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Efficacy of a Rehabilitation Treatment Using Action Observation Therapy Enhanced by Muscle Synergy-Derived Electrical Stimulation (OTHELLO) in Post-Stroke Patients: A RCT Study Protocol

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Abstract

Background: Action Observation Therapy (AOT) and Neuromuscular Electrical Stimulation (NMES) are widely adopted techniques for upper-limb rehabilitation in post-stroke patients. Although AOT and NMES are individually effective, studies investigating a potential synergistic effect on enhancing rehabilitative outcomes are lacking.
Objectives: This study aims at comparing the effect of AOT and NMES applied together (AOT-NMES) on muscle synergies with respect to either AOT alone or a Motor Neutral Observation treatment alone (MNO, involving neither AOT nor NMES) on motor function recovery of upper limb.
Design: Randomized Controlled Trial (RCT) with n = 60 post-stroke patients with resulting upper limb disability, randomly allocated (1:1:1 ratio) in three interventional arms: AOT-NMES (n = 20), AOT (n = 20) and MNO (n = 20).
Methods and Analyses: All rehabilitation treatments will consist of n°15 60 min-long rehabilitative sessions. Primary outcome measure will be upper limb motor function, assessed using the Fugl-Meyer Assessment scale for upper limb (FM-UL), collected at the baseline (T0), post-intervention (T1) and at follow-up (T2, 6-months after T1). Other outcome measures will be collected through a multidimensional evaluation including assessing stroke-associated quality of life, neurophysiological data, biomechanical and MRI measures. The innovative protocol will also be evaluated for usability and safety.
Discussion: We expect to determine the efficacy, usability and safety of the AOT-NMES rehabilitation approach for the recovery of upper limb motor function in post-stroke patients. The obtained results will also help reveal the neural underpinnings of motor recovery, as assessed by neurophysiological data, biomechanical and MRI measures.

Keywords

stroke, rehabilitation, action observation therapy (AOT), mirror mechanism, neuromuscular electro stimulation, MRI, upper limb, randomized controlled trial

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Introduction

Stroke is the third most common cause of death and disability worldwide, accounting for about 6% of disability-adjusted life-years.[1] Ischaemic strokes constituted 62.4% of all incident strokes in 2019,[1] with an expected increase in the future decades.[2] In 50-70% of the affected patients, stroke leads to persistent impairment of upper limb (UL) motor function,[3] reducing individual autonomy and quality of life that in turn result in a substantial economic social

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burden estimated in about $45.5 billion/year.[4] In this context, stroke-related diseases can be considered a key priority from several points of view, including healthcare, social, and economic. Effective rehabilitation treatments become thus fundamental to help the increasing number of people with stroke-related motor disorders to re-gain autonomy in everyday life activities, with direct impact on patients' wellbeing, social participation and quality of life.

From a physiological point of view, stroke can be defined as brain cell death attributable to a vascular insult resulting in structural and functional changes and is associated with clinical symptoms persisting for more than 24 hours.[5] Rehabilitation treatments have their roots in the brain's natural capacity to reorganize after diseases and injuries, a property called neuroplasticity.[6,7] Changes in brain structure and function constitute the basis for recovering at least partial independence following effective rehabilitation treatments.

In this regard, among various rehabilitation treatments, observing actions can induce lasting changes within the motor representation in people with stroke[8] and prevent corticomotor depression due to limb non-use.[9] The principle of functioning explaining such modulatory effects is the so-called *mirror mechanism*, a brain process that transforms the sensory representation of other's actions into the observer's motor representation concerning the same action.[10] Given this theoretical framework, it is not surprising that treatment strategies based on the alternation of action observation and motor execution – namely, Action Observation Treatment (AOT) – have been applied in the last decades for the acquisition,[11,12] maintenance[13,14] and recovery of motor skills[15-19] with substantial clinical evidence involving people suffering from stroke sequelae[15,16,20] and see 21 for a recent review). Several models of brain functioning might explain how action observation leads to motor improvements, including the facilitation of premotor-to-primary motor connections, recruitment of direct premotor descending pathways, and finally, the tuning of cortico-subcortical loops subserving movement execution.[12,21,22]

Upper limb rehabilitative interventions for post-stroke patients have also been developed that exploit a mechanism orthogonal to action observation, i.e., promoting plastic changes via inducing muscle contraction. Neuromuscular electrical stimulation (NMES) is a technique that induces muscle contraction by delivering electrical current transcutaneously using surface electrodes, either with or without voluntary participation.[23] Rehabilitation delivered via NMES was shown effective in clinical post-stroke populations,[24] and its use is recommended in clinical practice for people experiencing UL motor disturbances after stroke, especially when there is evidence of spared muscular contraction but poor motion against resistance.[25]

