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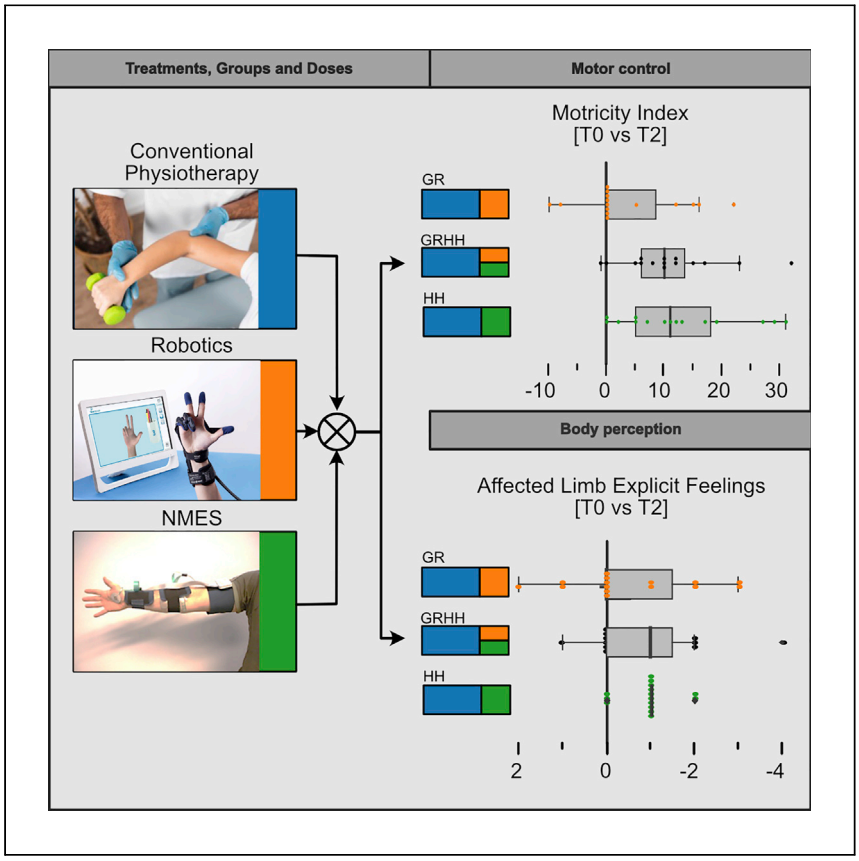

Crema et al. propose a new intervention based on a rich sensorimotor stimulation of the upper limb that is able to promote recovery of sensory and motor functions as well as body perception in chronic stroke patients.

Andrea Crema, Michela Bassolino, Eleonora Guanziroli, ..., Andrea Serino, Silvestro Micera, Franco Molteni

andrea.crema@epfl.ch

Highlights

Sensory recovery is often difficult to achieve in stroke survivors

A new NMES system improves sensory and motor recovery in chronic stroke patients

Novel assessment approaches highlight the impact of rehabilitation protocols

Motor improvements are correlated with the improved perception of the affected limb

Translation to Patients

Crema et al., Med 3, 58–74
January 14, 2022
https://doi.org/10.1016/j.medj.2021.12.001

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Neuromuscular electrical stimulation restores upper limb sensory-motor functions and body representations in chronic stroke survivors

Andrea Crema,[1,8,9,11,*] Michela Bassolino,[2,3,4,9] Eleonora Guanziroli,[5,9] Maria Colombo,[5] Olaf Blanke,[2,6] Andrea Serino,[3,4,10] Silvestro Micera,[1,7,10] and Franco Molteni[5,10]

SUMMARY

Background: A conventional treatment outcome is suboptimal for sensory impairments in stroke patients. Novel approaches based on electrical stimulation or robotics are proposed as an adjuvant for rehabilitation, though their efficacy for motor, sensory, and body representation recovery have not been tested.

Methods: Sixty chronic stroke patients with unilateral motor deficits were included in a pseudo-randomized open-label multi-arm control trial (ClinicalTrials.gov: NCT03349138). We tested the effects of a robotic glove (GloReha [GR]) and a new neuromuscular electrical stimulation system (Helping Hand [HH]) and compared them with conventional treatment (CT) in restoring motor and sensory functions and the affected limb perception. HH was designed to concurrently deliver peripheral motor activation and enhanced cutaneous sensation. Patients were split in four dose-matched groups: CT, GR, HH, and GRHH (receiving 50% GR and 50% HH). Assessments were performed at inclusion, halfway, end of treatment (week 9), and follow-up (week 13).

Findings: HH provided an earlier benefit, quantified by the Motricity Index (MI), than GR. At the end of the treatment, the amelioration was higher in groups GRHH and HH and extended to somatosensory functions. These benefits persisted at the follow-up. GRHH and HH also improved the perceived dimensions and altered feeling toward the affected limb. Interestingly, the reduction of altered feelings correlated with MI improvements and depended on the amount of HH.

Conclusions: We suggest that HH concurrently stimulates sensory and motor systems by generating an enhanced cutaneous sensation, coherent in location with the elicited motor recruitment, leading to ameliorated sensorimotor functions and bodily perceptions in stroke patients.

Funding: This work was supported by a Foundation advised by CARIGEST, by Fondazione CARIPLO, by the SNSF NCCR Robotics, and by the Bertarelli Foundation.

Context and significance

Patients after stroke show impairments in sensory abilities (e.g., feeling touch) and alterations in how they perceive their own body (e.g., "my affected arm feels as if it is not part of my body"). Both of these aspects are poorly addressed by current rehabilitative interventions.

The INCOGNITO study proposes a new wearable technological intervention based on electrical stimulation, stimulating at the same time the capacities of patients to move and sense the affected arm, as a supplement to conventional therapy. Results show that the new proposed intervention is able to improve sensory and motor functions as well as how patients perceive their body, even several months after stroke.

INTRODUCTION

Cerebrovascular accidents (CVAs) are one of the leading causes of disability, resulting in multiple functional impairments.[1] Upper extremity deficits, in particular, have been shown to have a significant impact on a person's ability to perform the activities of daily living,[2] thus negatively affecting their quality of life. Immediately after the stroke, 85% of patients suffer an impairment to upper-limb functions.[3] These impairments persist in 55%–75% of stroke patients in chronic conditions (i.e., at 6 months

after the event). Upper-limb impairments after CVAs are not limited to motor functions but also involve sensory functions, impacting multiple aspects of patients' behavior. Deficits in somatosensory function after stroke are common, with up to 53% of stroke patients showing impaired tactile sensations, up to 80% showing impaired stereognosis, and up to 64% showing impaired proprioception.[4] Motor and somatosensory deficits are also associated with disorders of body representations,[5,6] affecting the implicit and explicit perception of patients' bodies and impacting the way patients use their body to interact with objects in their environment. At the phenomenological level, body representation disorders can also result in a lack of ownership (i.e., the subjective experience that the body is one's own) and agency (the feeling of being in control of one's own body's movements), two fundamental components of "embodiment."[7]

Novel technologies based on robotics,[8] neuromuscular electrical stimulation[9] (NMES), non-invasive brain stimulation,[10] or virtual reality have been proposed in the last years as adjuvant tools for rehabilitation. Their clinical efficacy for multiple aspects of recovery (motor, sensory, body representation) is not yet clear, especially for chronic stroke survivors. In particular, although sensory deficits are very common in stroke survivors,[11] the possibility of obtaining effective sensory restoration after neurorehabilitation is still limited.[12] Moreover, technology-based approaches of evaluation and treatment are far from being fully integrated into the clinical practice because of their intrinsic limits in acceptability, due to their difficult set up, usability issues, and patients' and therapists' perceptions of the technology.[13]

INCOGNITO is an open-label clinical trial aimed at implementing an Integrated Cognitive, Sensory, and Motor Rehabilitation of Hand Functions. Study registration, ethical approval, and patient consent are reported in STAR Methods.

Detailed inclusion criteria, treatment protocol, primary and secondary outcomes, sample size determination, and a statistical analysis plan are also noted in the STAR Methods. The CONSORT diagram is reported in Figure 1. Informed consent was obtained from all subjects.

In this clinical trial, we tested the efficacy of different technological devices with chronic stroke survivors in comparison with conventional care. In addition to the assessment of motor improvements, we provide, for the first time, a comprehensive characterization of the effectiveness of these devices for sensory functions (tactile acuity [TA]) as well as on deficits in body representation (implicit perceived size of the contra- and ipsi- lesional upper limbs though body landmarks localization tasks and explicit feelings toward the affected limb via the affected limb explicit feeling questionnaire [ALEFq]; see Supplemental information and Sorrentino et al.[14]). In this study, we exploited a new wearable system for NMES (the "Helping Hand" [HH] system;[15,16] Figure 2) designed to provide a custom motor and sensory stimulation to extrinsic hand extensors through its electrode arrays and a semi-automatic procedure for therapist-driven calibration. The novelty of HH consists of the shape and the patterning of activation of the electrodes, empirically optimized to concurrently provide muscle activation and cutaneous stimulation (see Method details). We also exploited a hand robotic system (GloReha [GR]). Patients were allocated into four groups, each receiving a supplemental dose of treatment: CT for supplemental conventional treatment, GR for supplemental robotic treatment, GRHH for a supplemental half-dose of GR and half-dose of HH, and HH for supplemental NMES treatment.

[1]Bertarelli Foundation Chair in Translational Neuroengineering, Centre for Neuroprosthetics and Institute of Bioengineering, School of Engineering, École Polytechnique Fédérale de Lausanne (EPFL), Geneva 1202, Switzerland

[2]Laboratory of Cognitive Neuroscience, Center for Neuroprosthetics and Brain Mind Institute, School of Life Science, Swiss Federal Institute of Technology (EPFL), Geneva 1202, Switzerland

[3]Institute of Health, School of Health Sciences, HES-SO Valais-Wallis, Sion 1950, Switzerland

[4]Department of Clinical Neuroscience, Centre Hospitalier Universitaire Vaudois (CHUV), MySpace Lab, Lausanne 1011, Switzerland

[5]Villa Beretta Rehabilitation Center, Valduce Hospital Como, Costa Masnaga 23845, Italy

[6]Department of Clinical Neuroscience, University of Geneva Medical School, Geneva 1211, Switzerland

[7]The Biorobotics Institute and Department of Excellence in Robotics and AI, Scuola Superiore Sant'Anna, Pontedera 56025, Italy

[8]AGO Neurotechnologies, sàrl, Geneva 1201, Switzerland

[9]These authors contributed equally

[10]These authors contributed equally

[11]Lead contact

*Correspondence: andrea.crema@epfl.ch

https://doi.org/10.1016/j.medj.2021.12.001

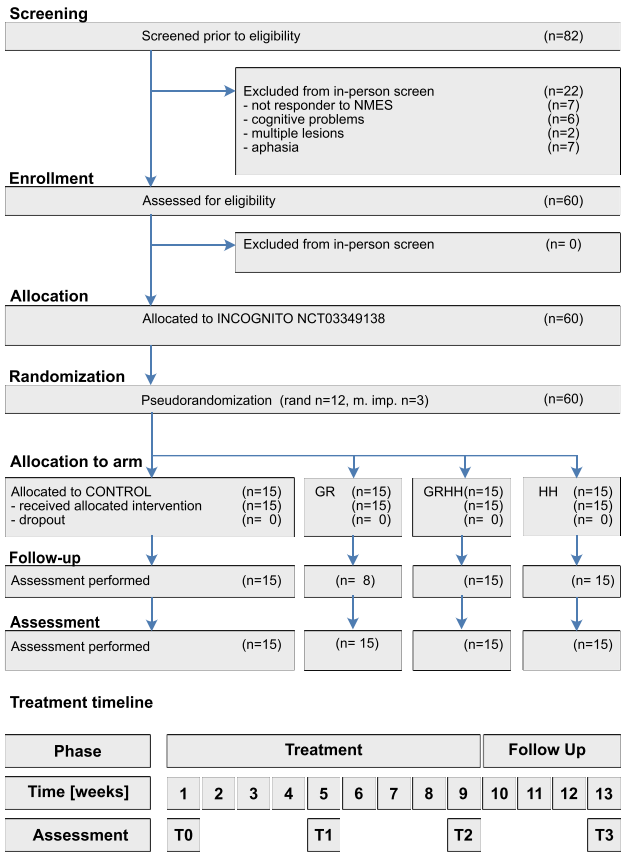

Figure 1. CONSORT and timeline

Top: Consolidated Standards of Reporting Trials diagram. Depiction of subject selection, group allocation, attrition, and data analysis. CT, conventional group; GR, GloReha group; HH, Helping Hand group; GRHH, GloReha and Helping Hand group. Bottom: treatment timeline diagram.

