TITLE: Brain-computer interface boosts motor imagery practice during stroke recovery

Running Head: BCI and motor imagery after stroke

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Abstract

Objective

Motor imagery (MI) is assumed to enhance poststroke motor recovery; yet, its benefits are debatable. Brain-computer interfaces (BCIs) can provide instantaneous and quantitative measure of cerebral functions modulated by MI. The efficacy of BCI-monitored MI practice as add-on intervention to usual rehabilitation care was evaluated in a randomized controlled pilot study in subacute stroke patients.

Methods

Twenty-eight hospitalized subacute stroke patients with severe motor deficits were randomized into 2 intervention groups: 1-month BCI-supported MI training (BCI group; n=14), and 1-month MI training without BCI support (CTRL group; n=14). Functional and neurophysiological assessments were performed before and after the interventions, including evaluation of the upper limbs by Fugl-Meyer Assessment (FMA; primary outcome measure) and analysis of oscillatory activity and connectivity at rest, based on high-density EEG recordings.

Results

Better functional outcome was observed in the BCI group, including a significantly higher probability of achieving a clinically relevant increase in the FMA score ($p < .03$). Post-BCI training changes in EEG sensorimotor power spectra (ie, stronger desynchronization in the alpha and beta bands) occurred with greater involvement of the ipsilesional hemisphere, in response to MI of the paralyzed trained hand. Also, FMA improvements (effectiveness of FMA) correlated with the changes (ie, post-training increase) at rest in ipsilesional intrahemispheric connectivity in the same bands ($p < .05$).

Interpretation

The introduction of BCI technology in assisting MI practice demonstrates the rehabilitative potential of MI, contributing to significantly better motor functional outcomes in subacute stroke patients with severe motor impairments.

Introduction

Mental practice in the form of movement imagination [motor imagery (MI)] has long been envisaged as a cognitive strategy to enhance poststroke motor recovery[1]. Significant efforts have been made toward identifying the neural mechanisms underlying MI and their relationship with improved motor recovery[2–5]. The rationale behind the application of MI to stroke rehabilitation is that mental practice with motor content engages areas of the brain that govern movement execution[6,7]. Such reiterated engagement of motor areas is intended to influence brain plasticity phenomena, improving functional outcomes[8,9].

Nevertheless, evidence for a clinical benefit of MI remains debatable. Although several studies have reported positive findings[10–12], a recent large, randomized, controlled trial in subacute stroke patients reported no significant clinical improvement of MI practice as add-on therapy to standard treatment or compared with mental practice without motor content[13].

Overall, these findings necessitate reappraisal of the content of MI training in stroke rehabilitation and its mode of delivery.

Brain-computer interfaces (BCIs) allow one to control external devices through direct brain activity *recognition* by a computer—ie, bypassing neuromuscular-based systems (voice, use of a mouse or keyboard)[14,15]. A widely adopted BCI paradigm uses the modulation of electroencephalographic (EEG) activity that is induced by the imagination of movement. MI elicits event-related desynchronization (ie, a reduction in spectral power) that occurs within certain EEG frequency oscillations and primarily over the scalp in sensorimotor cortical regions contralateral to the imagined part of the body (sensorimotor rhythms, mu rhythm)[16].

The hypothesis that such MI-based BCI systems can support motor rehabilitation[17–20] has increased the number of potential BCI users exponentially. Many groups have tested the applicability of sensorimotor BCIs in stroke rehabilitation[21–25]. A recent randomized, controlled trial (RCT) demonstrated significant clinical advantages in severely affected chronic stroke patients when BCI was combined with robotic therapy preceding intensive physiotherapy[26].

We hypothesized that the combination of MI practice with BCI technology facilitates the access of MI content under controlled conditions and our ability to track such cognitive motor task performance over time. In this pilot RCT, we examined the *efficacy* of a novel BCI-based MI training program, specifically implemented for upper limb motor recovery, the usability of which has been tested in a sample of hospitalized stroke patients[27].

This pilot RCT compared BCI-assisted MI training with MI training alone in a group of subacute stroke patients who were undergoing standard rehabilitation during admittance to our rehabilitation clinic. We assumed that BCI-supported MI training could reveal the rehabilitative potential of MI practice by providing therapists and patients with a tool to monitor MI execution; thus, we expected significantly better functional outcomes in the target group that performed MI with BCI support (primary outcome).

The volitional control of neural activity inherent to successful BCI operation[28] influences brain plasticity in healthy subjects[29,30] and stroke patients, further benefiting motor functional recovery[31,32]. To control for the effects of these phenomena, a neurophysiological assessment including high-density EEG recordings, was performed before and after both training interventions. We sought to investigate whether greater involvement of the affected hemisphere, expressed as changes in relevant EEG power oscillations and attributed specifically to BCI-assisted MI, would appear in the target group after training. We also explored whether changes in EEG-derived connectivity patterns at rest in a subgroup of patients were associated with both training modalities, based on evidence that functional connections in the motor network are disrupted after stroke in humans and in animal models[33,34].

Methods

Participants and study design

The trial comprised 28 stroke patients who were sequentially enrolled from those who were admitted to 3 stroke neurorehabilitation units of Fondazione Santa Lucia (FSL, Rome) over 2 years. The patients were

approached about the study in the first several days after admission. The physicians responsible for the clinical trial provided them and their relatives (when needed) with written information on the trial protocol.

The study was a randomized, controlled trial (consistent with indications on how to structure *pilot studies* to evaluate novel rehabilitative intervention[35]) and was approved by the local ethics board (Prot.CE/AG4-PROG.244-105), and written informed consent was obtained from each patient. All eligible and consenting patients were evaluated (PRE; see Functional and Behavioral and Neurophysiological Assessment sections) by the research team and assigned to 1 of 2 motor imagery (MI) intervention groups by blind randomized allocation. The randomization procedure and factors are shown in Supplementary File S1.

The intervention groups were an experimental patient group that received 1 month of "BCI-supported" MI training (*BCI group*, 14 patients) with 3 weekly sessions and a control patient group that received equally intensive MI training with no BCI assistance (Control [*CTRL*] *group*, 14 patients). The allocation was concealed from the evaluators (research physicians). The same research team responsible for the evaluation PRE training performed a post-training clinical and neurophysiological assessment (POST). Patients were informed about the group allocation by the research therapists who delivered the interventions.

