cortex).

To this end, we designed a prospective, multicenter, double-blind, sham-controlled, randomized trial, to determine the effects of an experimental intervention formed by dual-tDCS plus robot-assisted therapy on clinical and neurophysiological measures of upper limb functionality. Moreover, we evaluated whether MEP recording could be useful to identify those patients who could benefit more from the adjunction of a tDCS intervention to the robot-assisted therapy.

Methods

Inclusion and Exclusion Criteria

Among the 260 subjects screened between February 2017 and December 2020, 80 chronic stroke patients were enrolled in this study (for details please see flow chart, Figure 1). All eligible and consenting patients were screened (with a pre-clinical and neurophysiological assessment) by the research team. The following inclusion criteria were applied: single stroke confirmed by brain imaging (MRI or CT); ability to understand and follow the instructions given by doctors and therapists (Mini Mental State Evaluation $\geq$24)[21]; basal Modified Ashworth Scale score $<$3; basal Fugl–Meyer score $\geq$3 (so that the upper limb was not completely paralyzed); and stability of upper limb deficit in the 2 pre-treatment evaluations, in order to avoid "Hawthorne effect."[22,23] The exclusion criteria were: chronic paretic limb deformities; absence of voluntary movements at the proximal part of the upper limb (shoulder and elbow); severe neglect (assessed by Pizzamiglio's battery for unilateral spatial neglect[24]); epilepsy; contraindications to tDCS

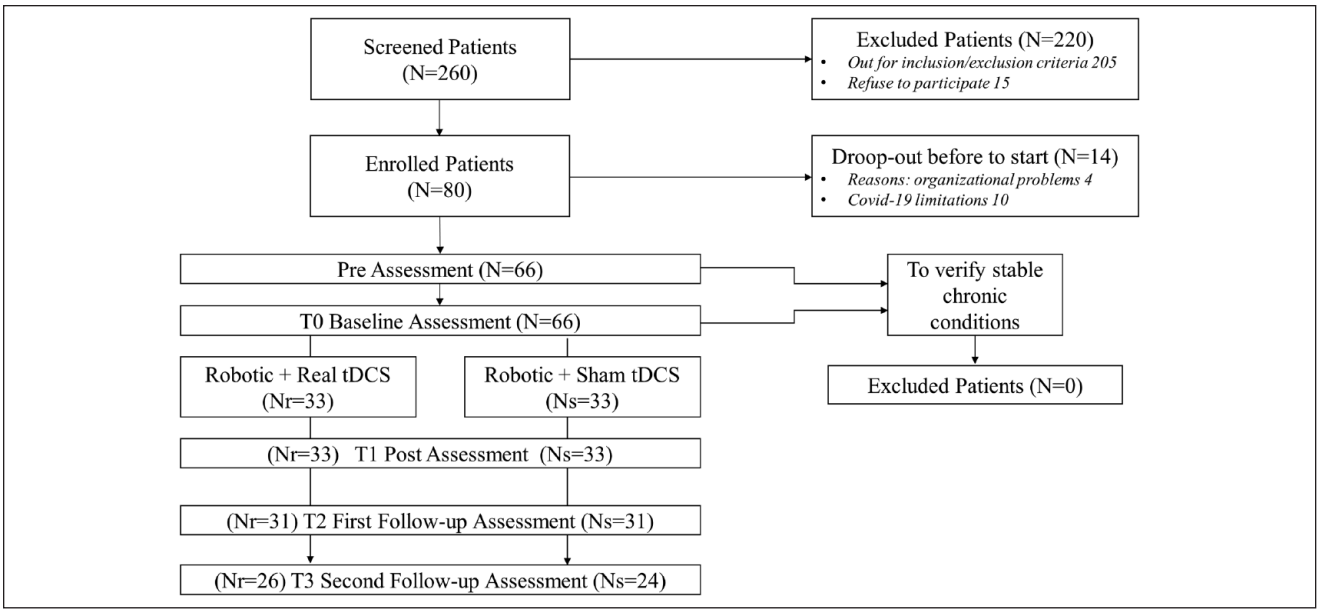

Figure 1. Flow chart of the study.
Abbreviations: tDCS, transcranial direct current stimulation.

and TMS (eg, pacemakers, metal implants); previous neurosurgical interventions; and severe upper limb osteoporosis, upper limb strength or joint movement limitation due to previous fractures or surgical interventions.

Trial Design and Treatments

A randomized clinical trial design was performed in 2 Italian hospital settings. Patients were enrolled among those admitted over a period of 5 years to the outpatient service of the Stroke Neurorehabilitation Unit of the Fondazione Santa Lucia (FSL, Rome) and the Neurology Unit of University of Campus Bio-Medico of Rome. All patients had undergone neurological rehabilitation in the acute and subacute phases. The physicians responsible for the clinical trial provided patients and relatives (when necessary) with written information on the trial protocol. The study protocol was approved by the local ethical committee board in both clinical centers and a written informed consent was obtained from each patient before treatment. The trial was recorded on Clinicaltrials.gov (number NCT03026712) (https://clinicaltrials.gov/ct2/show/NCT03026712).