The results show that, in addition to motor function, HH is able to restore sensory function and improve body representation. This result opens up important clinical opportunities to improve the quality of life of chronic stroke survivors.

RESULTS

Patients (n= 60) with unilateral stroke were recruited between September 29th, 2015, and March 14th, 2017, from a pre-existing pool of 82 chronic stroke patients, allocated to each of the four groups (n = 15), and included in the analysis as reported in Figure 1. No adverse effects were reported, and no dropout occurred.

Motor, sensory, and body representation baseline characteristics

The baseline demographic, motor, somatosensory, and body representations characteristics of the population are reported in Table 1. Baseline features' distributions are reported in Table S1. The main outcomes are summarized in Table 2.

The patients' distribution of all the different groups at inclusion was not homogeneous for the primary outcome of the study—the Motricity Index (MI; Table S1; MI total score Kruskal-Wallis, s = 13.3583 p = 0.0039)—or heteroscedastic, due to lower

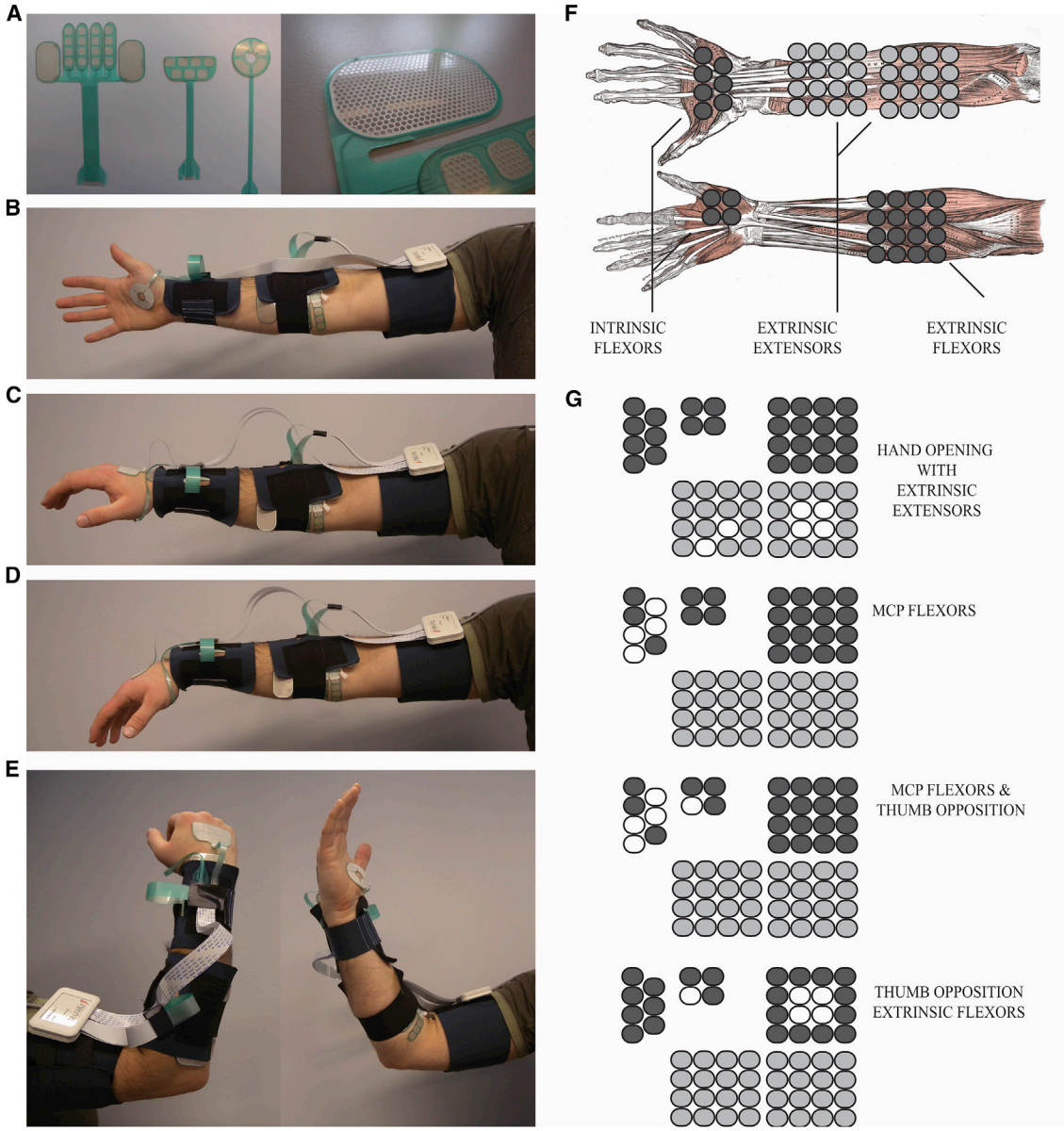

Figure 2. Helping Hand (HH) wearable NMES system

(A) Specialized electrode arrays designed for the study. (B–E) Details of the fully mounted device. (F) Detail of the targeted areas. (G) A possible activation configuration for extrinsic extensors; one virtual electrode (VE) targets the proximal extensors for eliciting extension of fingers 1 to 4, and a second VE targets the thumb extension. Stimulation maps sequenced for first eliciting extension grasp with the array on the dorsal side of the hand, then shift to tip/lateral grasp by eliciting the thumb through the array on the thenar eminence, and to finally reach power grasp with thumb support. The initial eliciting of intrinsic flexors aims at avoiding a claw grasp that may arise from targeting only the extrinsic proximal flexors.

scores in the CT group. However, by considering only the "technology-based" treatment groups (GR, HH, GRHH), motor impairment at baseline was not different (Table S1; MI total, s = 0.6907, p = 0.7080). This pattern was observed in all secondary motor outcomes. For this reason, data originating from the CT group are excluded by the motor outcome analysis.

The patients' distribution of all the different groups at inclusion was homogeneous for ALEFq, body landmarks localization task (BLT), and TA (Kruskal-Wallis, all p > 0.5500).

Table 1. Baseline characteristics of the intention to treat population

		Groups (n = 60)			
Scale	Feature	CT (n = 15)	GR (n = 15)	GRHH (N = 15)	HH (n= 15)
Sex	male (%)	11 (73)	10 (67)	9 (60)	10 (67)
	female (%)	4 (27)	5 (23)	6 (40)	5 (23)
Age		61 (54 to 67.5)	49 (40 to 62.5)	61 (47.5 to 65.5)	49 (40 to 62.5)
Stroke Type	hemorrhagic (%)	4 (27)	2 (13)	4 (27)	2 (13)
	ischemic (%)	11 (73)	13 (87)	11 (73)	13 (87)
Affected side of the body	left (%)	9 (60)	9 (60)	8 (53)	9 (60)
	right (%)	6 (40)	6 (40)	7 (47)	6 (40)
MI	total[0:100]	29 (24 to 42)	67 (49 to 75)	60 (47.5 to 69)	66 (55 to 75)
	pinch [0:33]	0 (0 to 5.5)	22 (15 to 24)	19 (11 to 22)	22 (11 to 26)
	elbow [0:33]	14 (9 to 19)	25 (14 to 25)	25 (16.5 to 25)	25 (22 to 25)
	abduction[0:33]	14 (14 to 16.5)	22 (14 to 25)	19 (19 to 22)	19 (16.5 to 25)
ARAT	total[0:57]	0 (0 to 3.5)	18 (8 to 46)	12 (4.5 to 45.5)	31 (9 to 48)
	grasp [0:18]	0 (0 to 0)	11 (0.5 to 15.5)	4 (0 to 16.5)	13 (1 to 18)
	grip [0:12]	0 (0 to 0)	4 (1 to 10)	4 (0 to 8)	8 (3 to 12)
	pinch [0:12]	0 (0 to 0)	0 (0 to 12.5)	0 (0 to 11)	3 (0 to 11.5)
	gross movement[0:9]	0 (0 to 3.5)	6 (3 to 7)	5 (4 to 7)	5 (4.5 to 8.5)
MAL	QOM	0 (0 to 0)	10 (0 to 23)	9 (0 to 37.5)	21 (2 to 36)
	AOU	0 (0 to 0)	12 (0 to 33)	9 (0 to 40.5)	22 (5 to 32)
BBT	contralesional	0 (0 to 0)	7 (0 to 18.5)	1 (0 to 21)	13 (1.5 to 17)
	ipsilesional	42 (31.5 to 52)	39 (28.5 to 53)	39 (36 to 49.5)	42 (35.5 to 51)
MRC	TOT [0:5]	11 (6.5 to 18.5)	31 (15 to 33.5)	25 (16.5 to 29)	28 (24.5 to 32.5)
	DA [0:5]	1 (1 to 3)	3 (2 to 4)	3 (3 to 4)	4 (3 to 4)
	DM [0:5]	2 (2 to 3.5)	3 (2 to 4)	3 (3 to 4)	3 (3 to 4)
	DP [0:5]	2 (1 to 2.5)	3 (2 to 4)	3 (3 to 3.5)	3 (3 to 4)
	EE [0:5]	1 (0 to 2)	3 (1.5 to 4)	3 (1.5 to 4)	3 (2 to 4)
	EF [0:5]	2 (1 to 3)	4 (2 to 4)	4 (2.5 to 4)	4 (3.5 to 4)
	WE [0:5]	0 (0 to 1)	3 (0.5 to 4)	1 (1 to 3)	3 (2 to 4)
	WF [0:5]	1 (0 to 2)	3 (1.5 to 4)	2 (1 to 3)	3 (1.5 to 3.5)
	FE [0:5]	0 (0 to 1)	2 (1 to 3)	1 (0.5 to 3)	2 (1 to 3)
	FF [0:5]	1 (0 to 2.5)	3 (2 to 4)	3 (2 to 3)	4 (3 to 4)
TA	arm	5 (3.5 to 6)	5 (4 to 5.75)	5 (3 to 6)	4 (3 to 6)
	CT = 12, GR = 14, GRHH = 15, HH = 13				
	forearm	4 (3.25 to 6)	4 (3 to 5.75)	5 (2.5 to 6)	4 (3 to 7)
	CT = 10, GR = 14, GRHH = 15, HH = 13				
	hand	3 (2 to 4.25)	4 (3 to 6)	2.5 (2 to 5.5)	3.5 (2.75 to 4)
	CT = 8, GR = 13, GRHH = 14, HH = 12				
ALEFq	Total (min:max)	4 (3 to 5)	3 (2.5 to 5)	4 (3 to 5)	3 (3 to 4)
BLT	ipsilesional ratio (%)	95.1 (81.4 to 114.45)	99.50 (86.0 to 112.30)	107.8 (92.6 to 113.9)	100.50 (84.00 to 108.05)
	contralesional ratio (%)	90.1 (85.85 to 106)	96.2 (77.9 to 101.1)	100.1 (78.75 to 112.35)	84.70 (77.30 to 100.90)

Data are n (%), median (IQR), or ratios (IQR). n = 60 or less. MI, Motricity Index; ARAT, Action Research Arm Test; MAL, Motor Activity Log; QOM, quality of movement; AOU, amount of use; BBT, Box and Blocks test; MRC, Medical Research Council scale; DA, deltoid anterior; DM, deltoid medialis; DP, deltoid posterior; EE, elbow extensors; EF, elbow flexors; WE, wrist extensors; WF, wrist flexors; FE, fingers extensors; FF, fingers flexors; TA, tactile acuity; ALEFq, affected limb explicit feelings questionnaire; BLT, body landmarks localization task.