The simultaneous application of two different therapeutic principles, is promising for maximizing rehabilitation outcomes. A combined protocol involving activation both central and peripheral stages is expected to achieve better results in motor recovery than approaches targeting a single domain.[26,27] This synergistic strategy has already been successfully applied by associating NMES with robot-assisted therapies[28] as well as combining action observation with peripheral electrical stimulation in stroke patients.[29] In this latter case, Lim and colleagues[29] showed stroke patients videos depicting repetitive grasping actions while the ulnar nerve of their hemiplegic arm was stimulated. The authors reported a facilitated sensorimotor cortical activation in the affected hemisphere (larger mu band suppression) in those treated with the combined approach. Given these findings, it is reasonable to hypothesize that a simultaneous use of AOT and NMES in stroke patients with UL motor impairment could lead to better functional recovery than AOT treatment alone. These data would align with preliminary evidence from a pilot RCT study based on a Brain-Computer Interface approach.[30]

In addition, it is possible to design the AOT-NMES treatment accounting not only for individual muscles but also for the multiplexed pattern emerging from the muscle synergies recruitment during a given action execution,[31,32] i.e., the set of muscles *operating together*, rather than in isolation. The correct, time-dependent combination of different muscle synergies is crucial to achieving a behavioural goal (i.e., object prehension and manipulation), optimizing the musculoskeletal control during action execution. Convergent evidence has shown that brain lesions, besides their observable impact on voluntary motor control, affect the ability to select, activate and flexibly combine muscle synergies.[33] Although it has been explicitly claimed that muscle synergies recruitment should be considered in neurorehabilitation protocols,[34] there is no evidence of their translational application to date.

Starting from this observation, here we will present a combined rehabilitation approach where post-stroke patients with UL impairment will be asked to observe hand actions related to activities of daily living. Following muscle synergies principles, during AOT, the timing of stimulation and the NMES target- muscles of NMES will be selected based on temporal and spatial muscles synergies activation coefficients derived from the same -*simultaneously* observed- action. Subjects will be then asked to attempt the repetition of the observed actions. We expect that the combined visuomotor and somatomotor action representation evoked by the synchronous use of both AOT and NMES would positively impact the treatment efficacy, inducing improvements larger than the treatments in isolation.

The main objective of this protocol is thus to test the efficacy of AOT-NMES rehabilitation therapy compared to AOT rehabilitation alone and a motor neutral observation (MNO) treatment. This aim will be pursued through an explanatory randomized controlled trial (RCT) with three interventional parallel arms.

Furthermore, this study will investigate the short and long-term changes driven by the AOT-NMES treatment spontaneous and evoked activity, as evidenced by high-field 3.0 T functional magnetic resonance imaging (fMRI), along with changes in neuroradiological and neurophysiological outcome measures.

Methods and Analysis

The protocol OTHELLO (action Observation THerapy Enhanced by muscLe synergy-derived eLectrical stimulation), is designed according to SPIRIT 2013 guidelines.[35] The study will last 36 months. OTHELLO received

approval by the ethical committee of the IRCCS Fondazione Don Carlo Gnocchi (Milan) and will be conducted in line with the Declaration of Helsinki. The study was registered to clinicaltrial.gov with ID number: NCT06055569.

OTHELLO is a randomized, controlled, patient- and assessor-blinded trial with three parallel arms. The trial work plan is shown in Figure 1.

Study Setting

The study setting will be IRCCS Fondazione Don Carlo Gnocchi ONLUS, Centro Santa Maria Nascente, Milan, Italy, where all data will be collected. Eligible patients will be consecutively recruited from the neurorehabilitation unit. Before recruitment, patients will be asked to sign informed written consent.

Eligibility Criteria

Patients eligible for the trial must fulfil the following inclusion criteria: (i) age between 50 and 85 years; (ii) Upper limb motor impairment after first episode of unilateral stroke as verified by MRI or CT; (iii) 2 weeks up to 6 months after stroke; (iv) Muscles force >2 at Medical Research Council scale (MRC) in upper limb muscles.