Randomization and Blinding

After enrollment, each patient was randomly assigned to one of the 2 experimental groups: (a) robot-assisted training coupled with real tDCS (Real-tDCS group, RTG); or (b) robot-assisted training coupled with sham tDCS (Sham-tDCS group, STG). Randomization was performed using a computer-generated list (www.random.org). Physiotherapists providing robotic treatment and assessors were blind to group allocation, as were patients and the statistician. The randomization list was managed by a collaborator who was not involved in the trial. The group allocation was revealed from time to time to the physician involved only in the tDCS treatment by the removal of an adhesive tape covering the list. Hence, patients were blind regarding the type of tDCS they received (real or sham).

Patients received 10 sessions of multi-joint and tridimensional exoskeleton robot-assisted therapy using Armeo® Power II (40 minutes for each daily session, 5 days per week for 2 consecutive weeks). This robot is an exoskeleton composed of an orthosis for the affected upper limb with 6 degrees of freedom: 3 for the shoulder, 1 for the elbow flexion, 1 for the forearm supination, and 1 for the wrist flexion. Each joint is powered by a motor and equipped with 2D sensors. The device can support the patient's upper limb weight, providing a feeling of fluctuation. It has an interface used for the exergaming and was designed to simulate upper limb gestures and provide a non-immersive digital environment.[7]

Therapy

Therapy was administered according to the recently published guidelines and recommendations for the upper limb robotic rehabilitation after stroke provided by the Italian Consensus Conference on Robotic in Neurorehabilitation.[5] Every session included exercises to enhance the range of motion (ROM) of shoulder, elbow, wrist, and hand coordination. The training characteristics (difficulty level, duration, visual stimuli) were set in conformity of residual ability of

the patient. Selected exercises may consist of single-joint movements in a single axis, combined movements of a single joint around 2 or 3 axes, selective exercises for the opening and closing hand, or multi-joint exercises.

Each patient received 20 minutes of dual tDCS stimulation daily, immediately before the session of robot-assisted training. Real tDCS stimulated the motor cortex at an intensity equal to 2 mA. The Eldith DC Stimulator® tDCS model (NeuroConn, Ilmenau, Germany) was supplied by 2 gel-sponge electrodes with a surface area of 35 cm^2 (5 cm × 7 cm), embedded in a saline-soaked solution. Stimulation was preceded by a few seconds during which the current increased gradually to the selected intensity (45 seconds of fade-in phase), eliciting transient tingling sensations that disappeared within several seconds and followed by the same few seconds during which the current was progressively reduced (45 seconds of fade-out phase).[25] These phases were the only ones also present during Sham stimulation to give the subject the impression that the device was active. The anode was positioned over the primary motor cortex area (C3/C4 according to the international classification system of EEG electrodes placement) of the injured hemisphere to stimulate it while the cathode was positioned over the contralateral motor cortex to inhibit it.

Clinical Assessment

A set of specific and validated clinical scales were administered 5 times, as described in the flow chart (Figure 1). A pre-assessment (Tpre) took place within 2 weeks after the enrollment; these data were compared with those of baseline (T0) assessed the day before the beginning of the intervention to verify the stability of the patients' conditions. Then, the scales were administered at the end of the intervention (T1) and after 1 (T2) and 3 (T3) months of follow-up.

The primary outcome measure was the arm section of the Fugl–Meyer Assessment scale (FM-UE).[26] The minimal clinically important difference (MCID) for this scale was set to 5.25 points for the overall arm function.[27] Secondary clinical outcome measures included: The National Institute of Health Stroke Scale (NIHSS) for signs and symptoms of stroke,[28] the Visual Analogue Scale (VAS) for pain in the upper limb, the Medical Research Council scale (MRC) for muscle strength of the upper limb, the Barthel Index (BI) for independence in performing activities of daily living, and the Modified Ashworth Scale (MAS) for spasticity of the upper limb.[29]

Neurophysiological Assessment

A neurophysiological assessment by means of motor cortex TMS for the evaluation of MEP was performed before (T0) and at the end (T1) of the intervention (at least 48 hours after the last training session) on a subgroup of patients (N = 48). TMS was carried out by a Magstim Rapid stimulator and a 70 mm figure-of-eight coil. The coil was placed tangentially on the scalp at a 45° angle from the midsagittal line, approximately perpendicular to the central sulcus. The coil was moved in steps of 0.5 cm around the fronto-central regions to find the "hot spot" for inducing maximal MEPs registered from the first dorsal interosseus muscle by EMG surface electrodes. After this step, we determined the resting motor threshold (RMT) as the lowest stimulus intensities produced stable MEPs with peak-to-peak amplitude $\geq 50\,\mu V$ in at least 5 of 10 consecutive trials. With the same coil orientation, we recorded the MEPs, stimulating at 120% of the RMT. We compared the MEPs at T0 and T1 in the 2 groups. Then, we used the presence of MEP >0 at T0 for subdividing the groups for a post-hoc analysis. The interhemispheric balance has been computed by the parameter MEPbal = ΔMEP of affected hemisphere – ΔMEP of unaffected hemisphere, with Δ the difference of MEP post-treatment and MEP pre-treatement.[15] At T0 and at T1, we also evaluated the volume reachable with the affected upper limb using a specific function of the exoskeleton that allowed for computing the specific joint ranges of motion.