However, a higher prevalence of patients with no measurable TA at inclusion was visible only in the CT group because of severe sensory deficits at baseline in this group but not in the other groups (e.g., TA hand, CT: 7 no measurable patients, while a maximum of 2 patients in GR, HH, GRHH). So, similar to motor outcomes, data originating from the CT group are excluded for TA but included for new experimental outcomes, BLT, and ALEFq.

Table 2. Longitudinal characteristics of population and main results

Scale	Feature	Time	Within-group analysis			Between-groups comparison
			GR	GRHH	HH	GR-GRHH-HH
MI total [0:100]	score	T0	67 (49 to 75)	60 (47.5 to 69)	66 (55 to 75)	–
		T1	67 (50.5 to 75)	64 (50.5 to 71.5)	73 (65 to 77)	–
		T2	73 (42.5 to 77)	70 (63.5 to 77)	77 (72 to 82.5)	–
		T3	73 (53 to 81)	70 (60.5 to 77)	77 (66.5 to 85)	–
	score variation	T0 versus T1	0 (0 to 2.5)	0 (0 to 4)	0 (0 to 9.5)	–
		T0 versus T2	0 (0 to 8.5)	10 (6 to 13.5)	11 (5 to 18)	–
		T0 versus T3	5 (0 to 14)	6 (2 to 15.5)	12 (5 to 17)	–
	Wilcoxon	T0 versus T1	p = 0.0679	p = 0.2354	p = 0.0273	–
		T0 versus T2	p = 0.1282	p = 0.0013	p = 0.0015	GR-HH, p = 0.0363
		T0 versus T3	p = 0.0253	p = 0.0035	p = 0.0024	–
MI pinch [0:33]	score variation	T0 versus T1	0 (0 to 0)	0 (0 to 1)	0 (0 to 2)	–
		T0 versus T2	0 (0 to 0)	0 (0 to 4)	4 (0 to 11)	–
		T0 versus T3	0 (0 to 4)	0 (0 to 4)	0 (0 to 4)	–
		T0 versus T1	s = 0, p = 0.1797	s = 8, p = 0.5961	s = 2, p = 0.1308	–
		T0 versus T2	s = 3, p = 1.0	s = 1, p = 0.0269	s = 4, p = 0.0094	–
		T0 versus T3	s = 0, p = 0.0339	s = 14, p = 0.5735	s = 5, p = 0.065	–
MI Abd	score variation	T0 versus T1	0 (0 to 0)	0 (0 to 0)	0 (0 t o0)	–
		T0 versus T2	0 (0 to 2.5)	0 (0 to 0)	6 (0 to 6)	–
		T0 versus T3	0 (0 to 0)	0 (0 to 7)	6 (0 to 6)	–
	Wilcoxon	T0 versus T1	s = 0, p = 0.1797	s = 0, p = 0.1025	s = 0, p = 0.0422	–
		T0 versus T2	s = 3, p = 0.2228	s = 0, p = 0.0036	s = 0, p = 0.0030	GR-HH, p = 0.0392
		T0 versus T3	s = 7, p = 0.1226	s = 0, p = 0.0041	s = 0, p = 0.0031	–
ARAT pinch	score variation	T0 versus T2	0 (0 to 2.5)	0 (0 to 0)	2 (0 to 2.5)	–
		T0 versus T3	2 (0 to 5)	0 (0 to 0)	1 (0 to 2.5)	–
	Wilcoxon	T0 versus T2	s = 0, p = 0.0422	s = 0, p = 0.1088	s = 0, p = 0.0047	–
		T0 versus T3	s = 1.5, p = 0.0079	s = 7.5, p = 1.0	s = 0, p = 0.0074	GR-GRHH, p = 0.0221 GRHH-HH, p = 0.0302
MRC-WF	score variation	T0 versus T1	0 (0 to 0)	1 (0 to 1)	0 (0 to 1)	–
		T0 versus T2	0 (0 to 0)	1 (0.5 to 1.5)	1 (0 to 1.5)	–
		T0 versus T3	0 (0 to 1)	1 (1 to 1.5)	1 (0.5 to 1)	–
	Wilcoxon	T0 versus T1	s = 0, p = 0.1573	s = 9, p = 0.0456	s = 0, p = 0.0339	–
		T0 versus T2	s = 4, p = 0.7055	s = 0, p = 0.0025	s = 0, p = 0.0041	GR-GRHH, p = 0.008 GR-HH, p = 0.0107
		T0 versus T3	s = 8, p = 0.0705	s = 5, p = 0.0032	s = 5, p = 0.0051	–
TA ARM (cm)	score variation	T0 versus T1	0 (−1.5 to 0)	0 (−1 to 0)	0 (0 to 0)	–
		T0 versus T2	−1 (−2 to 0)	0 (−1 to 0)	−1 (−1 to 0)	–
		T0 versus T3	−0.5 (−1 to 0)	−2 (−3 to 0.5)	0 (−2 to 0)	–
	Wilcoxon	T0 versus T1	s = 1.0, p = 0.0742	s = 0.0, p = 0.0339	s = 2.0, p = 0.2734	–
		T0 versus T2	s = 6.0, p = 0.0266	s = 0.0, p = 0.0235	s = 3.0, p = 0.0311	–
		T0 versus T3	s = 7.0, p = 0.1124	s = 0.0, p = 0.0029	s = 4.0, p = 0.0483	–
ALEFq	score variation	T0 versus T2	0 (−1.5 to 0)	−1 (−1.5 to 0)	−1 (−1 to −1)	–
	Wilcoxon	T0 versus T2	s = 11, p = 0.1673	s = 3, p = 0.0182	s = 0, p = 0.0011	–
BLT contralesional	score variation	T0 versus T2	4.4 (−10.8 to 14.2)	4.90 (−6 to 20.25)	20.8 (6.5 to 30.85)	–
	Wilcoxon	T0 versus T2	s = 38, p = 0.6002	s = 36, p = 0.1729	s = 0, p = 0.0010	–

Data are median (IQR) or p value. Wilcoxon for within group analysis, Dunn-Hommel for between-groups post-hoc. n = 15 per each treatment group. MI, Motricity Index total score, pinch, and abduction; MRC-WF, medical research council upper limb test, score for wrist flexors; TA, tactile acuity, total score; ALEFq, affected limb explicit feelings questionnaire; BLT, body landmarks localization task, contralesional limb.

Motor improvements emerge earlier with HH

Force recovery, as assessed by MI total score improvements (see Tables 2 and S2 and Figure 3), was visible at each time point after baseline (Wilcoxon, all groups, T0 versus T1, T0 versus T2, T0 versus T3; all p values ≤ 0.0010), showing that in general patients improved after all of the enriched treatments. Median improvements at

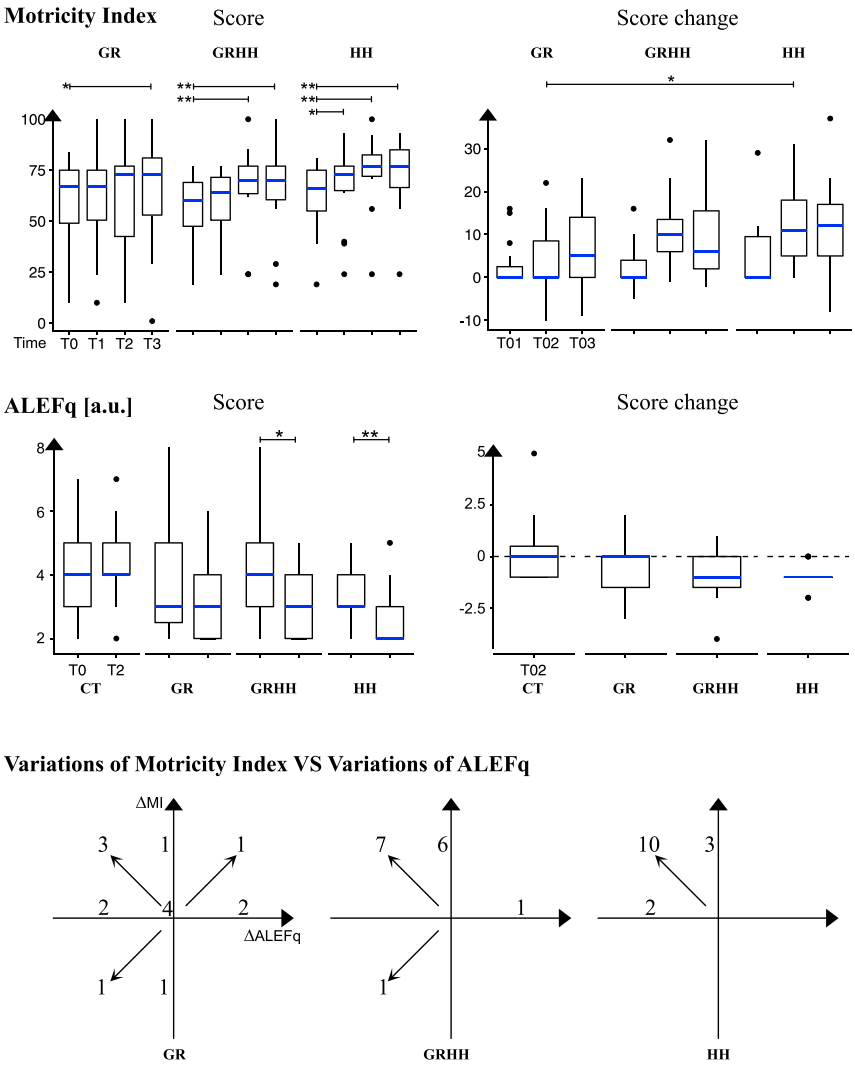

Figure 3. MI SCORE, ALEFq score, and joint variations

First row: MI score variation for total scores scales at all time points and ALEFq score variations at T0–T2. Boxplots show median, interquartile range (IQR), and confidence interval (CI) 95%. Left: absolute scores and significant in-treatment improvements for each group. Right: score variation and significant between-group improvements. Second row: ALEFq score. The total score has been calculated by summing all of the affirmative responses to the items of the questionnaire so that higher values (max score: 10) correspond to higher altered feelings related to the affected limb, while a score of 0 corresponds to no altered feelings. Left: score at inclusion (T0) and at end of treatment (T2) for each group. Right: score variation for each group. Significance levels: *p = 0.05, **p = 0.01; ***p = 0.001. Third row: count of the number of patients that had joint variations of MI and ALEFq during T0–T2. The top left quadrant indicates an improvement in both scales, single scale variations are counted on the corresponding axis, an d null variations are counted at the intersection of the axes.

the end of the treatment were GR: 0 (0 to 8.5), GRHH: 10 (6 to 13.5), and HH: 11 (5 to 18), whereas at follow up they were GR: 5 (0 to 14), GRHH: 6 (2 to 15.5), and HH: 12 (5 to 17).