Exclusion criteria will be: (i) other neurological/orthopaedic issues that would interfere with upper limb exercises; (ii) contraindications to Magnetic Resonance Imaging (MRI) examination or to transcranial magnetic stimulation (TMS); (iii) dermatologic issues that will interfere with neuromuscular stimulator; (iv) electronic subcutaneous implants; (v) epilepsy; (vi) peripheral neuropathy; (vii) pregnancy; (vii) presence of severe cognitive impairment, including language comprehension detected during the neurological evaluation.

Interventional Methods

Eligible patients will be allocated equally to one of three treatment groups: AOT-NMES, AOT, or MNO. All these interventions will be add-on treatments to the usual treatment performed while admitted to IRCCS Fondazione Don Carlo Gnocchi ONLUS.

The frequency and duration of treatment will be balanced across the three intervention, 15 sessions, intensive frequency with five sessions/week, each lasting 60 min.

The intervention specific type each group is detailed below.

AOT-NMES (Study Intervention)

During the AOT-NMES intervention, the subject will sit in front of a table with both hands still and parallel on the table, in front of the chest. A monitor (32 inches) will be placed on the table at 80 cm distance from the subject's eyes. Four actions will be trained for the same amount of time during each session. The entire protocol comprises 60 different daily-living actions proposed with an increasing difficulty in terms of dexterity and hand-object interaction.

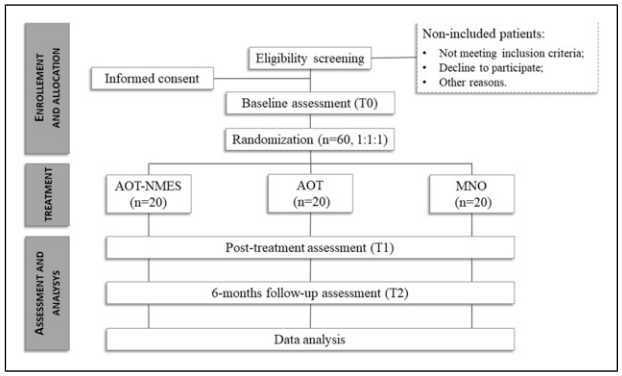

Figure 1. OTHELLO – Schematic Diagram Including Time Schedule of Enrolment, Interventions and Assessments for Participants. AOT, Action-Observation Therapy; NMES, Neuromuscular Electrical Stimulation; MNO, Motor-Neutral Observation.

Observation Phase

The subject will be required to watch a short video clip showing unilateral and transitive (i.e., object-directed) daily living hand action on a black background. The actions will be shown from both allocentric and egocentric points of view. While the former perspective will provide a more comprehensive information about the scene's elements, the latter is expected to induce a higher degree of embodiment in the observer, as indirectly suggested by its capacity to elicit a higher reactivity of fronto-parietal circuits during action observation.[36,37] During the observation phase, NMES will be delivered in an open-loop (pre-programmed) time-related fashion. NMES delivery will be programmed based on the temporal dynamic of the muscular synergies recruited by a healthy subject, whose electromyography (EMG) was acquired in parallel with video-recording of model actions (EMG: posterior, anterior and medio-lateral deltoid, triceps, biceps, flexor and extensor carpi radialis, flexor digitorum superficialis, extensor digitorum communis, abductor pollicis brevis, first dorsal interosseus, and abductor digiti minimi). For each action the specific muscles synergy pattern will be extracted from the recorded EMG signals of the normative reference, and the time activation profiles will define the electrical stimulation parameters.

The stimulation intensity will be set in a personalized way for each patient and each action before treatment initiation. The intensity will be set to the minimal value able to evoke small muscular recruitment. A programmable multichannel electrical stimulator will be used. Any adverse events will be reported in accordance with the guidelines for medical devices of the European Commission (under directives 90/385/EEC and 93/42/EEC, May 2015), and a dedicated adverse events collection document will be prepared.

Execution Phase

Subjects will be requested to imitate the previously observed action. A therapist will follow a specific protocol that includes reading a verbal description of the target action.

The observation-execution sequence will be repeated for 15 min for each action.

AOT

AOT group will follow the same procedure of AOT-NMES, except for the absence of NMES stimulation. NMES electrodes will be placed on the affected limb to maintain patient blinding to treatment allocation, but electrical stimulation will not be applied.

MNO

The MNO group will follow the same procedures of AOT, except for the content of video clips depicting non-action videos (i.e., landscapes). Patients will be verbally told which action should be executed after the landscape observation.

Outcome Measures

All patients will be assessed through a multidimensional evaluation. Outcome measures will be collected at T0 (before treatment), T1 (at the end of rehabilitation treatment) and T2 (follow-up, 6 months after enrolment). The schedule of enrolment, interventions and assessments is reported in Table 1.