Statistical Analysis

All data were summarized in terms of mean ± standard deviation or median and interquartile range or frequency. Percentage gain was computed as the difference between the last FM-UE score assessed by each patient (according to the last observation carried forward method) and relevant baseline value divided by baseline value and multiplied for 100. Normality of data was tested by Kolgomorov–Smirnov analysis. Data were not normally distributed and analyzed by non-parametric statistics. For between group analyses, Mann–Whitney *U*-tests were used to compare the ordinal data between both groups. The Wilcoxon test allowed to compare data between Tpre and T0, Friedman analysis was performed to assess the within-group differences from T0 to T3, followed by post-hoc Wilcoxon test with Bonferroni correction on the alpha level of significance. Chi-squared tests were used to compare the number of patients who achieved the MCID in terms of FM-UE score in the 2 groups. Unless otherwise specified, the significance threshold was set at $P < .05$.

Because the presence of upper limb MEPs in response to TMS predicts recovery in the acute phase of stroke,[30] in a post-hoc analysis we stratified patients into 2 subgroups based on the presence or absence of MEPs at T0 and compared the response to treatment between these patients. Despite the stratification not being included in the statistical plan, it seemed useful to statistically investigate this aspect for its potentially clinical implication.

Table 1. Mean ± Standard Deviation, Median (Interquartile Range), or Percentage Relative Frequency of Demographic and Clinical Data for All Patients, As Well As Those Without Motor Evoked Potential at the Beginning of Treatment (MEP(T0) = 0) Divided By Those Receiving Real Transcranial Direct Current Stimulation (tDCS) and Those Receiving Sham Stimulation. The *P*-Values of Their Comparison Obtained by the Mann–Whitney *U*-Test (for Age and Time From Stroke) or Chi-Squared Test (for the Other Variables) are Shown.

Demographical and clinical data	All patients			MEP(T0) = 0		
	Real tDCS	Sham tDCS	*P*-value	Real tDCS	Sham tDCS	*P*-value
Age (y)	59.7 ± 12.5 60 (16)	60.2 ± 16.1 64 (22)	.539	58.1 ± 13.0 56 (13)	58.9 ± 14.6 62 (23)	.761
Gender (males) (%)	50	48	.715	43	44	.928
Time from stroke (mo)	58.5 ± 60.6 35 (34)	57.6 ± 70.9 30 (53)	.386	58.7 ± 59.1 37 (25)	55.7 ± 59.3 27 (66)	.311
Ischemic stroke (%)	84	91	.451	82	93	.385
Side of stroke (left)	36	53	.225	33	36	.888
Drop-out at T2 (%)	6	6	.999	11	7	.702

Results

Preliminary Analysis

As shown in Figure 1, of the 80 enrolled patients, 66 completed the entire rehabilitation pathway and 50 were assessed again at both of the planned follow-ups. A preliminary analysis was conducted to confirm the stability of chronic conditions of patients by comparing data between Tpre and T0. No significant differences were found in the entire sample for any of the administered scales (FM-UE: $P = .655$, NIHSS: $P = .999$, VAS: $P = .785$, MAS: $P = .157$, BI: $P = .102$, MRC: $P = .317$), confirming the stability of neurological conditions before the beginning of treatment. A sample of 66 patients was treated (mean age: 60 ± 14 years old, gender: 34 males, 32 females, time from stroke 58 ± 65 months, damage in the left/right hemisphere: 30/36 patients, Ischemic/Haemorrhagic: 58/8). No significant differences were found at T0 between the 2 groups (RTG vs STG, as shown in Table 1) confirming that the random allocation provided 2 groups that were not different in terms of demographic (age: $P = .875$, gender: $P = .822$) and clinical characteristics (time from stroke: $P = .452$, FM-UE: $P = .127$, NIHSS: $P = .569$, VAS: $P = .335$, MAS: $P = .799$, BI: $P = .117$, MRC: $P = .332$). Finally, since all of the clinical scores were not normally distributed ($P < .05$) for at least one assessment time, non-parametric analyses were performed. Only percentage gain resulted normally distributed ($P = .458$) and parametric analysis was conducted for this variable.

Primary Outcome

The main result of our study was that no significant differences were noted between RTG and STG for FM-UE or for other clinical scale scores at each of the assessment times (Table 2). As post-hoc analysis, significant within subject improvements were noted in both groups between T0 and T1, with a further improvement of FM-UE score between T2 and T3 only for RTG but not for STG.

The number of patients who achieved an MCID of 5.25 points on FM-UE between their baseline and last assessment was higher for RTG (23 out of 33, 70%) than for STG (19 out of 33, 58%); however, this difference was not statistically significant ($P = .306$).

Secondary Outcomes

Similar results were also found for the secondary outcomes. Friedman's analysis showed statistically significant improvements in all the assessed scales both in the RTG and in the STG (Table 2), again without statistically significant differences between groups ($P = .05$). Post-hoc analysis showed a further significant improvement in VAS-scores between T1 and T2 for RTG but not for STG.

The volume related to the upper limb ROM measured using the robot significantly increased in RTG from T0 to T1 ($+33.9 \pm 40.3\,dm^3$, $P < .001$) but not in STG ($+13.8 \pm 48.4\,dm^3$, $P = .109$). These increments were mainly due to significant improvements in shoulder rotation (RTG: $P = .003$; STG: $P = .095$) and forearm prono-supination (RTG: $P = .004$; STG: $P = .077$).