Within-group analysis (Wilcoxon for each single group) showed an early onset of the improvement in patients assigned to the HH group, remaining stable along the longitudinal evaluation (T0 versus T1, T0 versus T2, and T0 versus T3, all

p values < 0.0273). Improvement for the GRHH group appeared only at the end of the treatment (T0 versus T2 and T0 versus T3, all p values < 0.0012), and finally, GR improved only at follow up (T0 versus T3, p = 0.025). Thus, only the use of HH was able to provide significant and early improvements within the treatment. Post hoc tests showed that, when compared with the GR group, significant improvements were found in patients who received the HH treatment alone (Kruskal Wallis, T0 versus T2, s = 7.4378, p = 0.0243, Dunn-Hommel: GR versus HH, p = 0.0363). The GRHH group had score improvements numerically, but not statistically, higher than the GR and lower than HH groups, as an effect of the partial training with both treatments. This improvement was reflected by significant between-group differences for MI abduction (Dunn, T0 versus T2, GR versus HH, p = 0.0392) and ameliorations in secondary outcomes (see Supplemental information).

The additional analysis with linear mixed modeling (LMM) comparing the dose of CT, GR, and HH (see Method details and Table S9) by analyzing all patients together provided more insight with respect to the time-weighted contribution of each kind of treatment in each allocation group.

Total score improvements at T1 were associated with the dosage of CT and at T2 with the dosage of CT and HH treatments, as well as an almost complete persistence at T3 (dCT: effect_T01 = +2.3, effect_T02 = 6.3, effect_T03 = 5.2, all p values < 0.0001; dHH: effect_T02 = +27.9, p < 0.0001; effect_T03 = 25.6, p = 0.0010).

When analyzing single MI scores, the MI pinch score improvement onset was always modest but significant for CT (dCT: effect_T01 = 0.7, effect_T02 = 1.9, effect_T03 = 2.2, all p values < 0.0020), whereas HH had larger effects, significant at T2 (dHH: effect_T02 = 11.0, p = 0.002). Elbow score improvements were associated with CT (effects = 1.2, 2.8, and 2.2, respectively, at T1, T2, and T3, all p values < 0.0001). Abduction score improvements were significant across all the phases for HH (effects = 6.86, 13.77, and 15.53, respectively, at T1, T2, and T3, all p values $\leq$ 0.0003) and significant at T2 for CT (dCT: effect = 1.6, p = 0.0025). Further information is reported in Method details, Figure S1, and Tables S2 and S9.

Motor improvement is associated with an improvement of antagonists' recruitment

When looking at functional recovery, within-group analysis did show that the Action Research Arm Test (ARAT) pinch improved over time in groups HH and GR (Wilcoxon, T0 versus T2 and T0 versus T3, all p < 0.0422) but not in group GRHH (all p > 0.1088), meaning that for finer movements, the mixed treatment GRHH was not successful. Post hoc analysis confirmed such differences (Dunn: GR-GRHH, p = 0.0221; GRHH-HH, p = 0.0302) and that no significant difference was found between groups GR and HH. This result suggests that from a fine manipulation perspective, both the GR approach and the HH approach are viable yet are potentially using different relearning mechanisms and pathways. However, each of these mechanisms could be requiring a minimum dose of treatment, which was not delivered to the GRHH group, or the two mechanisms could be competing against each other for this specific function.

Considering volitional muscle control, motor improvement was associated with an improved recruitment of the extrinsic wrist flexors, antagonists of the stimulation site of HH. Significant within-group improvements were found for Medical Research Council wrist flexors (MRC-WFs) in GRHH and HH (Wilcoxon, T0 versus T1, T0 versus T2, and T0 versus T3, all p < 0.0456) but not in GR, meaning that exposure to HH

contributed to an earlier onset of segmental motor modulation. Between-group differences were confirmed at T2 (MRC-WF, T0 versus T2, Dunn: GR-GRHH, p = 0.008; GR-HH, p = 0.0107), thus suggesting that a time course of 9 weeks was needed to provide consistent effects. Changes in the recruitment capabilities of other muscle groups, including the stimulation site, were not significant. These findings suggest that the patients in the HH group performed equivalently or better than the patients in the GR and GRHH groups in all of the subscales discussed above.

For a summary of the main results, please refer to Table 2. For a detailed explanation, please refer to the Supplemental information and Tables S2, S3, and S6.

TA improves with GR, and HH provides persistence

The results for the assessment of TA for the affected upper limb (arm) are provided in Figure 4. TA for the affected arm improved globally after all of the "technology-based" treatments (HH, GR, GRHH) (n= 42, T0 versus T2, Wilcoxon, s = 48.5, p < 0.0001) and for each treatment (all p values < 0.0311). Only for patients allocated to the HH and GRHH groups did the improvement at T2 remain persistent at T3 (Wilcoxon, T0 versus T3, GRHH: p = 0.0029; HH: p = 0.0483; GR: p = 0.1124). A difference emerged between the three treatment groups at T3 (Kruskal, all times, p = 0.0479). Similar results of better improvement (T0 versus T2) in GRHH or HH groups were also found for the TA evaluated on the forearm and the hand (but with only a trend, p = 0.0606, for GRHH regarding the hand). All the findings are reported in Method details and Table S7.

ALEFq scores are ameliorated by GR and HH

The treatment also resulted in a reduction of altered feelings related to the affected limb, collected through the questionnaire. Before the treatment, patients reported very frequent adherence to statements related to feelings of disembodiment, numbness, strangeness, lack of control, and uselessness of the affected limb (i.e., at least 2 out of the 10 altered feelings proposed in the questionnaire were reported by each patient; M.B., A.C., and M.C., unpublished data), which was comparable between groups (n = 60, Kruskal Wallis, T0, s = 1.1682, p = 0.7606). As reported in Figure 4, altered bodily feelings were reduced after the treatment. A main effect of the assessment was visible at the end of the treatment (Wilcoxon, T0 versus T2, s = 182.5, p = 0.0027), showing that in general, patients improved with all the treatments. A correlation analysis (Table 3) confirmed a global relation between improvement at the ALEFq and the MI (Spearman, r = −0.3184, p = 0.0330, negative correlation because, differently from the MI, lower scores indicated less altered feelings at the ALEFq after the treatment).

Within-group analysis showed improvement of the patients in the groups GRHH (Wilcoxon, T0 versus T2, s = 3, p = 0.0182) and HH (Wilcoxon, T0 versus T2, s = 0, p = 0.0011). There is a trend to a difference among groups (T0 versus T2, CT: 0 [−1 to 0.5], GR: −0 [−1.5 to 0], GRHH: −1 [1.5 to 0], and HH: −1 [−1 to −1]; Kruskal-Wallis, s = 7.42, p-0.0596) with an effect approaching significance in the comparison between the groups CT and HH (Dunn, p = 0.0561) where HH seems more effective in reducing negative feelings related to upper limb perception. For a detailed comparison, please refer to Tables S8 and S9.

ALEFq improvements, analyzed through mixed models, highlighted a positive net effect of the doses of GR and HH (T0 versus T2: dCT, effect = 0.33, p < 0.0001, dGR, effect = −2.40, p = 0.0045; dHH, effect = −3.60, p < 0.0001). For comparison, please refer to Figures 3 and S2 and Table S9.

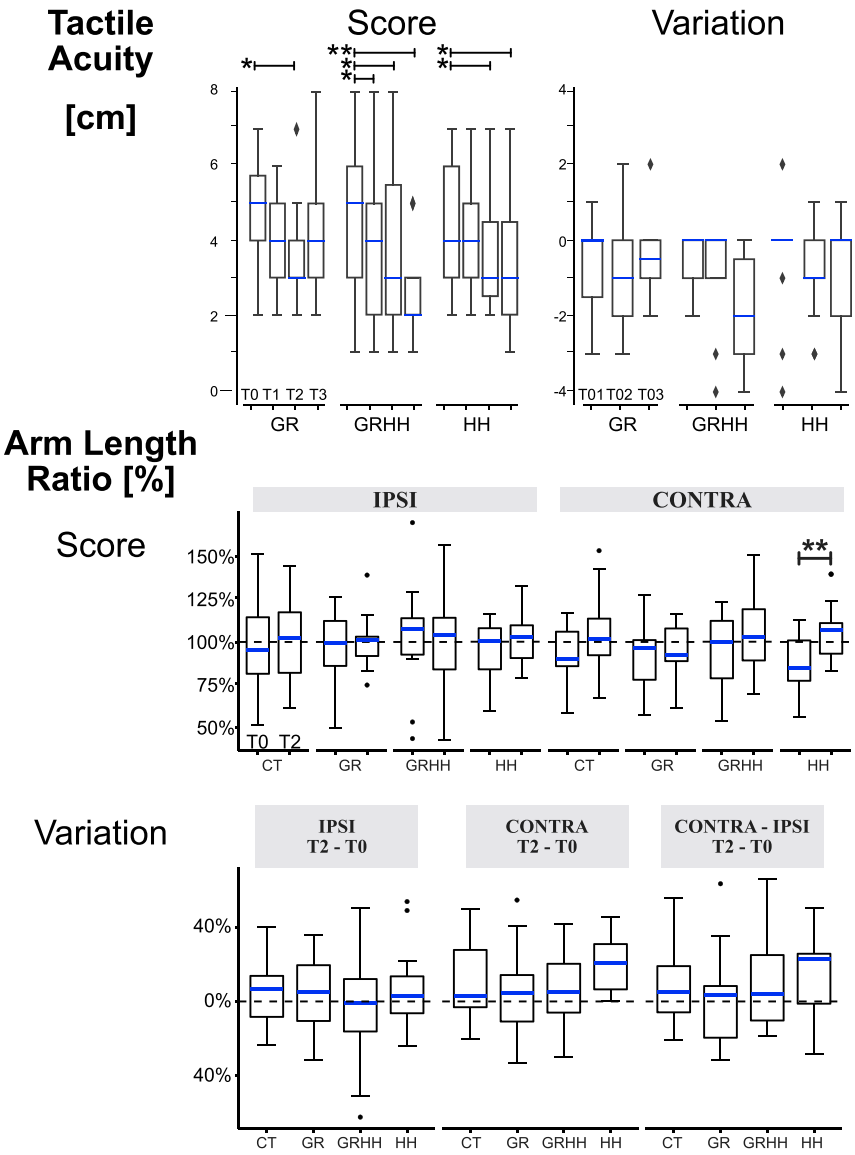

Figure 4. Distorted perception of the affected limb perception questionnaire, tactile acuity, and body landmarks localization task (BLT)

Boxplots show median, IQR, and 95% CI. Significance levels: *p = 0.05, **p = 0.01; ***p = 0.001. First row: improvement of tactile acuity as assessed by the two-point discrimination threshold for the arm. Lower values indicate better sensitivity. Left: tactile acuity scores over time. Right: tactile acuity variation per group over time. Second and third rows: BLT; distribution of the perceived arm length, expressed as a ratio of the perceived arm length versus real arm length, for the ipsilesional and the contralesional arms. A ratio of 100%, dashed line, indicates a correct estimation of the arm length, while values lower than 100% indicate that the estimated arm length is smaller than the real one. Bottom: perceived arm length variation from T0–T2; ipsilesional variations (%) on the left, contralesional variations (%) at the center, and the difference between contralesional and ipsilesional variation (%).