Primary Outcome

The primary outcome will be upper extremity motor performance as assessed using the Fugl-Meyer Assessment - Upper Extremity (FMA-UE) scale. This scale evaluates upper limb motor recovery across five domains (motor function, sensory function, balance, joint range of motion, joint pain), with items scored on a 3-point ordinal scale (0 = cannot perform, 1 = performs partially and 2 = fully performed, total maximum score = 66). In 2017, the International Stroke Rehabilitation and Recovery Roundtable[38] recommended to use FMA-UE as a core measure for the assessment of stroke-associated motor impairments. In this study, FMA-UE will be collected at T0, T1 and T2, thus allowing the longitudinal evaluation of motor functioning.

Secondary Outcomes

Upper limb functional recovery will be assessed using the Action Research Arm Test (ARAT)[39] evaluating performances in the execution of 19 different gestures scored on a Likert scale from 0 (no movement) to 3 (perfect execution), with a total score of 57 points (higher scores indicating best performances). The ARAT test will allow for the characterization of clinical state and for the measurement of spontaneous and therapy-induced recovery in post-stroke patients; it has been recommended as a core measure in stroke trials.[38] ARAT will be collected at all time points.

Changes in disability will be assessed at T0, T1 and T2 using the WHO Disability Assessment Schedule 2.0 (WODAS 2.0) across six functional areas: understanding and communicating, getting around, self-care, getting along with people, life activities, and participation in society.[40]

Quality of life will be evaluated using the EQ-5D-5L questionnaire, an instrument assessing health-related quality of life along five dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) on five levels[41]; its use has been recommended in stroke trials.[38] Specific domains in stroke-associated quality of life (i.e. strength, hand function, emotion, participation) will be further investigated using the Stroke Impact Scale – SIS.[42,43]

Global cognitive functioning will be evaluated at each time point using the Montreal Cognitive Assessment scale (MoCA).[44] This instrument allows a rapid screening evaluation of cognitive functions that cover multiple domains (short-term and delayed verbal memory, visuospatial abilities, executive functions, attention, concentration, working memory, language, orientation to time and place – score range 0-30). Higher scores indicate better cognitive performance.

Other Outcomes

Neurophysiological biomarkers associated with motor functions will be studied before (T0) and after (T1) the rehabilitation treatment. [1] recruitment curve of corticospinal excitability, will be assessed using motor potentials evoked via TMS in the first digital interosseous (FDI) muscle for both the healthy and affected sides. TMS will be administered using an ATES STM9000 magnetic stimulator (EB Neuro S.p.A., Florence, Italy) with the coil held tangentially to the skull, with its handle oriented 45° from midline. A single-pulse approach will be used for TMS, starting at an output corresponding to 80% of the subject-specific resting motor threshold and increasing by 10% each time. [2] The H/M ratio will be computed by stimulating both the radial and median nerves at increasing intensities and recording respectively from the extensor carpi radialis (ECR) and the flexor carpi radialis (FCR) using a Nicolet EDX system (Natus Neurodiagnostic Solutions). The ratio will be determined by comparing the maximal motor response (M max) in each muscle to the maximal amplitude of the H-reflex, for both the healthy and affected sides.

Upper limb kinematics of muscle synergies will be acquired in the three timepoints (T0, T1, T2). Subjects post-stroke and twenty healthy subjects will perform a set of motor acts derived from daily living activities, which will be not directly trained during post-stroke rehabilitation treatment. During this upper limb assessment, kinematic data and surface EMGs from 12 muscles per limb will be recorded. Kinematic data will be recorded using a 9 camera optoelectronic system during 3D motor acts. The system will measure the 3D coordinates of spherical markers attached to body landmarks to compute trunk, shoulder, elbow, wrist and fingers angles. Outcome measures will include: speed, smoothness, range of motion, multi-joint coordination index. Moreover, muscle synergies will be extracted from the EMG envelope of each subject using the non negative matrix factorization algorithm. Short (T1)- and long (T2)-term changes induced by treatment in the similarities of muscle synergies with respect to physiological patterns will be evaluated. The module similarity will be measured by the maximum scalar product of the

Table 1. Schedule of Enrolment, Interventions and Assessments of the OTHELLO Study.