Post-Hoc Evaluation Based on Neurophysiological Stratification

At T0, 18 patients in the RTG and 12 in the STG showed no elicitable MEP in the affected hemisphere, whereas the other patients showed MEP with mean amplitude values of $524 \pm 437\,\mu V$ (STG group) versus $969 \pm 995\,\mu V$ (RTG) ($P = .212$). In the unaffected hemisphere, the mean value of

Table 2. Mean ± Standard Deviation for the Clinical Scale Scores in the 2 Groups Real- Versus Sham-tDCS (RTG vs STG). N is the Number of Subjects for Each Assessment and the Comparative Analyses Were Conducted on the Number of Patients Present in Both Sessions, Excluding Drop-Outs. Friedman Analysis was Performed on Data from T0 to T3, followed by Post-Hoc Performed With Wilcoxon Test (*P*-Values are Reported in Bold If Statistically Significant: <.05 for Friedman's Analysis or <.025 for Post-Hoc Tests). The Results of Between Group Analyses Were Not Reported Because There was No Statistical Significance for Any of the Assessed Scores for Any Assessment Times.

Group	Time	N	FM-UE	NIHSS	VAS	MAS	BI	MRC
RTG	Tpre	33	25.8 ± 15.2	4.2 ± 2.5	2.5 ± 2.7	4.4 ± 2.3	85.1 ± 11.0	18.5 ± 7.8
			22 (21)	3 (3)	2 (5)	4 (3)	86 (12)	17 (9)
	T0	33	25.8 ± 15.3	4.2 ± 2.5	2.5 ± 2.6	4.4 ± 2.3	85.2 ± 10.8	18.5 ± 7.9
			22 (21)	3 (3)	2 (5)	4 (3)	86 (12)	17 (9)
	T1	33	30.7 ± 15.5	4.0 ± 2.8	1.7 ± 2.3	3.6 ± 2.1	86.2 ± 10.5	22.8 ± 8.9
			30 (24)	3 (4)	0 (3)	3 (3)	87 (13)	21 (9)
	T2	31	33.8 ± 15.4	3.6 ± 2.2	1.3 ± 2.0	3.2 ± 1.6	87.4 ± 8.5	24.1 ± 8.7
			34 (21)	3 (3)	0 (3)	3 (2)	87 (13)	24 (8)
	T3	26	33.9 ± 15.7	3.5 ± 2.2	1.7 ± 2.4	2.9 ± 1.7	86.2 ± 8.5	24.6 ± 8.5
			35 (22)	3 (2)	0 (4)	3 (1)	85 (14)	24 (8)
Friedman's *P*-value		26	<.001	<.001	<.001	<.001	<.001	<.001
Post-hoc test *P*-values	T0-T1	33	<.001	.014	<.001	<.001	.009	<.001
	T1-T2	31	<.001	.999	.021	.034	.174	.002
	T2-T3	26	.022	.999	.197	.095	.999	.005
STG	Tpre	33	30.7 ± 15.0	4.4 ± 2.4	1.9 ± 2.5	4.1 ± 1.7	79.9 ± 14.0	19.8 ± 7.6
			34 (24)	5 (2)	0 (2)	4 (3)	81 (18)	22 (12)
	T0	33	30.8 ± 14.9	4.4 ± 2.4	1.9 ± 2.5	4.1 ± 1.8	80.1 ± 14.0	19.8 ± 7.6
			34 (24)	5 (2)	0 (3)	4 (3)	81 (17)	22 (12)
	T1	33	36.8 ± 15.7	4.2 ± 2.4	0.9 ± 1.7	3.1 ± 1.6	80.8 ± 14.6	24.4 ± 85
			39 (23)	4 (3)	0 (1)	3 (2)	81 (18)	25 (14)
	T2	31	37.4 ± 16.3	4.1 ± 2.4	0.7 ± 1.5	3.0 ± 1.5	81.0 ± 14.0	25.0 ± 8.6
			40 (24)	4 (3)	0 (0)	3 (2)	81 (19)	27 (11)
	T3	24	36.1 ± 16.6	3.8 ± 2.6	0.7 ± 1.7	2.7 ± 1.4	79.3 ± 15.1	25.4 ± 8.8
			37 (24)	3 (3)	0 (0)	3 (3)	80 (85)	27 (24)
Friedman's *P*-value		24	<.001	<.001	<.001	<.001	.004	<.001
Post-hoc test *P*-values	T0-T1	33	<.001	.008	.003	<.001	.014	<.001
	T1-T2	31	.001	.346	.174	.182	.181	.003
	T2-T3	24	.170	.999	.999	.346	.999	.008

Abbreviations: tDCS, transcranial direct current stimulation; RTG, real-tDCS group; STG, Sham-tDCS group; N, sample number; FM-UE, Fugl–Meyer assessment scale for Upper Extremity; NIHSS, National Institute Health Stroke Scale; VAS, Visual Analogue Scale; MAS, Modified Ashworth Scale; BI, Barthel Index; MRC, Medical Research Council Scale.

MEP amplitude was 1516 ± 1616 μV versus 1165 ± 1149 μV ($P=.064$) for the 2 groups. Patients with MEP=0 in the affected hemisphere did not recover MEP after rehabilitation. However, these patients showed a significant ($P=.05$) reduction of 22% in the MEP of the unaffected hemisphere. However, the related MEPbal was not significantly different between RTG and STG groups ($P=.344$), or in the subgroups without MEP at T0 ($P=.598$).