The improvement in the perceived length of the affected arm is driven by HH

The analysis of the BLT has been conducted on 58 patients, while 2 patients of the GR group, unable to maintain the required posture, were excluded (Figure 4). At T0, the perceived length of the contralesional limb was lower than that of ipsilesional limb in all patients (Wilcoxon, all patients: s = 577.5, p = 0.0314, Table S8). At T2, the

Table 3. Spearman correlation between sensory, body representation, and motor outcomes changes

	ALEFq	BLT	MI
TA ARM	r = 0.2593, p = 0.09712, n = 42	r = 0.1701, p = 0.2938, n = 40	r = 0.0198, p = 0.9006, n = 42
ALEFq	–	r = 0.0414, p = 0.7917, n = 43	r = −0.3184, p = 0.0330, n = 45
BLT	–	–	r = 0.0372, p = 0.8126, n = 43

Data are r, p value, and sample numerosity. Scales changes are calculated between T0 and T2. Results show the global correlation of GR, GRHH, and HH groups (n = 45 or less). TA, tactile acuity ARM change; ALEFq, affected limb explicit feelings questionnaire score change; BLT, change of ratiometric score in the contralesional limb; MI, Motricity Index total score change.

significant difference between the perceived length of the two limbs was no more significant in all patients (Wilcoxon, s = 757.5 p = 0.4480), suggesting that the treatments reduced the perceptive distortions of the contralesional limb present at baseline. This effect was specific for the contralesional side (Wilcoxon, all groups, s = 392, p = 0.0006) and particularly evident for the HH group (Wilcoxon, T0 versus T2, s = 24, p = 0.0010). Perceptual variations on the ipsilesional side were not significant (Wilcoxon, s = 756.5, p = 0.4434). No other significant correlation emerged between the variations of BLT and of TA and ALEFq or of MI (see Table 3).

Body representation analysis with LMM showed significant changes of the distortions of the contralesional limb over time (T2 versus T0) associated with the dose of CT and were approaching significance with the dose of HH (dCT: effect = +11.7%, p < 0.0001; dHH: effect = +31.1%, p = 0.0530) but not with the dose of GR (p = 0.351). No significant effect emerged for the ipsilesional limb (all p values > 0.0952). For comparison, please refer to Figuress 4 and S2 and Tables S8 and S9.

MI and ALEFq improvements correlate when HH is applied

Joint changes of MI and ALEFq differed among groups (see Figure 3). The higher number of patients reporting joint changes of MI and ALEFq were in the HH group (i.e., 10 patients; the remaining 5 patients improved in MI or ALEFq). Only 7 patients in the GRHH group and 3 patients in GR group improved in both domains. This comparison suggests that HH could boost joint recovery in multiple domains, precisely in motor control and body perception.

Global correlations (Spearman) between motor improvement (T2-T0) and changes (T2-T0) in sensory functions (TA) and body representations (BLT and ALEFq) were analyzed (Table 3). A significant, although moderate, correlation between changes in MI and changes in ALEFq (Spearman, r = −0.3184, p = 0.0330, n = 45) emerged, meaning that the improvement in motor performance was associated with a reduction of the reported altered feelings of the affected arm. No other significant correlations emerged (all other p values > 0.09712).

Thus, to further investigate the interrelations between motor outcomes and changes at the ALEFq, we evaluated the changes of MI as a function of the changes in ALEFq in a global LMM regression in all patients belonging to the enriched group by considering treatment doses (first fixed factor: ALEFq score changes weighted by dHH; second fixed factor: the ALEFq score changes without dHH). This analysis showed an effect in patients treated with HH (coefficient [coeff] = −16.74, p = 0.0241) but not in the other cases (coeff = −0.49, p = 0.7209), thus suggesting

that the relation between the amelioration at MI and at ALEFq is due to the delivered dose of HH.

For details, please refer to Table S10.

DISCUSSION

The possibility to induce a significant recovery in chronic stroke survivors is often considered very limited because of the reduced cortical plasticity exploitable a few months after the event. Previous studies investigated the effect of sensory electrical stimulation, showing improvements in the somatosensory evoked potentials of the paretic limb[17] or of somatosensory cortical plasticity,[18] or corticomuscular coherence.[19] However, it is still unclear if these results are of clinical relevance.

In this paper, we show how the use of a novel technological system (HH), based on NMES as a supplement of conventional clinical rehabilitation protocols, can significantly change this situation by facilitating the recovery of sensory and motor function and improving body representation, compared also to novel robotics rehabilitation treatments relying on visual and motor relearning schemes.

Related to the motor function, our findings show that the HH device induced earlier and long-lasting motor recovery as compared with robotic glove therapy in chronic patients, as demonstrated by the primary outcome measure (MI) and other scales assessing the strength recovery (MRC). A specific improvement due to HH training has also been shown in functional tasks, as assessed by the ARAT. In addition, a general improvement of motor skills, independently from the type of treatment, was found at the ARAT, in the blocks and box test, and in the subjective evaluation of the quantity and quality of upper limb usage in everyday life (Motor Activity Log [MAL]). These generalized effects are likely due to the fact that HH and GR were used in addition to standard therapy, which is typically dedicated to train specific functional skills instead of recovering impairment. Consequently, patients might have acquired some compensatory strategies via standard rehabilitation that we did not specifically control for.[20]

Specifically for the sensory function, HH, alone or when associated with GR, induced a long-lasting improvement in TA (Table S7). This is likely due to the rich pattern of somatosensory stimulation provided by the HH and GR, which might have reactivated residual but silent patterns between the contralesional arm and the somatosensory cortices.

Indeed, the electrodes used for HH were designed with a form factor different from the usual electrodes used in functional electrical stimulation (FES) to provide more cutaneous stimulations in addition to the expected motor activation typically induced by NMES techniques. This would have delivered supplementary sensory inputs congruent with the movements by reinforcing the remaining body efferences. This is a novel and important finding considering that somatosensory perception is normally neglected by standard treatment[21] and very few studies focus on this aspect in CVA rehabilitation, despite the key role of sensory feedback for motor control.[22] Further studies focusing on more extended somatosensory assessments are necessary to expand this result.

Few studies assessed the presence of alterations in body representations[5,23–25] and demonstrated deficits in tasks assessing the so-called body schema (hand imagery[6]),

body image (hand recognition[26]), or body structural description (localization of touch on body parts or of body parts, e.g., autotopagnosia/heterotopagnosia[27]). However, whether these deficits improve with sensorimotor rehabilitation is an open question.[25] Here, we assessed longitudinally the implicit perceived length of the arm by a using a tool, the BLT,[14,28–30] which has never been used before with stroke patients. By assessing the perceived position of few key landmarks on patients' upper limbs in a static condition, we can derive an implicit measure of the perceived size and shape of the upper limb, which is necessary to plan and execute accurate movements.[31] Previous studies directly support the link between implicit body perception, as assessed by the BLT, and limb use, as assessed by the overuse of one limb during immobilization of the dominant arm[29] or the use of a tool to reach positions of space beyond the arm limits in healthy participants.[28] We found that patients showed an underestimation of their contralesional limb length as compared with the ipsilesional one before the treatment, suggesting an altered perception of arm dimension, probably linked to motor deficits, limiting the limb use and the role of sensorimotor signals in updating body representations (Table S8). Crucially, such bias reduced, and even normalized, after the treatment. Importantly, such a group effect was driven by stronger amelioration after HH. Higher strength induced by HH might have boosted the patients' ability to feel and move the contralesional upper limb, thus increasing the number of interactions and related multisensory inputs from the arm by contributing to restore its perceived length. On the other hand, it might be the case that the rich somatosensory stimulation provided by HH might have directly stimulated the corresponding cortical representation. Future studies should test these two non-mutually exclusive interpretations.

Independently from the underlying mechanism, the present data also show a significant improvement of the subjective feelings toward the contralesional arm. Before the treatment, patients reported to feel their arm was a stranger, numb, dead, or not under their control. Such statements were radically reduced (improved) after the treatment and, in particular, in patients who had received HH (alone or with GR). This amelioration in the subjective feeling was correlated with the motor improvement evaluated with the MI, suggesting that better motor performance could be associated with better feelings toward the affected limb. In general, deficits in body representations and subjective body feelings might have a detrimental effect on patients' recovery.[32] However, a specific, quantified, and rigorous assessment of body representations and feelings is not part of the usual clinical evaluation. Our data suggest that these are important factors to assess in the context of upper-limb rehabilitation. Future studies are needed to understand whether motor recovery and better use of the upper limb in everyday life improve body representations and bodily feelings, or, vice versa, if the amelioration of body representations and the associated body feelings, as induced by HH, is at the basis of additional beneficial improvements in motor recovery associated with this treatment.

The present study shows how, in the chronic phase of stroke, NMES delivered via HH induced an earlier and more long-lasting recovery of sensorimotor functions and body perception, as compared with a robotic intervention provided via GR. Indeed, within each considered time frame, the improvement in motor function due to HH was equivalent or—in training specific domains—superior to the improvement provided by GR. The recovery was associated with improved dexterity: global improvement in the use of the limb (MI abduction), improved control of finer manipulation (MI pinch and ARAT pinch), and improved recruitment modulation of the muscle group antagonist to the target stimulation area (extrinsic fingers flexors).

Very interestingly, motor recovery was associated with an improvement in sensory functions and of body perception, as assessed by implicit and explicit tasks. Specifically, improvements of TA, induced both by GR and HH, were persistent only after HH treatment. An improvement in the perceived length of the affected arm, as assessed by the BLT, was specific for HH. Finally, the ALEFq scores were ameliorated by GR and HH, but the joint improvement of MI and ALEFq was dominant when HH was applied.

Therefore, the HH system may facilitate a general multi-faceted rehabilitation with a potentially strong clinical impact. We speculate that these effects could be linked to the additional rich sensory stimulation provided by HH, perceptively coherent with the assisted motor activation, in long-desensitized patients, and are able to perceptually boost the impact of motor activation.

Our results suggest that these are important dimensions to consider in the implementation and evaluation of stroke treatments, and we propose novel measures that can be used in everyday clinical practice. The current findings extend mounting evidence[33] demonstrating the importance of providing rich sensorimotor rehabilitation with proper assessment, targeting multiple functions in the chronic phase of the disease.

Finally, the flexibility, wearability, and modularity of the stimulation approach of the HH device will allow, in the future, the ability to customize it for rehabilitation protocols in the sub-acute phase and for home treatment.

Limitations of the study

A first limitation of the study relies on the sample size for each group, limited by the patients' pool at the clinic. A second limitation derives from the separate time of patients' enrollment, due to the time necessary to obtain the authorization of using the HH prototype from the Italian Ministry of Health. A third limitation of our study lies on the lack of homogeneity in patients' severity at enrollment. Despite the pseudo-random procedure used to assign patients to the different treatment groups, patients assigned to the CT group showed lower motor abilities at the baseline, thus their lower pattern of recovery might be due to their more severe deficits. This bias was partially mitigated by performing novel analyses on the proportion of recovery rather than on the raw scores, which confirmed the main results of the study. However, we are aware that this approach cannot fully exclude the selection bias in the conventional group. For this reason, we repeated the analyses by excluding the CT group and comparing only the improvement induced by adjunct therapies consisting of HH and the robotic glove. The improvement was particularly evident when HH was administered as a unique adjoint to therapy or in combination with GR and was significantly higher than that induced by the glove alone. This analysis confirms that, regardless of the sampling bias of the CT group, the HH plays a fundamental role in inducing strength recovery .