	STUDY PERIOD			
	Enrollment	Post-allocation		
TIMEPOINT		***T0***	***T1***	***T2***
ENROLMENT				
Eligibility screen	X			
Informed consent	X			
Recruitment	X			
Allocation	X			
INTERVENTIONS				
AOT-NMES		⟷	⟷	
AOT		⟷	⟷	
MNO		⟷	⟷	
ASSESSMENTS				
Demographic data				
Age				
Education				
Sex		X		
NIHSS				
MRC				
Primary outcome measure				
FM-UE		X	X	X
Secondary outcome measures				
ARAT				
WHODAS 2.0				
EQ-5D-5L		X	X	X
SIS				
MoCA				
Surrogate outcomes				
Neurophysiological biomarkers		X	X	X
Kinematic and muscular synergies data		X	X	X
Brain Magnetic Resonance Imaging (MRI)		X	X	X
Assessment of the used technology				
SUS			X	
Treatment safety				

AOT, action observation therapy; NMES, neuromuscular electrical stimulation; MNO, motor-neutral observation; NIHSS, national institute of health stroke scale; MRC, medical research council scale; FM-UE, Fugl-Meyer assessment - upper extremity (FMA-UE) scale; ARAT, action research arm test; WHODAS, WHO disability assessment schedule 2.0; EQ-5D-5L, euro quality of life 5-dimension, 5-levels questionnaire; SIS, stroke-impact scale; MoCA, montreal cognitive assessment; SUS, system usability scale.

muscle weightings of each module between each participant and the normative reference, while the activation profile similarity will be measured by the Pearson's correlation coefficient of the activation profile of each module.

High-field MRI data of the brain will be collected at T0, T1 and T2 on a Siemens Prima 3.0 T scanner. Structural data will be acquired: [1] to investigate brain morphometry (T1w MPRAGE, 0.8 mm^3 resolution); [2] to characterize brain lesions (fluid-attenuated inversion recovery, FLAIR, 1 mm^3 resolution). [3] to investigate microstructural tissue properties (echo planar imaging multi-shell diffusion-weighted imaging (DWI), 5 b0 images, 50 encoding directions with b = 1000 s/mm^2 and 50 with b = 2000 s/mm^2 TR = 3600 ms, TE = 92 ms, resolution = 2 mm^3, 72 slices). Functional data will be also collected to: [1] investigate the sensory-motor pathway at rest (fMRI, multi-band and multi-echo gradient-echo, TR = 1000 ms, TEs = 15/33.7/52.4 ms, resolution = 2.5 mm^3, MB acceleration factor = 4, two spin-echo acquired in AP-PA) to correct for geometrical distortions.); [2] investigate sensory-motor pathways during a sensory-motor task according to 45 (fMRI, accelerated gradient-echo, TR = 2000 ms, TE = 30 ms, resolution = 3 mm^3). An

MRI evaluation of the spinal cord (according to 46) will be acquired in those willing to undergo additional imaging (optional evaluation).

Sample Size

The sample size was computed using G*Power software. According to the literature,[47] an increase of 6 points in the FM-UE scale (primary outcome of the present study) is expected for people with stroke treated with AOT (vs control treatment). Setting the standard deviation at 7.4 points, equal to the maximum variability within the groups[47] and under the assumption of an increased 20% effect due to the synchronous use of AOT and NMES, a moderate effect size of 0.46 among groups was estimated. Therefore, a dimensionality of 17 patients/group, given an alpha of 0.05 and beta 0.80, is sufficient to observe a significant difference in the treatments' effects. Considering the risk of drop-outs, we increased the sample size to 20 subjects/group.

According to sample size computation, in the present study will be enrolled n = 60 patients with a motor disease following stroke. Eligible patients will be randomly allocated (1:1:1) in one of the three groups: AOT-NMES (n = 20), AOT (n = 20) and MNO (n = 20).

Methods: Assignment of Intervention and Blinding

Considering the prognostic value of UL impairment at admission on rehabilitation-associated motor outcome,[48] a stratified randomization plan will be carried out to prevent imbalance between the three treatment groups, reducing type-1 errors and improving statistical power.[49] An independent researcher not involved in the study will produce separate randomization lists for each stratum before study initiation. Average MRC score for upper limb at T0 will be used as stratification variable.