A post-hoc analysis was performed stratifying patients according to their initial MEP. Neither those with any MEP at T0 ($P=.458$), nor the others ($P=.902$), showed significant differences between RTG and STG in terms of FM-UE scores. Despite not being statistically significant, the number of patients who overcame the MCID was higher in the RTG for patients without MEP in the lesioned brain hemisphere (RTG: 72% vs STG: 42%, $P=.094$) and was higher in the STG for those with MEP (RTG: 62.5% vs STG: 70%, $P=.737$). The percentage gain in terms of FM-UE score was significantly different among the 4 groups of patients when divided by type of tDCS (real vs sham) and MEP (present/absent) in the affected hemisphere ($F=4.6$, $P=.007$, ES=0.239). In fact, a significantly higher gain was observed in patients without MEP included in the RTG (67.8% ± 46.0%) compared to patients with MEP both of the RTG (22.8% ± 10.1%, $P=.018$) and of the STG (27.7% ± 24.0%, $P=.025$). The gain obtained by patients included in the STG without MEP was 41.6% ± 30.8% and was not significantly different from patients in STG with MEP ($P=.189$) nor from patients of RTG without MEP ($P=.123$). The distributions of changes in FM-UE scores

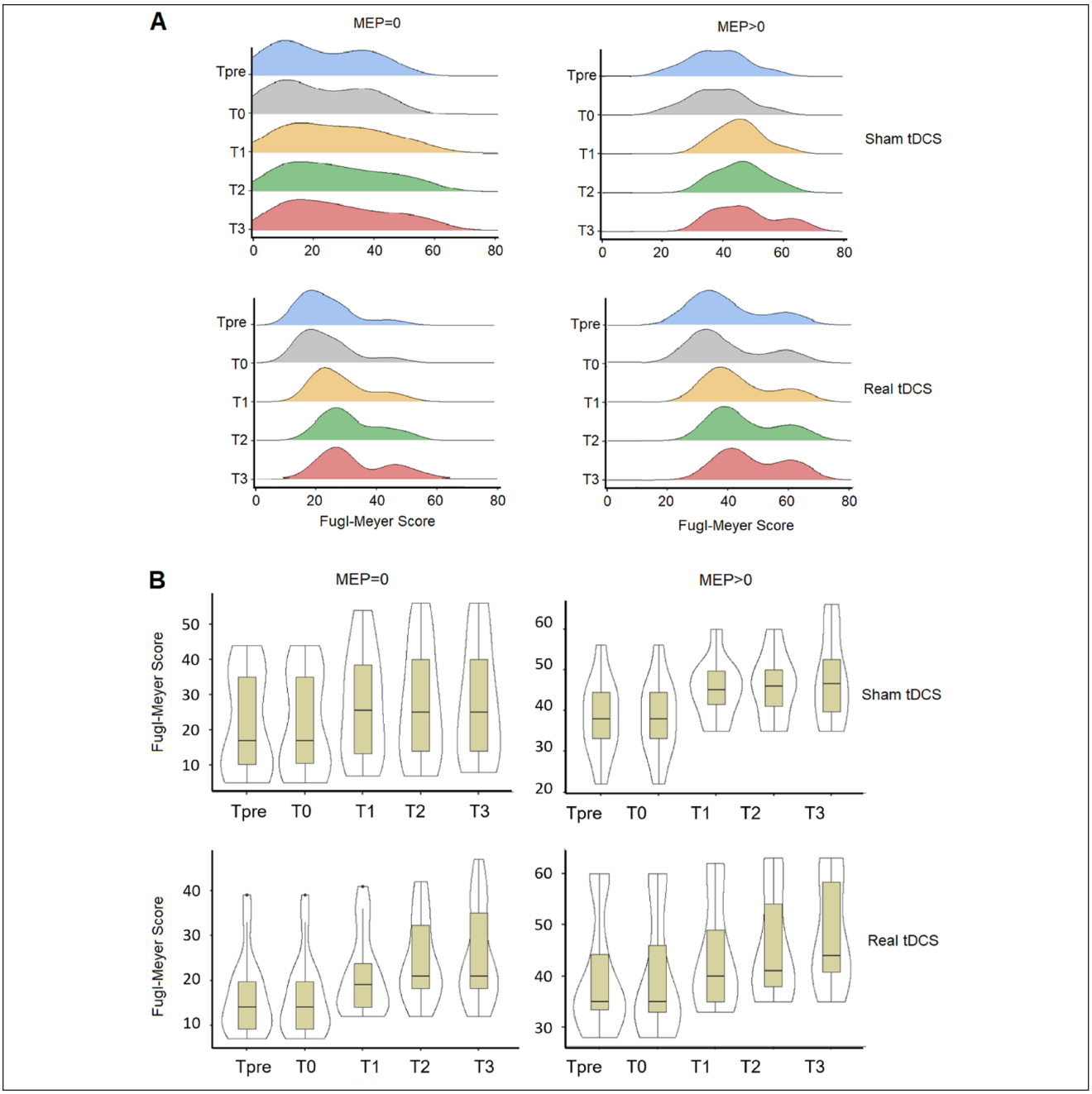

Figure 2. (A) Density distribution of the Fugl–Meyer scores in the 4 subgroups plotted with respect to the timing of assessment. (B) Box-whiskers violin plot of the same data: the boxes represent the data between first and third quartiles and the bold horizontal lines the medians of the data.