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.medj.2021.12.001.

ACKNOWLEDGMENT

The authors are grateful to the participants who participated in the clinical trial. This work was supported by a Foundation advised by Carigest S.A., by Fondazione Cariplo, by the Swiss National Science Foundation through the National Centre of Competence in Research (NCCR) Robotics, and by the Bertarelli Foundation.

AUTHOR CONTRIBUTIONS

A.C., E.G., A.S., F.M., O.B., and S.M. formalized research goals. A.C., M.B., E.G., A.S., O.B., F.M., and S.M. wrote the manuscript. A.C., M.B., A.S., and S.M. performed literature search. M.B., A.S., and O.B. designed the sensory assessment protocols. A.C. designed the mixed sensory and motor stimulation protocols. A.C. designed the wearable and programmed, deployed, and provided assistance with the HH prototype. A.C. prepared the figures and the tables. E.G., M.C., and F.M. performed the study design, recruited the patients, and performed data collection. E.G. and M.C. coordinated and assisted with the patients' training. F.M. supervised the clinical activities. M.B. and E.G. had unrestricted access to all data and annotated, filtered, and verified the underlying data. A.C., M.B., A.S., E.G., O.B., M.C., F.M., and S.M. interpreted data. S.M., F.M., and A.S. managed and coordinated the research planning and execution. A.S., S.M., F.M., and O.B. managed funding acquisition. A.C. and M.B performed formal data modeling and statistical analyses. A.C., M.B., S.M., and A.S. prepared the first draft of the manuscript, reviewed it, and edited it. All authors agreed to submit the manuscript, read and approved the final draft, and take full responsibility for its content, including the accuracy of the data and the fidelity of the trial to the registered protocol and its statistical analysis.

DECLARATION OF INTERESTS

A.C. reports grants from the CARIPLO Foundation, CARIGEST, and the Bertarelli Foundation during the conduct of the study and is a recipient of the NCCR Spin Fund 2018, sponsored by the Swiss National Center of Competence in Robotics. A.C. is founder and president of AGO Neurotechnologies Sàrl. M.B. reports a grant from SNSF Ambizione (PZ00P1_161210) during the conduct of the study. A.S. reports personal fees from MindMaze SA outside the submitted work. O.B. is cofounder and shareholder of Metaphysiks Engineering SA and is a member of

the board and a shareholder of Mindmaze SA. E.G., M.C., and F.M. report grants from the CARIPLO Foundation during the conduct of the study. S.M. reports grants from the Fondazione CARIPLO Foundation, CARIGEST, the Bertarelli Foundation, and the Swiss National Science Foundation through the National Centre of Competence in Research (NCCR) Robotics during the conduct of the study.

INCLUSION AND DIVERSITY

We worked to ensure gender balance in the recruitment of human subjects. We worked to ensure ethnic or other types of diversity in the recruitment of human subjects. We worked to ensure that the study questionnaires were prepared in an inclusive way.

Received: February 1, 2021
Revised: June 8, 2021
Accepted: November 30, 2021
Published: January 7, 2022

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STAR★METHODS

KEY RESOURCES TABLE

RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
Scipy (version 1.4.1)	Virtanen et al.[34]	https://github.com/scipy/scipy
Pandas (version 1.2.3)	McKinney[35]	https://github.com/pandas-dev/pandas
StatsModels (version 0.12.2)	Seabold and Perktold[36]	https://github.com/statsmodels/statsmodels
Scikit PostHocs (version 0.6.6)	Terpilowski[37]	https://github.com/maximtrp/scikit-posthocs
SeaBorn (version 0.11.1)	Waskom[38]	https://github.com/mwaskom/seaborn
NMES software	Andrea Crema, TNE, EPFL. Custom software for internal use.	N/A
Other		
Gloreha Sinfonia Plus	Idrogenet srl, Idro, Italy	https://www.gloreha.com/sinfonia/
IntFES V2	Tecnalia Serbia, Belgrade, Serbia	Custom adaptation of one prototype for EPFL
ALEFq test	EPFL, LNCO; MySpaceLab, CHUV	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the Lead Contact, Dr Andrea Crema (andrea.crema@epfl.ch).

Materials availability

This study did not generate new unique reagents. Further information and requests for resources should be directed to the Lead Contact.

Data and code availability

All data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Any additional information required to re-analyze the data reported in this paper is available from the lead contact upon request.

METHOD DETAILS

NMES development

Helping Hand (HH)[15] is an evolution of previous prototypes[16,39] from the same authors. With HH we improved the following features of our previous systems: (1) smaller electrode and new patterns for motor and sensory activations, (2) electrode-skin adhesion (3) wearability and reliability of cabling (4) stimulation apparatus (5) *ad hoc* GUI and control software.

Commercial and research-grade FES systems usually rely on standardized electrodes, and standardized patterns which can be modulated to produce the desired motor response. In the conventional case, the size and shape of electrodes, and stimulation patterns are standardized to maximize motor response, while maintaining comfortable stimulation conditions. In HH, the shape of the electrodes, and the patterning of the electrode activation were empirically optimized to both induce muscle activation and provide increased cutaneous stimulation. In previous tests performed on one neurologically intact subject used to NMES, electrodes of different shapes were arranged in arrays, and virtual electrodes (VE) tested with tonic stimulation patterns for assessing the minimum electrode sizes usable for eliciting motor response. To produce persistent conscious sensory excitation, usually

cognitively suppressed as an adaptation to repetitive stimuli, a set of patterning variants included additional spatially-distributed components aimed at generating tickling sensations. Stimulation variants were cross validated on four healthy subjects used to NMES. Subjects tested patterns for motor-only activation and for motor and sensory activation in 30 minutes long sessions with pseudorandomized sequences and, while watching an unrelated movie used as distractor, were asked to report when they perceived stimulation. While frequency of reporting for motor NMES decreased over time, some sensorimotor stimulation patterns were reported throughout the test with higher coherence. The stimulation variants best matching with descriptors of comfort, and perceptual cutaneous persistence were associated to VEs, to allow at different electrode scales different sensorimotor patterns.

Large electrodes conform badly to skin surfaces, especially in case of localized curvatures, and can comply badly with volumetric variations due e.g., to prono-supination. Electrode arrays included appropriate longitudinal cuts between electrodes to allow local bending and to better conform to the skin of the users.

The previous prototype[16] was designed for operating only in conjunction with a pre-existing lightweight upper limb exoskeleton and a large multiplexer, which imposed constrains to the location and type of cabling solutions. In the new prototype connectors were moved proximally to the elbow, their size and weight reduced, to allow the device to operate alone or with devices providing an arm weight support.

The updated prototype, to reliably support new stimulation features, included a different stimulation apparatus (IntFES, Tecnalia Belgrade, Serbia) which allows defining: the current intensity, independent for each electrode; the pulse-width, common to all the active electrodes; the activation sequence of electrodes; and the global stimulation frequency. The electrodes are controlled and activated by an independent controller, which makes the system portable and wireless.

Because the low-level control of the stimulation apparatus is not intuitive nor practical for clinical routine, we developed a graphical user interface mimicking the usual clinical approach. For each desired hand posture, the clinician could define a stimulation map (SM) containing one or more VEs of custom shape and size.

Appropriately sequenced SM allow eliciting transitions between different hand postures and grasps. The GUI and control system, implemented on a tablet pc (SurfacePro 3, Microsoft) via a touch user interface, allows to customize the stimulation maps with cursors, and to time and sequence each SM and - in accordance with clinical needs, allowing to mimic via NMES the exercises of the GloReha.

These solutions allow easy set-up and usability in the clinical context, and customization of evoked movements as a function of the therapy needs, while maintaining easy administration and reducing the amount of supervision needed during the treatment.

Assessment of body representations

We used the body-landmarks localization task, described elsewhere,[28,29] to measure the implicit perceived size of the contra- and ipsi- lesional upper limbs. The patients were asked to indicate when a visual marker moved by the experimenter over their hidden forearm, reached the felt position of one of some target anatomical landmarks (see Sorrentino et al.[14] for the protocol used here). We considered the following markers of interests: the tip of the index finger, the tip of the annular finger,

the internal part of the wrist (the radius styloid), the external part of the wrist (the ulnar styloid) and the elbow joint (the olecranon), to then calculate the length and width of the arm and the hand as the mean distance between two specific markers, e.g., the distances between the internal and external parts of the wrist and the elbow to calculate the arm length. Real positions of the landmarks were also acquired to then compute an index of the bias in the perceived dimension with respect to the actual one, i.e., the ratio between the perceived and the real dimension (%). In this way, values below 100% represent an underestimation of the perceived dimension with respect to the real one, and values above 100% indicate an overestimation.

From the data obtained at the body-landmarks localization task we observed at baseline (T0) a significant underestimation in the perceived length of the contralesional arm with respect to the ipsilateral one (M.B., A.C., and M.C., unpublished data). Thus, in the present work aiming at testing the effect of the treatment on alteration in body representations observed at baseline, we focus on that parameter that was altered before the treatment, i.e., the arm length.

In addition, to capture explicit disturbances in upper limb perception, we administered the novel "affected limb explicit feelings questionnaire," (ALEFq). This 10 items questionnaire was designed by adapting items from two previously proposed questionnaires for patients with chronic pain, i.e., the Feeling of foreignness questionnaire[40] and the Neurobehavioral Questionnaire.[41] Five questions propose adjectives related to the limb (e.g., clumsy, unsuitable, from the Feeling of foreignness questionnaire) and the other 5 items describe feelings such as dis-ownership or loss of agency (from the Neurobehavioral Questionnaire). Patients had to positively answer to the items if they experienced the described sensations about their affected limb.

We computed a total score by summing all the affirmative answers, so that a higher score indicates a higher number of reported altered feelings.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Patients

INCOGNITO is an open label clinical trial (NCT03349138, https://clinicaltrials.gov/ct2/show/NCT03349138) aimed at implementing an Integrated Cognitive, Sensory, and Motor Rehabilitation of Hand Functions. It was held at Villa Beretta Rehabilitation Center, Costa Masnaga, Lecco, Italy. Ethics approval was provided by local ethical committee (CE Interaziendale delle Province di Lecco Como e Sondrio, 48/2016), and the clinical trial for non CE-marked devices was approved by the Italian Ministry for Health (DGDMF/P/I.5.i.m.2/2014/1060). The study was not overseen by a data monitoring committee, and was retrospectively registered on ClinicalTrials.gov, NCT03349138. Informed consent was obtained from all subjects.

Patients were included if they had suffered one or more strokes, with unilateral functional impairments at the contralesional upper-limb (Motricity Index < 85) at least six months before the study enrolment. Exclusion criteria were: left-handedness; inability to understand the instructions, or cognitive deficits that could prevent them to undertake the evaluations and the interventions. Limitations to use the devices due to impairment of Passive Range of Motion; pain due to spasticity (Modified Ashworth Scale > 2); previous major neurological or psychiatric disorders; allergy to electrodes constituted. A total 60 patients were included in the study; the trial profile is reported in Figure 1. Patients were pseudo-randomly assigned to one of four

treatment groups using block randomization: i) conventional (CT group), ii) robotic glove, GloReha (GR group); iii) Helping Hand NMES (HH group); iv) combined Helping Hand and the GloReha system (GRHH group) (see below for description). Randomization was divided into two different sub-phases: 1) block randomization of control group and GR group was performed from September 2015 until April 2016 due to unavailability of the NMES system in the clinic; 2) block randomization of HH group and GRHH group was performed from May 2016 until the end of the study. Patient were enrolled by the clinical team of the Villa Beretta Rehabilitation Center (F.M., M.C.). The list of treatment codes was generated through a permuted-block randomization procedure by the Engineering Department of Hospital (EG). They kept the randomization sequence hidden and were responsible for assigning participants to interventions. The assessors, collecting the outcome measures, were blinded to the treatment allocation, while the physiotherapists, delivering the intervention, and the participants could not be blinded.