After intervention assignment, outcome assessors will not be involved in the rehabilitation protocol, and they will be blind to the treatment delivered. Patients will be blind to the intervention: all patients undergoing action observation will wear the NMES electrodes regardless of whether NMES is actually delivered, ensuring no explicit indication of inclusion in the AOT-NMES. For each patient, the muscles involved in the muscle synergies chosen for stimulation will be placed according to anatomical guidelines[50] and SENIAM specifications.[51]

Participants will be informed that the study involves multiple conditions, without emphasizing differences in stimulation mode, to minimize expectation biases. It will also be specified that the stimulation amplitude will be set below the motor threshold, meaning that direct movement will not be activated, and that the stimulation may be minimally perceived, with high individual variability in perception. Trained operators will interact with participants in a standardized, neutral manner to avoid inadvertently revealing group allocation. Therapists will also periodically and discreetly engage participants with neutral questions about their experience during sessions to reinforce the integrity of blinding.

Data Collection and Management

Clinical baseline assessment will include an evaluation of level of stroke severity using the National Institute of Health Stroke Scale (NIHSS).[52] This scale assesses stroke-associated neurological deficits and is recommended as core measure for stroke severity by the Stroke Recovery and Rehabilitation Roundtable.[38] Its score ranges from 0 to 42, with higher scores indicating more severe deficits.

Upper extremity muscles strength will be assessed at baseline using the Medical Research Council Scale (MRC).[53] Moreover, Demographic data (age, sex, years of education) will be collected.

Data on the usability of AOT-NMES technology and its safety will be collected post-intervention (T1). In particular, usability will be assessed through System Usability Scale (SUS)[54] and safety will be operationalized as the number of adverse events, in line with the literature.[55] Data about patients' adherence to treatment will be collected by the therapist, through an *ad-hoc* application, after each rehabilitation session.

Primary, secondary and surrogate outcome measures will be assessed at all timepoints (T0, T1, T2) by blinded assessors.

A schedule of enrolment, interventions and assessments is reported in Table 1.

Data Analyses

Statistical analyses will be performed using dedicated software (*i.e.,* MedCalc 20.111, Jamovi 2.2 https://www.jamovi.org). All the variables will be expressed in terms of appropriate descriptive statistics (e.g., frequencies, percentages, means, standard deviations) and summary statistics will be graphically expressed when appropriate. Normality of distribution will be tested for continuous variables using Shapiro-Wilk test, along with assessment of skewness and kurtosis. The time, group and time*group effects of AOT-NMES treatment, compared to other treatments, will be assessed on primary and secondary outcomes using a repeated measure ANOVA (between-factor, groups: AOT, AOT-NEMS, MNO, within-factor T0, T1, T2) according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines.[56] An Intention-to-treat approach will be used to address missing data. Time effect, group effect and interaction will be investigated. Potential bias related to demographic characteristics and heterogeneity of stroke severity will be addressed in the analysis by introducing covariates. Adjustments will be performed when necessary to account for stratified randomization procedures.[57]

The changes over time (T0-T1) of the recruitment curve of corticospinal excitability (indexed by the area under the curve - RC_{AREA}) and the H/M reflex amplitude will be evaluated with two separate repeated measures ANOVAs, with "TIME" as within-subject factor (T0, T1) and "GROUP" as between-subject factor (AOT-NMES, AOT, MNO).

MRI data will be analysed as follow: [1] to investigate brain morphometry, dedicated software will be used (e.g., FreeSurfer software suite, https://surfer.nmr.mgh.harvard.

edu/). When appropriate, and according to each peculiar brain lesion, specific tools will be considered to facilitate best segmentation quality and data analysis (e.g., Virtual Brain Grafting[58]); [2] brain lesions will be characterized in terms of localization and lesion load, also taking advantage of specific software to investigate the effect of the lesion site on the entire brain system (e.g., Lesion Quantification Toolkit,[59] NeMO toolkit[60]) and visualize them (i.e., SPIDER-net software[61]); [3] after pre-processing, microstructural brain features will be investigated using a dedicated pipeline (including FSL libraries https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). Functional resting-state and task-related brain data will be analysed using dedicated software (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Spinal MRI data will be analysed according to 46.

Discussion

The principal aim of the present study is to test the efficacy of AOT-NMES treatment in the rehabilitation of UL motor disorders following stroke. The adopted approach will be characterized by using NMES stimulation with onset and intensity levels based on muscle synergies proper of the observed action. We expect to find an increased efficacy of this combined rehabilitation protocol over either AOT-alone or MNO treatments.