Abbreviations: MEP, motor evoked potential; tDCS, transcranial direct current stimulation.

are shown in Figure 2: for RTG without MEP, not only did the mean peak move toward higher FM-UE scores, but there was also the formation of a secondary peak around a FM-UE score of 45. Friedman's analysis showed significant improvements in all 4 groups, as shown in Figure 2 ($P < .001$). The same analysis conducted on the secondary outcomes was significant for all 4 groups and all of the scales ($P < .05$) except for VAS-score for RTG with MEP ($P = .510$) and for BI-score for STG without MEP ($P = .117$) and RTG with MEP ($P = .194$).

Discussion

Our clinical trial showed that dual tDCS does not enhance the effect of a short, intensive, upper limb robot-assisted therapeutic intervention in chronic stroke patients. In fact, except for the increase in the ROM of upper limb (shoulder rotation and prono-supination), no other statistically different effects were found in clinical and neurophysiological assessments between real and sham stimulations coupled with robot-assisted therapy.

This not relevant effect of tDCS coupled with robot-assisted therapy found in our trial is in line with a recent meta-analysis conducted on randomized controlled trials that did not highlight any significant improvement provided by tDCS to the effects of robot-assisted therapy.[12]

The lack of synergistic effect between tDCS and robotic-rehabilitation observed in this trial could have different causes. Recovery of upper limb functionality after stroke is a very complex phenomenon that varies widely between subjects. Different individual factors (such as age, gender, stroke location and size, genetics) can influence this process and should be considered in the choice of a better strategy to promote recovery. In our trial, according to the interhemispheric competition model,[9] we used dual tDCS to restore the interhemispheric balance by simultaneously inhibiting the unaffected hemisphere and exciting the affected hemisphere. However, this model can be oversimplified or even incorrect in some circumstances. For instance, it has been demonstrated that in patients with a smaller stroke, the most effective strategy is to improve the functionality of affected hemisphere, while it is better to improve the functionality of the contralateral, unaffected hemisphere in patients with a larger stroke.[9] Moreover, it is possible that preliminary exposure to dual tDCS aimed at increasing the excitability of affected motor cortex can interfere with motor learning induced by robotic rehabilitation (by a ceiling effect). Indeed, according to homeostatic metaplasticity rules,[31] an effective strategy to boost learning is to decrease rather than increase neuronal activity in the motor cortex before practice. This is also suggested by the results of a proof of principle study evaluating the effects of physical therapy associated with an NIBS protocol suppressing the excitability of the affected hemisphere with the aim of promoting motor recovery through a homeostatic interaction between brain stimulation and motor training.[32] We should highlight the absence of a control group performing the same amount of conventional (non-robotic) therapy, so there is the need for caution when affirming the efficacy of a short intensive robotic intervention, as it is not clear whether this efficacy was due to the assistance provided by the robot. However, in a previous study, it was demonstrated that an exoskeleton robot might enhance motor function in a chronically impaired paretic arm after 24 sessions.[33] Despite authors finding an estimated marginal mean difference of 0.78 (higher than our effect size of 0.32), we have showed for the first time that a shorter, less intensive training, with 10 sessions in 2 weeks, is also effective in improving motor function.

Another interesting result is that the improvement in motor function continued at the 1-month follow-up for both groups (real and sham) and that this improvement was maintained at the 3-month follow-up for the sham group and further increased in the RTG. The long-term maintenance of the motor learning of the tDCS after 3 months or more was recently described by a systematic review with metanalysis.[34]

In the secondary analysis, we evaluated the potential effects of robot-assisted therapy coupled with dual tDCS on other clinical measures exploring neurological status (NIHSS), upper limb strength (MRC), pain (VAS), spasticity (MAS), and independence in the activities of daily living (BI). We found that all of the administered scales improved after experimental intervention, without any significant differences between groups.

Moreover, in the evaluation of the ranges of motion of the affected UL through a specific function of the exoskeleton, we found a significant increase in shoulder rotation and forearm prono-supination in the group of patients treated with real tDCS. This finding suggests that dual tDCS, associated with robotic rehabilitation, can enhance the motricity of the upper limb in chronic stroke patients. However, this improvement in the ROM does not strictly reflect clinical or functional gain.[35]

The adjunctive benefits induced by tDCS when coupled with robot-assisted therapy were statistically significant only in patients with specific features and neurophysiological measures can be useful to identify these best responders. Indeed, our analysis showed that all subgroups (RTG with MEP, RTG without MEP, STG with MEP, STG without MEP) improved but the highest gain was observed in patients without MEP in the affected hemisphere for whom robot-assisted therapy was associated with real tDCS. It should be noted that the higher gains observed in patients without MEP with respect to those with MEP could be affected by the fact that these patients were more severely affected and the percentage increase was computed with respect to their lower baseline values. However, this potential methodological bias should also be observed in the STG; instead, in these patients, the gain was no different between those with and without MEP. This suggests a real effect of tDCS in patients without MEP.