All patients received the standard treatment for stroke in terms of medical care and rehabilitation (intensive treatment, including motor, occupational, and cognitive therapy) for approximately 3 hours per day; thus, the interventions were intended as "add-on" therapy. The following inclusion criteria were applied: (i) a history of first-ever unilateral, cortical, subcortical, or mixed stroke, caused by ischemia or hemorrhage (confirmed by magnetic resonance imaging), that occurred 6 weeks to 6 months prior to study inclusion; (ii) hemiplegia/hemiparesis that was caused by the stroke; and (iii) age between 18 and 80 years. The exclusion criteria were the presence of chronic disabling diseases, such as orthopedic injuries that could impair reaching or grasping; spasticity of the shoulder, elbow, or wrist, scored 4 or 5 on the modified Ashworth Scale[36]; a Mini-Mental State Examination score < 24[37]. A neuropsychological evaluation was

routinely performed for diagnostic purposes, and patients with severe hemispatial neglect, severe aphasia, and apraxia were excluded.

Functional and behavioral assessment

A set of specific functional scales was administered before and after the experimental and control interventions. The primary outcome measure was the arm section of the Fugl-Meyer Assessment (FMA) [38,39]. A minimal clinically important difference (MCID) for this scale was set to 7 points[40]. Other functional outcome measures included the National Institute of Health Stroke Scale (NIHSS)[41], the upper limb section of the Medical Research Council scale for muscle strength (MRC), and the upper limb section of the Modified Ashworth Scale for spasticity[36]. To account for the high variability in impairments, we quantified the parameter "effectiveness" for FMA, NIHSS, and MRC, defined as the proportion of potential improvement that could be achieved after the intervention and calculated as POST score minus PRE score, divided by the maximum score minus PRE score, multiplied by 100. Thus, if a patient achieved the highest possible score after the intervention, the effectiveness was 100%[42]. This approach allowed us to normalize the data, accounting for baseline differences.

All statistical between-group differences in epidemiological and clinical characteristics at baseline were analyzed by Mann-Whitney U-test. The changes from pre- to post-training assessment in primary and secondary outcome measures in both intervention groups were analyzed by Wilcoxon matched-pairs rest. Between-group changes in the effectiveness parameter were analyzed by t-test (independent, by variables). The probability of a patient achieving an MCID for the primary outcome measure, FMA score, was examined by relative risk analysis (ie, analogous to the odds ratio for prospective studies[43]).

Finally, the perceived subjective workload that was associated with both training modalities was analyzed by NASA TLX[44], an instrument that has been use in BCI applications as a measure of efficiency—ie, the costs that have been invested in relation to how accurately a task can be performed[45]. This scale was administered at the end of the first and last BCI training sessions.

Between- and within-group differences in NASA TLX score were analyzed by repeated measures analysis of variance (ANOVA) with “group” (BCI and CTRL) as an independent variable and “session” (first and last) as a dependent variable. The threshold for significance was set to $p < 0.05$.

Neurophysiological assessment

As part of the clinical trial, we conducted an extensive neurophysiological assessment by high density-EEG and single-pulse transcranial magnetic stimulation (TMS). All patients were evaluated before (PRE; these data were also used to extract EEG features for BCI control in the *BCI group*, see section "Experimental intervention") and at the end of both training interventions (at least 48 hours after the last training session, POST).

Data Acquisition

During the EEG data acquisition (PRE and POST sessions), all patients were comfortably seated in an armchair in a dimly lit room with their upper limbs resting on a desk. Visual cues were presented on a screen on the desk. Scalp EEG potentials were collected from 61 positions, assembled on an electrode cap (according to an extension of the 10-20 International System) and band pass-filtered between 0.1 and 70 Hz, digitized at 200 Hz, and amplified by a commercial EEG system (BrainAmp, Brainproducts GmbH, Germany).

The sessions were divided into runs, each of which comprised 30 trials. Each trial began with a cursor appearing in the lower center of the screen and moving toward the top at constant velocity on a line. The total trial duration was 9 s, with an intertrial interval of 1.5 s. Patients were instructed by the therapist to perform 2 tasks: MI or rest. The timing of the tasks was cued visually. During MI task trials, a green rectangle appeared at the top of the screen; its width was 100% of the screen width, and its length equaled approximately 57% of that of the screen, occupying the last 4 s of the cursor’s trajectory,[2,4] and patients had to start performing the cued motor task when the cursor reached the green rectangle and continue it until the end of its trajectory.

Each run was dedicated to a specific motor task that involved their unaffected or affected hand. Task A consisted of imaging a sustained grasping movement, whereas Task B entailed sustained complete extension of the finger. Tasks A and B were then trained during both MI interventions (BCI and CTRL). The choice of a double task was agreed with clinicians, in order to comply with standard physical therapy (ie to stimulate agonist and antagonist muscles aiming to prevent spasticity of forearm flexor muscles). During rest trials, the patients were simply asked to watch the cursor's movement on the screen. The command sequences were randomized; thus, the runs included 15±1 rest and 15±1 MI trials, respectively. Five minutes of EEG recordings at rest (relaxed, eyes closed) were acquired at the beginning of the PRE and POST EEG screening sessions.

Because the intervention required the patients to engage in active motor imagery[46], they were instructed to perform kinesthetic MI, which is defined as MI that implies somesthetic sensations that are elicited by the action[47]. The guiding principle is that sensorimotor integration favors brain plasticity phenomena that potentially underlie better motor outcomes[9]. To facilitate *correct* performance of such MI, patients were allowed to execute tasks A and B with the unaffected hand several times in a row (ie, task timing acquisition) and were invited during the MI to rehearse the sensations that were felt during the actual execution of the same movements. All patients were thus instructed to perform only MI in tasks A and B; to verify muscle relaxation and avoid movement attempts in patients with residual motor ability, electromyography (EMG) values were recorded through surface electrodes on the hands and forearm muscles and visualized online.

Kinesthetic, but not visual, MI engages the motor system, enhances motor cortical excitability, as measured by TMS[48]. Moreover, the kinesthetic type of MI increases the motor evoked potential (MEP) amplitude[6,48], which correlates with the ability to perform MI[49]. Thus, TMS of the primary motor areas was performed during the MI tasks to verify the patients' compliance with the tasks, as reflected by the changes in MEP amplitude.

The TMS session was performed before training (PRE) within 48 hours of the EEG recording session on a separate day. The protocol was similar to that of the EEG session, except that the number of trials per run was 20 versus 30, with a longer intertrial interval (6.5 s *vs* 1.5 s). TMS stimuli were delivered by the experimenter approximately 2 s after any given command (either rest or MI task). The electromyographic (EMG) activity from the first dorsal interosseous (FDI) muscle was recorded through Ag/AgCl surface electrodes in a belly-tendon montage (Galileo-NT; Italy). The amplified and bandpass-filtered (0.1 Hz to 2 kHz) raw EMG signal was digitized at a 20-kHz sampling rate and stored for offline analysis.