Pilot studies suggest that the combination of AOT with functional electrical stimulation (FES) associated with the Brain-Computer Interface[30,62] is more effective in enhancing recovery from UL motor deficits than other proposed singular approaches. Specifically, Lee et al[30] showed that AOT was associated with a brain-computer interface-controlled FES delivered to the extensor carpi radialis muscle, with a stimulation intensity set to produce full wrist extension with a duty cycle of 15 s on and 3 s off, while watching the video clip. Conversely, Kim et al[62] delivered a peripheral electrical stimulation to the ulnar nerve above the sensory (but below the motor) threshold of the first dorsal interosseous (FDI) muscle. During AOT, such stimulation was applied continuously or alternating on/off periods according to the user's attention, as classified by EEG-based steady-state visual evoked potential. In both studies, the combination of AOT with electrical stimulation showed better efficacy than the control condition in facilitating UL motor function[30] and corticospinal excitability.[62]

In the OTHELLO protocol we aim to unveil the potential of AOT-NMES interaction in enhancing functional recovery of post-stroke patients with upper limb deficits. Several novelties are introduced relative to the previous literature. First, instead of stimulating a single muscle, we will implement a multi-channel neuromuscular stimulation for muscle groups contributing to distinct muscle synergies involved in the actions observed in the AOT. Secondly, instead of using continuous fixed stimulation or a mere on/off stimulation, the amplitude and timing of the stimulation of each muscle synergy will be modulated synchronously to the spatial and temporal activation profile of the same muscle synergies underlying the observed action. For these reasons, we expect that using the OTHELLO protocol, the synergistic effect of visuomotor and somatomotor representations elicited by the AOT and NMES should enhance the physiological recovery of movement after stroke compared to the two treatments used individually. In other words, on one hand action observation will drive motor recovery *top-down*, accessing the motor system from a "backdoor entrance" (i.e., via *mirror mechanism*), on the other hand NMES will provide a *bottom-up* counterpart, stimulating the muscular synergies involved in the observed action. As a result of such a *"double hit"* approach, a more holistic motor stimulation will be provided, leading to a more favourable motor outcome. The combined administration of AOT and NMES is further supported by recent evidence demonstrating that the amelioration of the motor performance induced by AOT parallels the degree of convergence of the motor pattern of the observer toward that exhibited by the model, even at the muscular, peripheral level.[22] This finding suggests that motor resonance supports the efficacy of AOT at the cortical level, and is also reflected at the muscular level, i.e., one step nearer to the behaviour along the descending motor chain.

The activation of affected motor-related networks is an important indicator of good motor recovery because the motor cortex can reorganize and modulate the interactions between the affected and unaffected motor cortices following a stroke.[63] The combination of AOT plus NMES will facilitate activations of neural networks, including the peripheral nervous system and corticospinal motor neurons, and will simultaneously reward brain networks associated with learning. In particular, the combination of AOT with NMES will provide a relevant real-time somatosensory feedback using stimulation pattern according to the degree of physiological synergistic activation. Restoring physiological muscle synergies in stroke rehabilitation could potentially reduce the severity of motor impairments by addressing the disrupted patterns of muscle coordination typically seen in post-stroke patients. Research suggests that stroke survivors often experience alterations such as "merging" or "fractionation" of muscle synergies, which negatively impacts motor function.[33] For example, in patients with severe chronic stroke, muscle synergies in the more affected arm are less similar to those in healthy individuals, and these changes are associated with poorer motor function.[33] Restoring muscle synergies, based on physiological patterns, might help to counteract compensatory movements and promote more efficient motor control.[64,65] The Othello AOT-NMES approach aims to reduce these compensatory mechanisms by fostering physiological muscle contraction-coordination during action repetition that follows action observation, compared to the other two groups. These two aspects are fundamental points for achieving a long-lasting functional recovery after a stroke and for this reason we expect a clear improvement following AOT plus NMES.

With the present study we will also address the safety and usability of the AOT-NMES approach in the post-stroke population. Whereas it is well-known the safety of NMES in our population is well known,[66] to our knowledge no studies have investigated this important aspect in rehabilitation approaches involving both AOT and peripheral muscular stimulation.

We will investigate how the synchronized stimulation according to physiological synergic pattern in the AOT-NMES combined approach will influence the sensorimotor mechanisms subserving muscle synergies recruitment by

examining the similarity of the EMG activations between post-stroke subjects after training and healthy subjects. Within this framework we expect to find positive changes (higher level of similarity) parallel to improvement in clinical outcomes which in turn implies the good functioning of motor learning mechanisms.

The collection of neurophysiological indexes across the evaluation time points will allow us to assess the treatment's role in inducing lasting changes in corticomotor and spinal excitability. If correlated with individual behavioural improvements, such biomarkers would provide novel insights into the functional reorganisation of the central nervous system induced by AOT alone or in combination with NMES.