An explanation of the higher effect of tDCS in patients without MEP could be related to the fact that stroke not only leads to reduced excitability in the primary motor cortex (M1) of the affected hemisphere, but also often to the increased excitability of the contralateral M1. In healthy subjects, the 2 cerebral hemispheres find themselves in a state of balanced mutual inhibition. Stroke often impairs this interhemispheric balance, leading to the decreased inhibition of the contralesional hemisphere by the affected

hemisphere and, in turn, to the increased inhibition of the affected hemisphere by the contralesional one. Permanence of healthy hemisphere hyperexcitability in chronic phase after stroke is usually an index of little functional recovery and can be correlated with greater ipsilateral structural damage. Accordingly, in our sample, the lack of MEP in the affected hemisphere could identify those patients in which there is a greater inhibition of the affected hemisphere by the contralesional one. In this situation, dual tDCS could be more effective because inhibition of the unaffected hemisphere (by cathodal stimulation) can reduce its pathological inhibition toward the affected hemisphere and potentiates the direct activation of anodal stimulation on the affected hemisphere. According to Lindenberg et al, a bihemispheric brain stimulation and the consequent increased excitability of the ipsilesional hemisphere leads to the activation of alternative tracts, for example, the intact ipsilesional primary motor regions and adjacent non-primary motor regions. In addition, a dual brain stimulation concurrent to a peripheral sensorimotor training, in our study performed with the robot, might potentiate motor outcome and the related plastic changes.[36,37] However, in other cases, inhibition of the unaffected hemisphere (by cathodal tDCS) could have a detrimental effect because it reduces the part of the functionality depending on this hemisphere.[38]

The findings of the present study should be carefully taken into account in light of the limitations of our study. First of all, our analyses, stratifying patients bases on the MEP at T0, was not planned a priori, but was instead a post-hoc analysis; therefore, further studies with a greater sample size are needed to better investigate this point. Second, the absence of a control group performing the same amount of conventional (non-robotic) therapy prevents us from drawing definite conclusions about the efficacy of a short intensive rehabilitative robotic intervention, mainly because this was not the aim of the current trial.

The most important strengths of our study were the design (randomized clinical trial with parallel assignment and quadruple masking), the sample size (previously statistically evaluated), and the use of neurophysiological tools to identify the potential responders of experimental intervention.

Conclusions

In conclusion, the association of dual tDCS did not generally enhance the efficacy of robot-assisted therapy, but some of our results may suggest a slight effect on a few of the patients that could be identified by means of neurophysiological tools. In particular, in patients without MEP in the affected hemisphere (usually related to a more severe motor impairment), robotic rehabilitation could be empowered by a dual tDCS protocol administered before robotic training. Further studies on more samples are mandatory to confirm these results and identify specific subgroups of patients that could benefit from the association of dual-tDCS with robot-assisted therapy.

Author Contributions

Conceptualization, V.D.L., G.M., S.P., and M.I.; methodology, V.D.L., M.G., S.P., and M.I.; formal analysis, M.I.; investigation, A.M.C., A.C., F.C., N.B., and C.C.; writing—original draft preparation, F.C., A.M.C., G.M., and M.I.; writing—review and editing, V.D.L. and S.P.; All authors have read and have agreed the manuscript.

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References

1. Kwakkel G, Kollen BJ, van der Grond J, Prevo AJ. Probability of regaining dexterity in the flaccid upper limb: impact of severity of paresis and time since onset in acute stroke. *Stroke*. 2003;34:2181-2186.
2. Koh CL, Pan SL, Jeng JS, et al. Predicting recovery of voluntary upper extremity movement in subacute stroke patients with severe upper extremity paresis. *PLoS One*. 2015;10:e0126857.
3. Joy MT, Carmichael ST. Encouraging an excitable brain state: mechanisms of brain repair in stroke. *Nat Rev Neurosci*. 2021;22:38-53.
4. Joy MT, Carmichael ST. Learning and stroke recovery: parallelism of biological substrates. *Semin Neurol*. 2021;41:147-156.
5. Morone G, Palomba A, Martino Cinnera A, et al; "CICERONE" Italian Consensus Conference on Robotic in Neurorehabilitation. Systematic review of guidelines to identify recommendations for upper limb robotic rehabilitation after stroke. *Eur J Phys Rehabil Med*. 2021;57:238-245.
6. Micera S, Caleo M, Chisari C, Hummel FC, Pedrocchi A. Advanced neurotechnologies for the restoration of motor function. *Neuron*. 2020;105:604-620.
7. Gandolfi M, Valè N, Posteraro F, et al; Italian Consensus Conference on Robotic in Neurorehabilitation CICERONE. State of the art and challenges for the classification of studies on