Protocol for motor function restoration

Patients received 27 treatment sessions, one session 3 times a week (on non-consecutive days), for 9 weeks. Each treatment session included 60 minutes of conventional treatment followed by 30 minutes of supplemental treatment in accordance with group assignment: CT additional conventional therapy; GR GloReha (Idrogenet s.r.l.) hand robotic treatment; HH HelpingHand NMES wearable; GRHH half of each session with the robotic glove and half with the NMES wearable.

The "conventional" rehabilitation pathway for upper-limb included: upper-limb passive motion, arm cycle ergometer, upper-limb exercises using augmented or virtual reality environment, occupational therapy exercises, upper-limb active movement (reaching, grasping, elevation, spatial orientation), repetitive task training.

The GR treatment was based on the use of a robotic glove (GloReha), a neuromotor rehabilitation electrically powered device that mobilizes the metacarpophalangeal, proximal interphalangeal and distal interphalangeal finger joints. GloReha, thanks to its modular composition, is a highly flexible device which adapts to the patient's characteristics. The weight and dimensions of the accessories assembled on the patient's hand and forearm are negligible: these are braces and gloves specifically designed for GloReha application, to maximize the patient's comfort and optimize flexion and extension generated by the device. During therapy, the patient's hand was moved by the robotic glove, while arm supported was provided. GloReha enables a multitude of hand exercises, programmable by the therapist, to offer personalized therapy based on the patient's clinical requirements. On mobilization, visual and sound effects, coupled with the exercise, are also delivered. To provide ecologically valid multisensory stimulation. GloReha enables passive mobilization exercises to be carried out accompanied by simultaneous, three-dimensional representation on a screen, functional tasks with real objects and treatments according to the Action-Observation Therapy scheme.

The HH treatment relied on an NMES system *ad hoc* developed for this trial[15] and aimed at providing a balanced muscles recruitment and enhanced sensation of stimulation on the target area. The Helping Hand system consisted of an array of 59 active electrodes, embedded in a flexible and easy to set polymeric matrix, to be placed on the patient's forearm (see Figure 2). The electrodes target selectively and specifically hand extrinsic and intrinsic muscles involved in hand pre-shaping and grasping, in order to stimulate and facilitate patients' upper-limb functional

movements during rehabilitation. In the trial, HH was clinically set only to target extrinsic hand extensors.

Outcomes measures

The primary outcome for Motor recovery was upper-limb strength as assessed by the Motricity Index (MI). Secondary motor outcomes of the protocol were: the Action Research Arm Test (ARAT),[42] the Blocks and Block Test (BBT),[43] the Motor Activity Log (MAL),[44] the Medical Research Council Upper-Limb Muscle Scale (MRC).

Recovery in sensory function was explored by focusing on the assessment of the Tactile Acuity test (TA) by means of the two-points discrimination threshold.[45,46] In order to evaluate body representations, we used an implicit task, the body-landmarks localization task (BLT,[14] see above) and a questionnaire capturing explicit disturbances in upper limb perception, ALEFq (see above). MI, MRC, and TA were assessed at baseline (T0), halfway during the training period (T1), at the end of the training period (T2), and one month after the end of the treatment (T3). ARAT, MAL, and BBT were assessed at T0, T2, and T3. BL and ALEFq were assessed at T0 and T2. Clinicians supervised the treatments for ensuring safety and reporting adverse events.

QUANTIFICATION AND STATISTICAL ANALYSIS

The patients sample size was chosen in order to guarantee an adequate significance level and, at the same time, the sustainability of the study on the base of previous similar studies comparing different treatment for the rehabilitation of the upper limbs.[47–51] These studies show a difference between 12 and 15 points in upper limb scale of the primary outcome (with standard deviation ranging between 25 and 45). Thus, considering a power of 0.8 with an alpha of 0.05, a minimum sample size of 50 patients was required to show a significant improvement between T0 and T2. Thus, a total of 60 patients have been included. For MI, ARAT, MRC, MAL, BBT, TA and the ALEFq, we used non-parametric tests since data did not follow a Gaussian distribution (Shapiro test: all p values < 0.0007 for MI, ARAT, MRC, MAL, BBT, and ALEFq; $p = 0.0413$ for TA); BLT was added for completeness (Shapiro, contralesional side, $p = 0.1435$). We first performed a baseline analysis between the groups of the patients' characteristics (Kruskal-Wallis) at inclusion (T0) (see Table S1). Then, to compare the level of global improvement and of within-group improvements, score variations collected during the different assessments (T0, T1, T2, T3, or T0, T2, T3, or T0, T2) were compared between the groups of patients exposed to the different treatments (CT, GR, HH, GRHH) using Wilcoxon test. For the BLT we included an analysis of the ipsilesional, of the contralesional side, and of the difference between ipsilesional and contralesional side. For all between-groups analyses, Kruskal-Wallis test and Dunn method with Hommel correction was used for post hoc comparisons.[52] Possible correlations (Spearman) between motor improvement (T2-T0) and changes (T2-T0) in sensory functions and body representations have been explored.

We applied the non-parametric analysis described in the main text to all the clinical scales.

For the analysis on the data obtained at the MI, BLT and ALEFq we also applied linear mixed modeling (LMM) to quantify the impact of different dose allocation of the three treatments on the score variation over time (T0 versus T1, T0 versus T2, and T0 versus T3, or T0 versus T2). In detail, our study included four groups of

participants, each with different dose (d) allocation of the three treatments (dCT, dGR, and dHH). Patients received the following average treatment per session: CT [1 dCT, 0 dGR, 0 dHH], GR [2/3 dCT, 1/3dGR], GRHH [2/3 dCT, 1/6 dGR, 1/6 dHH], and dHH [2/3 dCT, 1/3dHH]. In the analysis, the score variation over time of one feature (e.g., MI) was modeled as the weighted sum of the averaged doses distribution, with dCT, dGR, and dHH considered as fixed factors, and patients as random factors. This approach allowed to differentiate the effects associated with each allocation group, shown through non-parametric analysis, and the correlated dose-dependent effect of each treatment. For fixed effects, p values were obtained by likelihood ratio test as reported by Lindstrom and Bates.[53] In addition, in the present study, the use of linear mixed model was supported by a model selection based on Akaike's Information Criterion (AIC) and Bayesian information criterion (BIC), revealing LMM always better than ANOVA.

Moreover, to refine the correlation analysis between motor outcome and sensorimotor function described in the main text, we modeled MI score change as the sum of the sensorimotor score changes, with BLT, ALEFq, and TA considered as fixed factors, and patients as random factors. The model was further refined by hierarchically conditioning the correlated features with the dose of treatment as supplemental fixed factor.

Further outcomes

This section contains detailed analysis of subscales of the primary study outcome, or secondary outcomes, that could not fit into the main body. These analyses, while not mandatory, allow the reader to better navigate through the Tables S3–S6. This subset analysis shows that e.g., while distal functional improvements are related to fine manipulation, improvements in volitional recruitment of distal muscles were not detectable. On the contrary, improvements followed an agonist-antagonist scheme in the treatment area and show that the improvement associated with NMES is less likely associated with a direct motor recruitment improvement.

In detail, MAL results show a generalized improvement on the perception and usage of the affected limb after the treatments, whereas MRC specifically shows that wrist flexors score improved globally at the end of treatment and improvements within groups associated to GRHH and were significantly different from GR.

This analysis may allow to speculate that functional changes derived from a boosted limb awareness, and globally improved sensorimotor control.

MI subscales

Given the fact that different approaches could target different types of upper limb movements, we then investigated the effects at level of single movements for the hand and upper arm separately. Considering Pinch, a main effect of the treatment was visible at the end of the treatment GR: 0 (0 – 0), GRHH: 0 (0 – 4), HH: 4 (0 – 11) (Wilcoxon, T0 versus T2, s = 25, p = 0.0016) and at follow up GR: 0 (0 – 4), GRHH: 0 (0 – 4), HH: 0 (0 – 4) (Wilcoxon, T0 versus T3, s = 49, p = 0.0187). Within-group analysis did show that the improvement of the HH group was achieved at the end of the treatment (Wilcoxon, T0 versus T2, s = 4, p = 0.0094); the GRHH group achieved an improvement at the end of treatment (Wilcoxon, T0 versus T2, s = 1, p = 0.0269); finally, the GR group achieved improvement at follow up (Wilcoxon, T0 versus T3, s = 0, p = 0.0339). No significant difference appeared between the groups (Kruskal Wallis, all p > 0.0687). Considering Elbow, a main effect of the assessment was visible at the end of the treatment (T0 versus T2, s = 1.5, p = 0.0005) and at follow

up (T0 versus T3, s = 14.5, p = 0.0002), showing that in general patients improved after all the enriched treatments. Average improvements at the end of the treatment were GR: 0 (0 – 2.5), GRHH: 6 (0 – 8), HH: 0 (0 – 0), whereas at follow up were GR: 0 (0 – 8), GRHH: 0 (0 – 8), HH: 0 (0 – 8). Within group analysis showed the improvement in patients assigned to the GRHH group at the end of the treatment and at follow up (Wilcoxon: T0 versus T2, s = 0, p = 0.0105; T0 versus T2, s = 0, p = 0.0015; T0 versus T3, s = 0, p = 0.0167), and a delayed improvement for the GR group (Wilcoxon: T0 versus T3, s = 1.5, p = 0.0311). No difference between the groups emerged (Kruskal Wallis, all p > 0.1676). Considering Abduction, a main effect of the assessment was visible halfway through the treatment (T0 versus T1, Wilcoxon, s = 0, p = 0.0047), at the end of the treatment (T0 versus T2, s = 4.5, p < 0.0001) and at follow up (T0 versus T3, s = 25.5, p < 0.0001), showing that in general patients improved after all the enriched treatments. Average improvements at half of the treatment period were GR: 0 (0 – 0), GRHH: 0 (0 – 0), HH: 0 (0 – 5.5), at the end of the treatment were GR: 0 (0 – 0), GRHH: 6 (0 – 6), HH: 6 (2.5 – 8), whereas at follow up were GR: 0 (0 – 7), GRHH: 6 (0 – 6), HH: 6 (2.5 – 8). Within group analysis showed an early onset of the improvement in patients assigned to the HH group (Wilcoxon: T0 versus T1, s = 0, p = 0.0422; T0 versus T2, s = 0, p = 0.0030; T0 versus T3, s = 0, p = 0.0031), and a more delayed improvement for the GRHH group (Wilcoxon: T0 versus T2, s = 0, p = 0.0036; T0 versus T3, s = 0, p = 0.0041. A significant difference between groups emerged at the end of the treatment (T0 versus T2, Kruskal Wallis, s = 6.7364, p = 0.0345).

For comparison, please refer to Figure S1 and Table S2.