Single-pulse magnetic stimuli were delivered through a round coil that was connected to a Magstim 200 (Magstim Company, Whitland, UK) over the motor cortex in the optimal position to elicit motor-evoked potentials (MEPs) in the FDI muscle of the imagined hand. Due to the severity of the motor deficit, MEPs from the affected hand could not be elicited in certain patients, in which case only MI of the unaffected hand was performed. The motor threshold (MTH) at rest was defined as the lowest intensity that produced MEPs greater than 50 uV in at least 5 of 10 consecutive trials in the FDI muscle[50]. During the session, the intensity of the stimulator was set to 120% of the MTH. The MEP amplitude from FDI muscles was measured peak to peak.

The mean MEP amplitude values (± standard deviations) at rest were compared with those during MI of hand movements for each patient (tasks A and B were analyzed together). Due to the high variability in MEP amplitude between individuals, the data were normalized, and amplitude changes during the MI tasks were expressed as percentages of the amplitudes at rest. Differences in MEP amplitude increases that were associated with MI task between groups were analyzed by t-test for independent variables. Transcranial magnetic stimulation could be administered to 23 of 28 patients (BCI group, n= 12 patients; CTRL group, n=11 patients), based on compliance and safety issues (eg, pacemaker or other metallic implants). Of the 23 patients, 9 had recordable MEPs on the affected-side FDI muscle (3 in the BCI group, 6 in the CTRL group). Unless otherwise noted, the threshold of significance was set to $p < 0.05$.

The same procedure was applied for BCI and CTRL patients.

Data Analysis

Power Spectral Density Analysis

Power spectral density (PSD) analysis of the EEG data that were recorded during the PRE and POST sessions was performed offline to describe the differences between the BCI and CTRL groups. EEG data were downsampled at 100 Hz and band pass-filtered (1-45 Hz). Ocular artifacts were removed by independent component analysis (ICA)[51,52], and residual artifacts (muscular, environmental, etc) were removed using a semiautomatic procedure, based on the definition of a voltage threshold (± 80µV). The preprocessed EEG signals were then segmented, considering the last 4 s of each MI and rest trial as the period of interest. After common average reference (CAR) spatial filtering, the PSDs of EEG signals that were acquired during the task and rest trials were computed by Welch method[53] for each channel. Individual alpha frequency (IAF; 9.45 ± 0.54 Hz) was determined for each subject to account for the between-subject variability of the alpha peak in the spectrum[54]. The IAF was used to define 5 frequency bands: theta (IAF-6 Hz through IAF-2 Hz), alpha (IAF-2; IAF+2), beta1 (IAF+2; IAF+11), beta2 (IAF-11; IAF+20), and gamma (IAF+20; IAF+35). The PSD values for each frequency of the range of interest (1-45 Hz) were averaged within the 5 EEG frequency bands.

Thereafter, statistical PSD maps were generated for each patient's dataset, as follows. Single-subject statistical comparison (independent two-sample t-test) was performed between MI and rest PSD values for each channel and frequency band for MI tasks that were performed with the affected and unaffected hand. Due to the similarity between the spatial and frequency patterns elicited by Task A and B (ie, power spectra desynchronization of scalp sensorimotor areas), data from tasks A and B were pooled for further analysis. The tests returned negative t-values in the case if desynchronization occurred (ie, a decrease in power) and a positive t-value in the case of synchronization (an increase in power).

For the group analysis, we eventually flipped the functional (EEG time series) and anatomical (scalp electrode positions) data of patients with right-sided lesions along the midsagittal plane, so that the ipsilesional side was common to all patients[2,55,56].

To evaluate any significant between-group (BCI and CTRL) differences in the PSD maps from each patient (considering each patient as "repetition") during the PRE and POST conditions, we performed independent two-sample t-test (significance level of 0.05). In this analysis, Bonferroni correction for multiple comparisons was applied to avoid type I errors[57,58].

To analyze the EEG data that were recorded during the BCI training, "EARLY" (the second session) and "LATE" (a session from the final week of training) sessions were identified for each patient. The online EEG data on MI in tasks A and B were preprocessed per the procedure above. The PSD values, relative to the MI and baseline epochs, were computed and averaged within the 5 frequency bands. One-tailed paired sample t-test (significance level of $p < 0.05$) was used to compare negative t-values (desynchronization) of EARLY and LATE sessions for each frequency band on EEG channels that were selected for BCI control. Thus, this analysis included only central and centroparietal channels in the affected hemisphere to highlight the reinforcement of desynchronization patterns. FDR correction for multiple comparisons was applied[59].

Resting State Connectivity

Here, we adopted the effective connectivity[60–62] estimation method to describe the cortical network properties under resting conditions (resting state). Partial directed coherence (PDC)[62], a well-established, full multivariate spectral measure that determines the directed influences between a pair of signals in a multivariate dataset, was used as a measure of effective connectivity. PDC has many advantages, such as high accuracy, stability, and robustness to noise[61,63–65]. PDC prevents false-positives from appearing compared with other connectivity measures (such as ordinary coherence and other pairwise approaches) and distinguishes between direct and cascade causal effects[61,65].

In this study, the squared formulation of PDC was applied[64] to further ensure its accuracy and stability[65]. The PDC matrices were computed per methods that have been detailed elsewhere [61,62,64]. In brief, spurious connectivity values due to random correlation between the data were discarded by asymptotic statistical procedure[66–69], returning a *significance* threshold for each PDC value and thus allowing only significant

connections (ie, PDC values) within the (adjacent) matrices to be selected. This technique prevents conclusions that are based on random properties of the network from being drawn[69].

The PDC matrices were computed for EEG data that were recorded at rest during the PRE and POST sessions from a subgroup of BCI (n=11) and CTRL (n=9) patients. To reduce the computational complexity, PDC values were calculated from 51 of 61 EEG channels (omitting the most peripheral electrode leads: Fpz, AF7, AF8, FT7, FT8, TP7, TP8, PO7, PO8, and Oz) for each (5) frequency band.

The obtained connectivity networks, expressed as PDC matrices, were then examined with a graph theoretical approach to provide synthetic measures that described the topological properties of the network[60].

The following 2 indices were considered to summarize the chief network properties: *Density* and *Weighted Density*.

Network Density is the more general property of the network[70] and is defined as the number of significant connections divided by the total number of possible connections:

$$Density = \frac{L}{N(N-1)} \quad (1)$$

where *L* is the number of significant connections that is returned by the (asymptotic) statistical assessment and *N*, in this case, is the number of electrodes. Density ranges from 0 to 1; the sparser the network the lower its value.