electromechanical and robotic devices in neurorehabilitation: a scoping review. *Eur J Phys Rehabil Med*. 2021;57:831-840.
8. Wu J, Cheng H, Zhang J, Yang S, Cai S. Robot-assisted therapy for upper extremity motor impairment after stroke: a systematic review and meta-analysis. *Phys Ther*. 2021;101:pzab010.
9. Di Pino G, Pellegrino G, Assenza G, et al. Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat Rev Neurol*. 2014;10:597-608.
10. Fuentes Calderón MA, Miralles AN, Pimienta MJ, Estella JMG, Ledesma MJS. Analysis of the factors related to the effectiveness of transcranial current stimulation in upper limb motor function recovery after stroke: a systematic review. *J Med Syst*. 2019;43:69.
11. Cassani R, Novak GS, Falk TH, Oliveira AA. Virtual reality and non-invasive brain stimulation for rehabilitation applications: a systematic review. *J Neuroeng Rehabil*. 2020;17:147.
12. Reis SB, Bernardo WM, Oshiro CA, Krebs HI, Conforto AB. Effects of robotic therapy associated with noninvasive brain stimulation on upper-limb rehabilitation after stroke: systematic review and meta-analysis of randomized clinical trials. *Neurorehabil Neural Repair*. 2021;35:256-266.
13. Morone G, Iosa M, Bragoni M, et al. Who may have durable benefit from robotic gait training? A 2-year follow-up randomized controlled trial in patients with subacute stroke. *Stroke*. 2012;43:1140-1142.
14. Morone G, Bragoni M, Iosa M, et al. Who may benefit from robotic-assisted gait training? A randomized clinical trial in patients with subacute stroke. *Neurorehabil Neural Repair*. 2011;25:636-644.
15. Di Lazzaro V, Profice P, Pilato F, et al. Motor cortex plasticity predicts recovery in acute stroke. *Cereb Cortex*. 2010;20(7):1523-1528.
16. Di Lazzaro V, Dileone M, Capone F, et al. Immediate and late modulation of interhemipheric imbalance with bilateral transcranial direct current stimulation in acute stroke. *Brain Stimul*. 2014;7:841-848.
17. Di Lazzaro V, Manganelli F, Dileone M, et al. The effects of prolonged cathodal direct current stimulation on the excitatory and inhibitory circuits of the ipsilateral and contralateral motor cortex. *J Neural Transm (Vienna)*. 2012;119:1499-1506.
18. Kuo IJ, Tang CW, Tsai YA, et al. Neurophysiological signatures of hand motor response to dual-transcranial direct current stimulation in subacute stroke: a TMS and MEG study. *J Neuroeng Rehabil*. 2020;17:72.
19. Zappasodi F, Musumeci G, Navarra R, Di Lazzaro V, Caulo M, Uncini A. Safety and effects on motor cortex excitability of five cathodal transcranial direct current stimulation sessions in 25hours. *Neurophysiol Clin*. 2018;48:77-87.
20. Zappasodi F, Musumeci G, Navarra R, Di Lazzaro V, Caulo M, Uncini A. Safety and effects on motor cortex excitability of five anodal transcranial direct current stimulation sessions in 24hours. *Neurophysiol Clin*. 2019;49:19-25.
21. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12:189-198.
22. French JRJ. Field experiments: changing group productivity. In: Miller JG, ed. *Experiments in Social Process: A Symposium on Social Psychology*. McGraw-Hill; 1950:81-96.
23. French JRJ. Experiments in field settings. In: Festinger L, Katz D, eds. *Research Methods in the Behavioral Sciences*. Holt, Rinehart and Winston; 1953:98-135.
24. Matano A, Iosa M, Guariglia C, Pizzamiglio L, Paolucci S. Does outcome of neuropsychological treatment in patients with unilateral spatial neglect after stroke affect functional outcome? *Eur J Phys Rehabil Med*. 2015;51:737-743.
25. Fusco A, Iosa M, Venturiero V, et al. After vs. Priming effects of anodal transcranial direct current stimulation on upper extremity motor recovery in patients with subacute stroke. *Restor Neurol Neurosci*. 2014;32:301-312.
26. Gladstone DJ, Danells CJ, Black SE. The Fugl-Meyer assessment of motor recovery after stroke: a critical review of its measurement properties. *Neurorehabil Neural Repair*. 2002;16:232-240.
27. Page SJ, Fulk GD, Boyne P. Clinically important differences for the upper-extremity Fugl-Meyer Scale in people with minimal to moderate impairment due to chronic stroke. *Phys Ther*. 2012;92:791-798.
28. Brott T, Adams HP Jr, Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke*. 1989;20:864-870.
29. Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther*. 1987;67:206-207.
30. Stinear CM, Barber PA, Petoe M, Anwar S, Byblow WD. The PREP algorithm predicts potential for upper limb recovery after stroke. *Brain*. 2012;135:2527-2535.
31. Ziemann U, Siebner HR. Modifying motor learning through gating and homeostatic metaplasticity. *Brain Stimul*. 2008;1:60-66.
32. Di Lazzaro V, Rothwell JC, Talelli P, et al. Inhibitory theta burst stimulation of affected hemisphere in chronic stroke: a proof of principle, sham-controlled study. *Neurosci Lett*. 2013;553:148-152.
33. Klamroth-Marganska V, Blanco J, Campen K, et al. Three-dimensional, task-specific robot therapy of the arm after stroke: a multicentre, parallel-group randomised trial. *Lancet Neurol*. 2014;13:159-166.
34. Navarro-López V, Del Valle-Gratacós M, Fernández-Matías R. The long-term maintenance of upper limb motor improvements following transcranial direct current stimulation combined with rehabilitation in people with stroke: a systematic review of randomized sham-controlled trials. *Sensors (Basel)*. 2021;21:5216.
35. Iosa M, Martino Cinnera A, Capone F, et al. Clinical interpretation of working volume and weight support in upper limb robotic neurorehabilitation after stroke. *Appl Sci*. 2021;11:12123.
36. Lindenberg R, Renga V, Zhu LL, Nair D, Schlaug G. Bihemispheric brain stimulation facilitates motor recovery in chronic stroke patients. *Neurology*. 2010;75:2176-2184.
37. Shultz AM, Lee S, Guaraldi M, Shea TB, Yanco HC. Robot-embodied neuronal networks as an interactive model of learning. *Open Neurol J*. 2017;11:39-47.
38. Casula EP, Pellicciari MC, Bonnì S, et al. Evidence for interhemispheric imbalance in stroke patients as revealed by combining transcranial magnetic stimulation and electroencephalography. *Hum Brain Mapp*. 2021;42:1343-1358.