ARAT

ARAT Total score of the enriched groups improved globally and a main effect of the treatment was visible at the end of the treatment and at follow up (Wilcoxon, T0 versus T2: s = 53, p < 0.0001; T0 versus T3, s = 76, p < 0.0001). Median improvements at the end of the treatment were GR: 3 (1 – 5), GRHH: 1 (0 – 3), HH: 3 (0 – 6), whereas at follow up were GR: 3 (1 – 15.5), GRHH: (−0.5 – 3), HH: 3 (0.5 – 8.5). Within group changes were visible for GR and HH at the end of the treatment (Wilcoxon, T0 versus T2; GR: s = 2.5, p < 0.0001; HH: s = 4.0, p = 0.0097) and at follow-up (Wilcoxon, T0 versus T3; GR: s = 0.0, p = 0.0014; HH: s = 4.5, p = 0.0067. No difference between the groups emerged at the end of the treatment; at follow up a trend separating GR from GRHH was noticeable but not significant (T0 versus T3, Kruskal-Wallis s = 6.0651, p = 0.0482, Dunn p = 0.0513).

The feature able to best explain the change was Pinch. Global improvements were visible at the end of the treatment and at follow up (Wilcoxon, T0 versus T2: s = 0, p < 0.0001; T0 versus T3: s = 30.5, p < 0.0001). Within group changes were visible for GR (T0 versus T2: s = 0, p = 0.0422; T0 versus T3: s = 1.5, p = 0.0079) and HH (T0 versus T2: s = 0, p = 0.0047; T0 versus T3: s = 0, p = 0.0074). Post hoc analysis showed a weak group difference between GRHH and HH at end of treatment (T0 versus T2, Kruskal s = 5.3279, p = 0.0690; Dunn GRHH versus HH, p = 0.0665), and follow up confirmed significant group differences of GR and HH, versus GRHH (T0 versus T3, Kruskal s = 8.7627, p = 0.0125; Dunn GR versus GRHH, p = 0.0221, Dunn HH versus GRHH, p = 0.0302).

Grasp score of the enriched groups improved globally (Kruskal Wallis, T0 versus T2: s = 36, p = 0.0031; T0 versus T3, s = 24, p = 0.0024). Median improvements at the end of treatment were GR: 0 (0 – 3.5), GRHH: 0 (0 – 1), HH: 0 (0 – 1); at follow up were GR: 0 (0 – 4), GRHH: 0 (0 – 0.5), HH: 0 (0 – 2.5). Within group changes were

visible for GR (Wilcoxon, T0 versus T2: s = 1.5, p = 0.0204; T0 versus T3: s = 0, p = 0.0171). No difference emerged through post hoc analysis.

Global improvements of the Grip score of the enriched groups was only detectable at follow up (Wilcoxon, T0 versus T3, s = 63, p < 0.0023). Median changes were GR:0 (0 – 4) GRHH: 0 (0 – 2.5), HH: 0 (0 – 2.5). Within group changes were visible for GR (T0 versus T3: s = 1, p = 0.0166) and HH (T0 versus T3: s = 7.5, p = 0.0398). No difference emerged through post hoc analysis.

GrossMT score of the enriched groups improved globally at the end of the treatment (T0 versus T2, s = 24.5, p = 0.0400), and at follow up (T0 versus T3, s = 22, p < 0.0001). Median score changes at T2 were GR: 0 (0 – 0.5), GRHH: 0 (0 – 0.5), HH: 0 (0 – 0.5); score variations at T3 were GR: 1 (0 – 2), GRHH: 0 (0 – 0.5), HH: 0 (0 – 1). Within-group late-changes were visible for GR (T0 versus T3: s = 5.5, p = 0.0215) and HH (T0 versus T3: s = 2.5, p = 0.0473). No difference emerged through post hoc analysis.

For comparison, please refer to Table S3.

BBT

Analysis of BBT results did not highlight significant group differences. On the contralesional side, a main effect of the assessment was visible at the end of the treatment (T0 versus T2, Wilcoxon, s = 121, p = 0.0360), and at follow up (T0 versus T3, s = 101.5, p = 0.0007), showing that in general patients improved after the treatment. Score changes at T2 were GR: 0 (−0.5 – 4), GRHH: 0 (0 – 2.5), HH: 0 (−1 – 1.5); score changes at T3 were GR: 2 (0 – 7), GRHH: 0 (−0.5 – 1.5), HH: 0 (0 – 5.5). Within group analysis showed an onset of the improvement in patients assigned to the GR group (Wilcoxon: T0 versus T2, s = 10, p = 0.0040; T0 versus T3, s = 6.5, p = 0.0178). Post hoc analysis did not highlight between group differences (Kruskal Wallis, all p > 0.4179). On the ipsilesional side, supposedly less affected by the injury, a main effect of the assessment was visible at the end of the treatment (Wilcoxon, T0 versus T2, s = 221, p = 0.0066) and at follow up (T0 versus T3, s = 166, p = 0.0004). Score changes at T2 were GR: 3 (1 – 5.5), GRHH: 1 (0 – 7.5), HH: 2 (−3 – 7.5), and at T3 were GR: 2 (0 – 4), GRHH: 6 (−0.5 – 14), HH: 5 (1.5 – 10). Within group analysis did show late improvements for GRHH (Wilcoxon, T0 versus T2: s = 9.5, p = 0.0365; T0 versus T3: s = 19.5, p = 0.0383) and for HH (Wilcoxon, T0 versus T3: s = 15, p = 0.0105). Post hoc analysis did not confirm significant differences between the groups.

For comparison, please refer to Table S4.

MAL

Significant improvements at the end of treatment and at follow up were a main effect of the treatment (Wilcoxon, all p < 0.0011) showing that in general patients had improved perceptions of the limb after the treatment, and reported improved confidence in using it. Within group improvements associated with HH appeared in the "quality of movement" (QOM) scale (Wilcoxon, T0 versus T2, s = 4, p = 0.006) and in the "amount of use" (AOU) scale (Wilcoxon, T0 versus T2, s = 7, p = 0.0071, T0 versus T3, s = 11, p = 0.0091). No significant between-group differences emerged, confirming that a trend to improvement was close to significance for the other groups. For comparison, please refer to Table S5.

MRC

A main effect of the assessment was visible halfway through the treatment (T0 versus T1, Wilcoxon, s = 68.5, p = 0.0002), at the end of the treatment (T0 versus T2, s = 64.5, p < 0.0001) and at follow up (T0 versus T3, s = 64.5, p < 0.0001), showing that in general patients improved after the treatment with all the enriched treatments. Within group analysis showed an early onset of the improvement in patients assigned to the GRHH group (Wilcoxon: T0 versus T1, s = 12.5, p = 0.0205; T0 versus T2, s = 3, p = 0.0012; T0 versus T3, s = 0, p = 0.0006), and the HH group (Wilcoxon: T0 versus T1, s = 2, p = 0.0057; T0 versus T2, s = 0, p = 0.001; T0 versus T3, s = 2, p = 0.0015), and finally GR improvements significant only at follow up (Wilcoxon: T0 versus T3, s = 20.5, p = 0.0243). However, post hoc analysis revealed the amount of improvement was not equal between all the groups (T0 versus T2, Kruskal, s = 6.2096, p = 0.0448), a trend of improvement appeared in the patients who received the HH treatment at T1 (GR versus GRHH, Dunn-Hommel, p = 0.0546) and T2 (GR versus HH, Dunn-Hommel, p = 0.0728). Median improvements at T2 were GR: 0 (−0.5 – 4.5), GRHH: 5 (3 – 8), HH: 4 (2 – 8.5).

Considering the shoulder area, global changes were detected for deltoid anterior (DA), deltoid medialis (DM), and deltoid posterior (DP) muscles (Wilcoxon, all p < 0.0097). In DA, the predominant within-group change at the end of treatment and at follow-up was associated with the GRHH group (Wilcoxon, T0 versus T2, s = 0, p = 0.0047; T0 versus T3, s = 0, p = 0.0016) with a median variation of 1 (0 – 1) both at T2 and T3. In DM, consistent changes were associated with the HH group (Wilcoxon, T0 versus T2, s = 0, p = 0.0039; T0 versus T3, s = 0, p = 0.0023) with a median variation of 1 (0 – 1) both at T2 and T3. In DP within-group changes at end of treatment and follow-up were associated with the groups GRHH and HH (Wilcoxon, T0 versus T2 and T0 versus T3, all p < 0.0339) and median improvement at T2 of 1 (0 – 1). No significant between group difference was found.

Considering elbow, global changes were detected for flexor muscles (EF) and extensor muscles (EE) (Wilcoxon, all p < 0.0339). Changes in EF muscles recruitment was associated with GR at T3 (Wilcoxon, T0 versus T3, s = 5, p = 0.0196) and with GRHH (Wilcoxon, T0 versus T2 s = 5, p = 0.0083; T0 versus T3 s = 5, p = 0.0067). Changes in EE muscles recruitment was detected in GRHH (Wilcoxon, T0 versus T2 s = 4.5, p = 0.0129; T0 versus T3 s = 0, p = 0.0094) and HH (Wilcoxon, T0 versus T2 s = 2.5, p = 0.0461; T0 versus T3 s = 0, p = 0.0139) with median changes of 1 (0 – 1) for GRHH and of 0 (0 – 1) for HH. No significant difference between groups was found.

Improvements of the wrist extensors (WE) - the only direct target area of the Helping Hand system – displayed significant global improvements at each stage of testing (Wilcoxon, T0 versus T1 p = 0.0046; T0 versus T2 p = 0.0040; T0 versus T3 p = 0.0011), but this improvement was only observable in GRHH (Wilcoxon, GRHH: T0 versus T1 p = 0.0588, T0 versus T2 p = 0.0209, T0 versus T3 p = 0.0348) or HH (Wilcoxon, GRHH: T0 versus T1 p = 0.0339, T0 versus T2 p = 0.0139, T0 versus T3 p = 0.0339). Between groups differences were not confirmed by post hoc analysis (T0 versus T2, Kruskal Wallis, s = 5.545323, p = 0.0625; Dunn GR versus GRHH p = 0.0735, GR versus HH p = 0.0997).

Wrist flexors (WF) displayed significant global improvements at each stage of testing (Wilcoxon, T0 versus T1 p = 0.0018; T0 versus T2 p < 0.0001; T0 versus T3 p < 0.0001), but this improvement was only observable in GRHH (Wilcoxon, GRHH: T0 versus T1 p = 0.0456, T0 versus T2 p = 0.0025, T0 versus T3 p = 0.0032) or HH

(Wilcoxon, GRHH: T0 versus T1 p = 0.0339, T0 versus T2 p = 0.0041, T0 versus T3 p = 0.0051). Between groups differences were confirmed by post hoc analysis (T0 versus T2, Kruskal Wallis, s = 11.1833, p = 0.0037; Dunn GR versus GRHH p = 0.0080, GR versus HH p = 0.0106).

Fingers extensors (FE) displayed significant global improvements at end of treatment and follow up (Wilcoxon, T0 versus T2 p = 0.0071; T0 versus T3 p = 0.0203), but within group improvement was only observable in the HH patients (Wilcoxon, GRHH: T0 versus T1 p = 0.0339, T0 versus T2 p = 0.0041, T0 versus T3 p = 0.0051). Between groups differences were not confirmed by post hoc analysis (T0 versus T2, Kruskal Wallis, s = 5.3323, p = 0.0695; Dunn GR versus HH p = 0.0628).

Fingers flexors (FF) displayed significant global improvements at end of treatment and follow up (Wilcoxon, T0 versus T1 p = 0.04550, T0 versus T2 p = 0.0004; T0 versus T3 p = 0.0027). Within group improvement was observable in GR halfway through the treatment (Wilcoxon, T0 versus T1, s = 0 p = 0.0455), in HH at the end of treatment (Wilcoxon, T0 versus T2 s = 10 p = 0.0293), and in GRHH from the end of the treatment onward (Wilcoxon, T0 versus T2, s = 4 p = 0.0348, T0 versus T3 s = 0 p = 0.0114).

For comparison, please refer to Table S6.