We initially aimed to identify the relevant differences in network size[71,72] that could be associated with the experimental and control interventions at baseline (PRE) and after training (POST). Thus, the between-group differences in connectivity network *density* were examined by t-test for independent samples ($p<0.05$) in the PRE and POST conditions across frequencies. Significant *density* variations between PRE and POST were also examined in each group by paired-sample t-test ($p<0.05$).

The *Weighted Density* index is the average value of network connections and is obtained by totaling the values of all significant PDC values divided by the number of all significant connections *L*.

This index was used to describe possible training-related changes in the estimated networks at the intrahemispheric level; hence, *weighted density* was computed separately for the affected and unaffected hemispheres. Per the definition of *weighted density*, the 2 intrahemispheric networks were obtained as follows:

$$Weight_{Hem} = \frac{\sum_{Hem} PDC}{L_{Hem}} \quad (2)$$

where $\sum_{Hem} PDC$ is the sum of PDC values of a given hemisphere and L_{Hem} is the number of significant connections within that hemisphere. Here, we examined whether the possible changes in the intrahemispheric networks correlated with the behavioral (primary) outcome measure, the FMA. Specifically, we assumed that a change toward a functional improvement, expressed as FMA scale effectiveness (see Functional and Behavioral Assessment), would be (positively) associated with a change toward an increase in the intrahemispheric *weight* of the affected (trained) hemisphere in the BCI with respect to MI alone.

To effect consistency between the correlated measures, the variation in *intrahemispheric weight* was expressed as the percentage of changes between the POST and PRE condition in the BCI and CTRL groups:

$$\Delta weight_{Hem} = \frac{weight_{Hem}^{POST} - weight_{Hem}^{PRE}}{weight_{Hem}^{PRE}} * 100 \quad (3)$$

where the superscripts *POST* and *PRE* denote the 2 conditions—before and after the intervention. The index in equation (3), considered to be analogous to FMA effectiveness, was calculated for each patient for both hemispheres and across frequency bands. A descriptive statistic, Pearson's correlation (p <0.05), was then applied to determine the existence of a significant positive correlation (one-tailed test) between $\Delta weight_{Hem}$ and FMA effectiveness for the experimental (BCI) and control (MI alone) interventions.

A subsequent analysis was performed, in which we focused on the interhemispheric connections (IHCs), based on evidence that changes in connectivity between hemispheres are linked to functional motor recovery after stroke[34]. Our assumption was that a change toward an increase in IHC (PDC) values was associated with the proposed (BCI) training intervention. Accordingly, one-tailed paired-sample t-test ($p<0.05$; FDR-corrected) was performed in each group (BCI and CTRL) to determine whether significant differences (ie, increases in PDC) in each estimated connectivity value (ie, without thresholding) could be detected after the experimental (BCI) and control (MI alone) interventions. Consequently, one adjacent matrix was generated for each intervention group and band frequency, and the number of the IHCs that were significantly "reinforced" after training (POST vs PRE) was extracted (see Fig. 4).

To validate the procedure and eventually determine the significance of the derived IHC values, we computed their empirical distribution in the null case by randomly shuffling the PDC values over the entire connectivity network (thus disrupting the network topology) in the PRE and POST conditions for each patient and across frequencies. Then, the distribution of the null case was obtained, evaluating the significant POST-PRE increase in IHC values of the *random* matrices, and the number of randomly "reinforced" IHCs was counted. This procedure was reiterated (up to 1000 times), and the significance threshold at the 95th percentile was computed for each experimental group.

Experimental Intervention: Brain-Computer Interface-Assisted Motor Imagery Training

A dedicated BCI prototype was developed to support MI training in the BCI group[27], based on a sensorimotor BCI training system (www.bci2000.org) that was modified slightly to address the specific aims of a rehabilitation session. First, we envisaged the presence of a therapist, who received continuous feedback on the patients' sensorimotor rhythm modulation by through a common sensorimotor feedback interface (ie, motion of a cursor on a screen). The patients received discrete feedback of successful trials through a specifically developed visual interface that was ecological and congruent with the imaginative task (a representation of their affected hand). Also, patients were guided continuously by the therapist,

who was allowed to monitor mental activity and muscle relaxation online (see Figure 1 and Video, Supplementary).

This online monitoring system also prevented movement attempts in patients with residual motor ability; all patients were trained to perform MI only of the affected hand movements that consisted of grasping and finger extension (as described in Neurophysiological Assessment, Data Acquisition) to achieve control of the same movements by the “virtual hand." The training lasted 4 weeks, with 3 weekly sessions; each training session comprised 4 to 8 runs (depending on the patient’s compliance) and lasted approximately 30 minutes (exclude the EEG cap montage time). Each run consisted of 20 trials.

Online EEG signals for BCI training were recorded from 31 electrodes, overlying the frontocentral, central, and centroparietal regions. Data acquisition, online EEG processing, and feedback to the therapist were performed using BCI2000 (www.bci2000.org); feedback to the subject was provided throughout a UDP connection between the BCI2000 and “virtual hands” software. EEG data were also stored for offline analysis. For further details on the BCI training paradigm, please refer to [27].

The control features that were to drive the visual feedback (to the therapist) and operate the “virtual hand” software (feedback to patients) in real-time during the BCI training sessions were extracted through offline analysis of the MI-related EEG data from the initial screening session (PRE, see Neurophysiological Assessment). EEG data were re-referenced to the common average reference and divided into epochs of 1 s, and spectral analysis was performed by using a maximum entropy algorithm with a resolution of 2 Hz. All possible features in a reasonable range (0–60 Hz in 2 Hz bins) were extracted and analyzed simultaneously. A feature vector was extracted from each epoch and labeled according to the experimental condition (MI and rest). This vector comprised the spectral amplitude at each frequency bin for each channel. Using all epochs of the recording session, the coefficient of determination R2 (ie, the proportion of total variance of the signal amplitude that was accounted for by the target position[73]) was calculated to determine significant differences in the values of each feature in the 2 conditions. At the end of this process, R2 values

were compiled in a channel-frequency matrix with head topography and evaluated to identify the set of candidate features that best separated rest versus task.

Relevant control features were selected by an expert neurophysiologist, who was aware of the procedures that were used to evaluate the patient's ability to perform MI tasks, from the central and centroparietal electrodes that were distributed only over the affected hemisphere that showed desynchronization patterns (ie, a decrease in spectral power) at EEG frequencies that were typical for the modulation of sensorimotor rhythms (see Supplementary File S4). Thus, through BCI training, we aimed to reinforce the individual EEG patterns of reactivity that most resembled the *physiological* activation that was relevant to movement imagination of the contralateral hand[16]. A similar physiologically driven, rather than data-driven, approach in BCI control feature extraction has recent garnered attention in BCI applications that promote motor rehabilitation after stroke[26].