In this study, the collection of longitudinal high-field MRI data will also address the investigation of neuroplastic brain mechanisms aimed at compensating motor impairment associated with vascular insult. Whereas, at the group level, clinical improvements due to rehabilitation are known to be associated with structural and functional brain reorganization patterns (for a review see 67), data collected here will give the opportunity to evaluate how these reorganization patterns are seen in individual subjects according to the treatment performed. In particular, MRI structural data will be collected with a specific focus on lesion characterization. As the correlation between brain lesion and functional recovery is well known (as between corticospinal tract damage and functional recovery[68]), in the present study, the acquisition of MPRAGE, FLAIR and DWI sequences will render it possible to characterize lesions and their impact both on structural connectivity (for example using[59,60]) and on clinical motor recovery associated with treatment. The relationship between brain lesions, functional and structural changes with respect to rehabilitation will also be investigated. Specifically, functional connectivity patterns will be analysed to investigate the sensorimotor areas that serve the recovery of UL motor function. We will collect functional data both at rest (rsfMRI) and while executing sensory-motor tasks (as in 45,69) activating the fronto-parietal network subserving the *mirror mechanism*.[70,71] We expect to verify adaptive functional plasticity mechanism associated to rehabilitation treatment. Literature data also demonstrate that rehabilitation treatment might induce specific structural brain modifications (for a review see 67). With the longitudinal acquisition of DWI images, we will investigate white matter microstructural modification, attributable to rehabilitation-associated plastic changes.

The collection of surrogate outcomes (i.e., neurophysiological and neuroradiological) will also facilitate future data analyses, aimed at identifying surrogate biomarkers predicting the efficacy of rehabilitation treatment, as recently done.[72] Discovery and validation of prognostic markers of treatment success will support the implementation of tailored rehabilitation strategies, based on clinical data (i.e., phenotype at admission, individual patient's level of physical disability) and/or neurophysiological and imaging markers, thus identifying those patients that are more suitable for specific rehabilitative approaches such as AOT-NMES intervention, thus paving the way for the design of personalized rehabilitation treatments.

This study has some limitations that may affect the generalizability of its findings. The inclusion criteria, such as age range (50-85 years), specific motor impairment level, and a single stroke episode, limit the applicability of results to broader populations, including younger patients or those with different types of motor deficits. Moreover, the study focuses on patients in the subacute phase (2 weeks to 6 months post-stroke), excluding those in chronic stages. Future research should evaluate the effectiveness of this protocol in other timeframes and clinical scenarios. Finally, the use of normative muscle synergy patterns for NMES may not fully account for individual variations. Personalized approaches and larger inclusion criteria should be explored in future studies to validate and expand the applicability of this rehabilitation protocol.

Conclusion

Taken together results obtained from this study will provide insights into the efficacy and feasibility of an innovative, combined AOT-NMES treatment for the recovery of UL motor function in subjects following stroke. Moreover, the outcome measures collected will allow for the study of the underpinnings of motor recovery subsequent to rehabilitation and, together with the collected clinical and demographic data, will pave the way for the designing of tailored rehabilitative interventions.

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Ethical Approval

OTHELLO protocol received approval by the ethical committee of the IRCCS Fondazione Don Carlo Gnocchi (Milan), (ID 02_16/12/2022, approved Jan 4th, 2023).

Consent to Participate

Before recruitment, patients will be asked to sign an informed consent form, approved by the Ethical Committee, for study participation.

Author Contributions

Monia Cabinio: Conceptualization; Formal analysis; Methodology; Project administration; Writing - original draft; Writing - review & editing.
Tiziana Lencioni: Conceptualization; Formal analysis; Methodology; Writing - original draft; Writing - review & editing.
Arturo Nuara: Conceptualization; Formal analysis; Methodology; Writing - original draft; Writing - review & editing.
Federica Rossetto: Conceptualization; Formal analysis; Methodology; Writing - original draft; Writing - review & editing.
Valeria Blasi: Conceptualization; Methodology; Writing - review & editing.
Gaia Bailo: Conceptualization; Methodology; Writing - review & editing.
Rebecca Cardini: Conceptualization; Methodology; Writing - review & editing.

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Pietro Avanzini: Conceptualization; Methodology; Writing - review & editing.
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Luca Fornia: Conceptualization; Methodology; Writing - review & editing.
Francesca Baglio: Conceptualization; Methodology; Supervision; Writing - review & editing.

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Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Trial Registration
Registration: This study was registered on clinicaltrial.gov (NCT06055569).

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