The outcome measure of BCI training was the subjects' performance, calculated as the percentage of correct trials per run. t-test for dependent samples was used to examine the changes in (BCI) group performance across BCI training sessions. The online performance of the second and last training sessions was considered for statistical analysis, because during the first BCI session, patients were instructed primarily on the BCI prototype setting and functioning. The significance threshold was set to $p < 0.05$.

Chance level was estimated under a no-control condition (subject at rest), in which modulation of sensorimotor rhythms was attributed solely to physiological variability. In these conditions, statistical properties (average and standard deviation) of the BCI transducer's output were estimated. Under the hypothesis of Gaussian distribution, a corrective factor was applied, such that the cursor would hit the target in only 5% of the trials (false positives). Empirical tests confirmed that after this correction, approximately 1 trial per run ended with an unintended hit.

Control Intervention: Motor Imagery Training

An MI training program (without BCI support) served as a control condition (CTRL group). The training room was equal to the BCI-supported MI training area with regard to the size and arrangement of furniture.

Patients were seated in a comfortable chair or directly on their wheelchair, with the hands resting on a desk in front of them, where an adjustable forearm orthosis provided support. Under the supervision of a qualified research therapist, the patients were instructed to imagine the same movements as in the BCI-based MI training (grasping and finger extension) with their affected hand. The visual cues and timing were provided on a screen in front of the patient, displaying hand representation similarly as in the BCI training. For the BCI group, training lasted 4 weeks, with 3 weekly sessions (each session was approximately 30 minute duration, comprising 4 to 8 runs, depending on the patient's compliance). Each run consisted of 20 trials.

Results

Baseline Differences

Between January 2011 and December 2013, we enrolled 32 patients consecutively; 4 patients dropped from the study, and thus, the data were analyzed for 28 patients (for details, see Supplementary File S1). The demographic, clinical, and functional data of both groups are summarized in Table 1. The same information is given for individual patients in Supplementary File S2. No significant differences between groups were noted at baseline with regard to demographic and clinical patient characteristics or functional outcome measures. Also, the analysis of MEP amplitudes (expressed as percentage increase) during unaffected hand MI (unaffected FDI muscle) revealed no significant between-group differences ($p>0.05$), indicating that the BCI and CTRL groups performed the required MI task equally well at baseline. The small sample of patients with recordable MEPs in the affected FDI muscle (n=9 patients in total) prevented us from performing the same between-group analysis during MI of the affected hand (See Supplementary File S5).

Functional Outcome

The BCI and CTRL groups experienced a significant improvement in mean FMA, MRC, and NIHSS values from the baseline (pretraining assessment; PRE) to outcome (post- training assessment; POST) assessment

(see Supplementary File S3). This improvement (with the exception of mean MAS scale values), regardless of the type of MI training, was predictable, based on concomitant factors, such as the patient's subacute stage of stroke and participation in a conventional intensive rehabilitation program.

In the statistical analysis, the effectiveness of the primary outcome measure, FMA (arm section), was significantly higher in the BCI versus CTRL group (44 ± 34.7 vs 19.8 ± 19.8; p = 0.03; Fig. 2). As shown in Figure 2, similar results were obtained for the effectiveness of the secondary outcome measures, MRC (BCI group: 36.8 ± 24.4; CTRL: 12.4 ± 16.2 SD; p = 0.004) and NIHSS (BCI group: 11.5 ± 6.1 ; CTRL: 4 ± 4.3; p = 0.0009). Further, the probability of achieving an MCID (7 points) for FMA was significantly higher in the BCI group compared with the CTRL group (11 vs 3, respectively; relative risk 33.7, 95% CI 1.2-10.3, z=2.4, p = 0.01).

MI-based BCI Training

Information on BCI training control features can be found in Supplementary File S4. All patients acquired confidence in controlling the system, and no significant changes in average performance were observed from the second (66 ± 25.7%) to final (65.1 ± 24%) BCI training session (dependent-sample t-test, p>.05). A comparative analysis (one-tailed paired-sample t-test; significance level of $p < 0.05$) of negative t-values, which reflected the desynchronization patterns that were associated with MI tasks in the EARLY and LATE training sessions, was performed for each frequency band in EEG channels that were selected for BCI control (central and centroparietal electrodes). Significant differences were noted only in the beta1 band (Table 2).

By repeated-measures ANOVA of NASA-TLX scores, there was a significant effect of the factor *group* ($F(1, 26)=6.4561$, p=.01737), with higher scores in the BCI versus CTRL group. We did not observe any significant effect of the factor *session* or any significant interaction between *group* and *session*.

Neurophysiological Outcome

EEG oscillatory patterns

As shown in Figure 3 (left panel), no significant differences were seen between the BCI and CTRL groups in desynchronized activity that was related to MI of the affected (paralyzed) hand in the PRE condition in any frequency band (Fig. 3, left panel, upper row). In contrast, under the POST training conditions, we noted significantly more robust desynchronization (p<.05, Bonferroni-corrected) in the BCI versus CTRL group in the alpha and beta1 bands (Fig. 3, left panel, lower row).

These significant differences were germane only to the centroparietal regions of the ipsilesional hemisphere (ie, CP5 and CP3 electrodes; Fig.3 left panel, lower row) and the central midline (Cz electrode) in the alpha oscillatory band. At the higher frequency (beta1), these differences still involved mainly the ipsilesional hemisphere (C1, CP1), but also C2 (contralesional hemisphere) and Cz electrodes were involved (Fig.3 left panel, lower row).

Similar to what was observed for the MI task of the paralyzed hand, the MI task with the unaffected hand was not associated with significant differences in the PRE screening session. In the POST training condition, however, there was a significant difference (BCI > CTRL; p < .05, Bonferroni corrected) only over the contralesional hemisphere (C4 and FC2 electrodes) in the alpha and beta1 bands (Fig. 3, right panel, lower row).

We did not observe any significant differences in synchronization patterns that were related to affected and unaffected hand grasping MI in PRE or POST training.

Functional Brain Networks

The network *density*, which quantifies the size of empirical networks[72], did not differ significantly between (t-test for independent samples) or within (paired-sample t-test) groups before or after training. This lack of significance in connectivity density held true for the overall, inter-, and intrahemispheric networks—ie, the

basic topological network characteristic (ie, the number of nodes and edges) was consistent across intervention groups and conditions.

This analysis focused on intrahemispheric connectivity, measured as the *Weighted Density* index, and detected a significant positive correlation between $\Delta weight_{AH}$ (ie, the post-training percentage increase in the index value, computed for the affected hemisphere [AH]) and the effectiveness of the FMA scale in the BCI group in the beta1 (Pearson's correlation coefficient R = 0.568, p = 0.034), beta2 (R = 0.604, p = 0.024), and gamma (R = 0.609, p = 0.023) ranges of frequency. The same index, computed for the unaffected (UH) hemisphere ($\Delta weight_{UH}$), was not significantly linked in any of the EEG frequency bands. No significant associations ($\Delta weight_{AH}$ and $\Delta weight_{UH}$) were observed in the CTRL group.

Focusing on IHC, we noted that IHC patterns varied after training as a function of the oscillatory frequency bands in the BCI and CTRL groups. As illustrated in Figure 4, the extracted number of "reinforced" IHCs after training (ie, the number of connections with post-training PDC values that rose significantly by one-tailed paired-sample t-test) exceeded that estimated for the null hypothesis network in the beta1 and beta2 frequency bands (30 and 35, respectively; Fig.4, upper row) for the BCI group, whereas this pattern occurred in theta and alpha bands for the CTRL group (29 and 26, respectively; Fig. 4, bottom row).

Discussion

This pilot RCT highlights the significant improvement in our primary functional outcome measure, FMA (arm section), following hand MI that is assisted by an EEG-based BCI system in first-ever, unilateral, subacute stroke patients. Such training reinforced the desynchronization in EEG sensorimotor oscillatory activity that occurred with greater involvement of the damaged hemisphere when the paralyzed trained hand was imagined.

Functional outcome

The positive relationship between MI practice and clinical improvement has been reported in RCTs that combined MI and physical practice in stroke patients with moderate hand-arm motor deficits[11,12]. These

positive findings, however, were challenged by the lack of efficacy when MI was implemented in a regimen that was independent of physical training, such as in a large RCT of early stroke patients with moderate motor impairments[13].

In our study, the BCI system intends to provided the patient (and therapist) with a means to control and monitor MI tasks and promote his adherence to a purely mental practice with visually enriched feedback, consistent with imagery content[74]. In this scenario, the clinically significant increase in arm FMA scores is substantial evidence that when MI practice is embedded in a closed-loop BCI paradigm, severely motor impaired subacute stroke patients benefit from such mental motor practices.

The chief element of the proposed BCI-driven MI intervention is that it establishes online, positive rewarding *output* of the MI tasks by providing feedback in the form of a visual representation of the patient's paralyzed hand closing or opening. This *time-locked* association between the mental task and its visual representation is mediated by the voluntary modulation of ipsilesional brain activity. An exogenous explicit link between the modulation of MI-related neural activity and the sensory (visual) consequences is thus engrained. Accordingly, a significant reinforcement of EEG features that were selected for BCI control[21,75] was observed between EARLY and LATE training sessions (Table 2). Thus, this experimental framework made it especially likely that the BCI allowed patients to learn to perform an *optimal* (kinesthetic) MI practice—ie, more effective recruiting of the MI sensorimotor neural substrates [17,18,20,76]—compared with the same MI tasks in an open-loop condition (ie, without the BCI).

The re-establishment of an instantaneous and contingent link between the patient's brain activity—related to motor intent/attempt—and the actual paretic arm movements, supported by a hand orthosis (haptic feedback) in a motor relearning context, has been hypothesized to be an essential component of the BCI that promotes motor function recovery in chronic stroke[26,77]. Despite the substantial differences between various BCI-based approaches (eg, motor execution *vs* imagery), it is conceivable that a similar *contingency* between MI tasks (and the related brain signals) and the congruent visual feedback could occur in our MI-assisted BCI intervention and account for the superior outcome of MI with the BCI. This hypothetical

mechanism would strengthen the function of the extrinsic feedback, which is relevant for motor relearning after stroke[78].

Our results also show that the clinical benefit of the combination of BCI and MI as an add-on to standard physical therapy was not confined to the FMA target function (upper limb)— general clinical outcome scales also improved. We attribute the latter to the positive effect of BCI training in facilitating the patient's adherence to the task performance which, in turn, would positively affect his response to the physical rehabilitation therapy itself [79,80].

Other *less specific* aspects that might account for the benefit of our experimental BCI intervention, such as motivational and psychological factors[79,81], can not be dismissed. We noted that MI training with BCI support was perceived to be more demanding than MI training alone—a difference that was evident only for the global score (ie, no significant between-group differences in single NASA TLX domains were observed). This finding might reflect spontaneous, greater engagement of patients with the BCI with respect to the control intervention, thus accounting for the effect of the BCI on recovery. Notably, we did not observe a higher rate of patient dropouts in the MI-alone group—the 1-to-1 sessions with therapists were equivalent between the 2 intervention groups, and the medical staff that oversaw the patients was blinded to the intervention. Based on these methodological considerations, we believe that psychological components explain our results marginally.

The subjective perception of workload remained stable across training sessions, confirming that the BCI-assisted intervention was well tolerated by stroke patients[27]. Nevertheless, the extent to which BCI-based interventions that are combined with complex multisensory feedback is suitable for elderly, often cognitively impaired stroke patients, remains unknown. The unselected sample of stroke patients (for whom the exclusion criteria were only severe neuropsychological deficits) in the current pilot RCT is a relevant factor in evaluating the experimental intervention. Whether the resulting clinical benefit is worth the cost with regard to workload remains to be determined in larger controlled studies that incorporate

techniques for standardizing task-related practice intensity, which is critical when testing the efficacy of novel therapeutic interventions[82].

Task-specific training was recently reported to induce long-term improvements (up to 6 months from the intervention) in upper limb motor function after stroke[83]. We speculate that the BCI-based rewarding[28,84] of MI promotes longlasting retention of motor performance with respect to MI practice in an open loop condition. The persistence of regained motor function requires evaluation in a follow-up study (eg, in a home-based context).

Neurophysiological Outcome

After training, MI (with feedback withheld) of the affected hand in the BCI group effected significantly greater desynchronization only in the sensorimotor rhythms, which involved the mesial (central) area and primarily the postcentral regions of the damaged hemisphere. Greater engagement of the lesioned hemisphere was our chief interest in determining the neurophysiological effects of BCI-assisted MI practice, and our results highlight the value of the BCI closed loop in facilitating greater "physiological" recruitment of the stroke-affected hemisphere with respect to MI practice without feedback. Enhanced laterality of the event-related sensorimotor oscillations has recently been shown in healthy subjects during real-time neurofeedback-guided MI training[85].

We observed, however, that the recruitment of the lesioned hemisphere mainly involved non-primary motor-associated areas.

Whereas (kinesthetic) MI activates a large network of cortical and subcortical areas [86–88], the extent and magnitude of M1 activation during MI vary (for review, see [89]). The contribution of M1-generated signals has also been questioned in MI-based BCI tasks[90] and real-time fMRI-based neurofeedback training[91]. Although we can not exclude that this factor is partially responsible for the inconsistency of central (M1) activity in our topographical maps (Fig. 3), the variable extent of stroke lesions might have resulted in an overall group map that *hides* the activity that is generated by scattered survival portions of M1. Moreover, the imagery of the untrained, unaffected hand also elicited significantly stronger sensorimotor rhythm

desynchronization that peaked in the contralateral central (and frontocentral) area in the BCI group (Fig. 3), which is compatible with M1 activity. Based on previous observations that the learning effect of unilateral MI training can be transferred to the untrained contralateral limb[92], the MI of the untrained hand reflects M1 engagement during MI by our stroke patients.

Several imaging studies have reported that human brain reorganization following a stroke in the area of the middle cerebral artery engages a widespread network, comprising primary and nonprimary motor areas in the ipsilesional and contralesional hemisphere, especially in the early phases of recovery (for review see[93]). The compensatory or restorative nature of these plastic changes and their relationship with functional recovery depend largely on the lesion size[93,94]. In most of our patients, the stroke lesion likely involved a substantial portion of the primary motor area (M1) and the tracts that descend from M1 (and the premotor dorsal cortex), as indicated by the severity of the motor impairment and by the lack of recordable MEPs from the affected upper limbs (14 of 23 patients). Based on these conditions, it is plausible that the BCI promoted the activity of sensorimotor areas (the ipsilesional parietal area and mesial premotor and supplementary motor areas) other than M1 that are stimulated during MI [5,86], implying that the better clinical outcomes in the BCI group were mediated by compensatory changes rather than the restoration of M1 activity.

It is important to stress that the explorative nature of our resting state brain network investigation (in a subsample of patients) advocates cautious interpretation. We hypothesize that the observed positive correlation between the increase in ipsilesional connectivity at rest in the beta and gamma oscillations and functional improvement supports our interpretation that proposed BCI training intervention effectively harness the sensorimotor rhythms in the affected hemisphere, the recruitment of which enhances the clinical improvement in the BCI group. Similarly, we also speculate that the post-training increase in IHC at rest in the BCI group and only in the beta-range frequency (Fig. 4) reflects a higher coupling between hemisphere which is related to the selective engagement of the stroke hemisphere promoted by the BCI intervention. The observed increase in IHC within the lower frequency oscillations in the CRTL group remains rather undefined. Some speculative interpretations arise from the assumption that the practice of

MI under an open-loop condition might have favored inter-subjects variability in MI performance. This variability might have lead to a (small size) group pattern of "reinforced" IHC that is representative of other components of the hand MI such as attention focusing[95] and/or of diverse imagery contents[96].

In conclusion, prioritizing the clinical evaluation, our pilot study in a rehabilitation clinic demonstrates that a low-cost technique (eg, EEG-based BCI) can be exploited to deliver an MI-based intervention more effectively. We believe that the clinical benefit in this pilot RCT is attributed primarily to compensatory changes in the motor system that are induced by MI, provided that the mental rehearsal of paralyzed hand movements is enhanced by BCI.

Figure Legends

Figure 1: In the prototype setting, the patient is seated with his hands resting on a desk, with an adjustable forearm orthosis that provides support. The hands are covered by a white blanket, on which the cue and feedback for the patients are projected via a custom software program, providing a visual representation of the patient's hands ("virtual hand"). During the session, the therapist is allowed to monitor the patient's mental "activity" continuously through instant BCI feedback, displayed on a dedicated screen: the degree of desynchronization of selected electrodes/frequencies (see Supplementary File S4) determines the vertical velocity of the cursor on the therapist's screen—once the cursor reaches a target in the upper part of the screen, the "virtual hand" performs the imagined movement (feedback to patients in successful trials). The therapist is also allowed to monitor the patient's extent of muscle relaxation based on the EMG signal, recorded from the hand and forearm muscles and displayed on a screen.

Figure 2: Bar diagram of the effectiveness of clinical outcome measures (FMA, MRC, NIHSS) in the two groups (BCI group, blue; CTRL group red). * denotes significant differences between groups (independent-samples t-test, $p<.05$).

Figure 3: Statistical scalp maps associated with tonic grasping movement imagery of the affected (left panel) and unaffected hands (right panel). T-tests were performed to analyze the desynchronization between the BCI and CTRL groups in the PRE (upper row) and POST (lower row) sessions in the alpha and beta1 frequency ranges. The scalp model is seen from above, with the nose pointing toward the upper part of the page, and the affected hemisphere (ah) is shown on the left side of the scalp. The color of each pixel represents the corresponding p-value: gray indicates non significant differences; white-yellow indicates stronger desynchronization ($p<.05$, Bonferroni-corrected) in the BCI group; and black denotes stronger desynchronization ($p<.05$, Bonferroni-corrected) in the CTRL group.

Figure 4: Statistical connectivity patterns estimated for the BCI (upper row) and CTRL groups (lower row) in the resting state. The PRE and POST conditions were contrasted to highlight significantly stronger connections in the POST session (one-tailed paired-sample t-test, $p< .05$, FDR-corrected). The scalp model is

seen from above, with the nose pointing toward the upper part of the page, and affected hemisphere (ah) is shown on the left side of the scalp. Connections between electrodes are represented by arrows (orange for interhemispheric connections [IHC]; burgundy for others). The number of significantly reinforced IHC is reported in brackets when above the null case.

Tables

	BCI	CTRL
Age (years)	64.1 ± 8.4	59.6 ± 12.7
Time from the event (months)	2.7 ± 1.7	2.5 ± 1.2
Side of lesion (r/l)	7r / 7l	5r / 9l
Site of lesion (c/s)	5c / 9s	5c / 9s
Etiology (i/h)	12i / 2h	12i / 2h
NIHSS	9 ± 2.6	8 ± 2.3
FMA	23.4 ± 17.3	24.2 ± 18.2
MRC	55.9 ± 11	57.2 ± 12.2
MAS	2.4 ± 2.7	2.8 ± 3.1

Table 1: Demographic and clinical characteristics of the patients (means ± standard deviations). NIHSS: National Institute of Health stroke scale, ranging from 0 (least affected) to 42 (most affected); FMA: Fugl-Meyer Assessment scale, upper limb section, ranging from 0 (most affected) to 66 (least affected); MRC: Medical Research Council Scale for Muscle Strength, upper limbs ranging from 0 (most affected) to 80 (least affected); MAS: Modified Ashworth Scale for spasticity in the upper limb joints, ranging from 0 (least affected) to 24 (most affected).

Channel	Theta	Alpha	Lower Beta	Upper Beta	Gamma
C1 / C2	0.519	0.242	0.019 *	0.16	0.386
C3 / C4	0.625	0.15	0.014 *	0.126	0.339
C5 / C6	0.731	0.213	0.071	0.333	0.721
CP1 / CP2	0.063	0.052	0.028 *	0.214	0.571
CP3 / CP4	0.857	0.154	0.01 *	0.104	0.677
CP5 / CP6	0.785	0.396	0.084	0.483	0.681

Table 2: P-values of the statistical comparison (paired-sample t-test) between EARLY vs LATE training sessions for channels over the affected hemisphere motor cortex of patients in the BCI group. FDR correction was applied on the significance level. Significant results ($p<.05$) are marked with *.

Potential Conflict of Interest

Nothing to report.

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Authors Contribution

- F.Pichiorri, MD: clinical trial responsible; design of experimental training; EEG experimental data analysis management; interpretation of data; manuscript writing;
- Giovanni Morone, MD: patient recruitment and randomization procedures; experimental training execution; patients' evaluation and clinical data collection and analysis;
- M. Petti, biomedical engineer: EEG data collection and analysis;
- J. Toppi, PhD: implementation and validation of EEG data analysis methodology (effective connectivity and graph theoretical approach);
- I. Pisotta, Psychologist: control intervention design and execution; related data analysis;
- M. Molinari, MD, PhD (neurorehabilitation ward director): prototype design; patients recruitment supervision; clinical trial design and supervision; data analysis validation;
- S. Paolucci, MD (stroke neurorehabilitation ward director): patients recruitment supervision; clinical trial design and supervision; ethical procedure supervision;
- M. Inghilleri, MD, PhD: EEG and TMS experimental procedure implementation and validation; interpretation of neurophysiological data;
- L. Astolfi, biomedical Engineer, PhD: EEG-derived brain network data analysis supervision and validation; data interpretation;
- F. Cincotti, electronic engineer, PhD: BCI Prototype design and development; validation of neurophysiology experimental procedure; data analysis supervision and interpretation of results;
- D. Mattia, MD, PhD: responsible of study; study design and management; overall data interpretation; manuscript writing management.

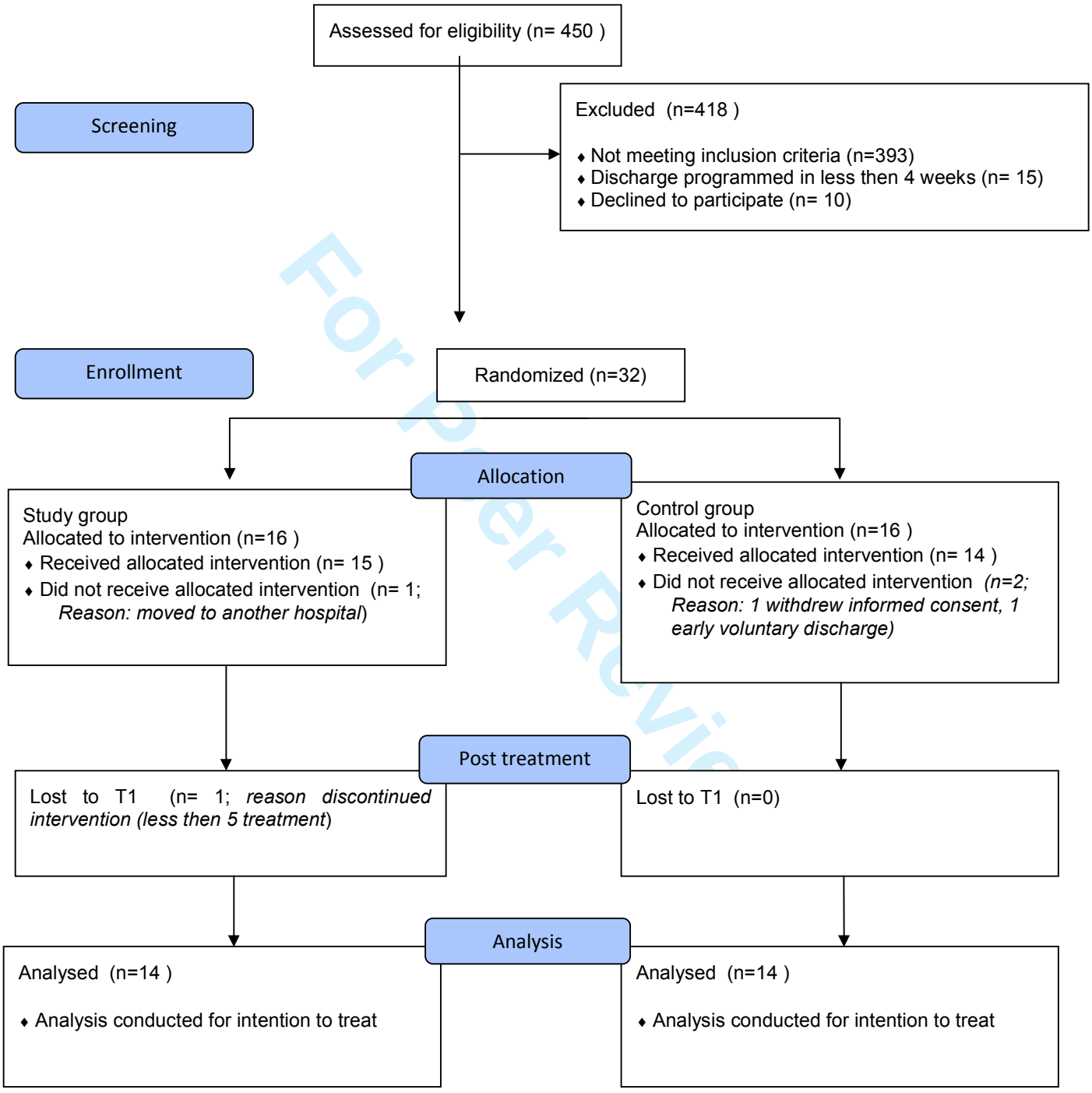
Brain-Computer Interface boosts motor imagery practice during stroke recovery.

Supplementary Material

Index

Study Flowchart (S1)

Consort flow diagram for the clinical study



Baseline Characteristics (S2)

Table S2 shows demographical and clinical characteristics of the patients included in the study. Age is expressed in years. Time from the stroke event is expressed in months. NIHSS: National Institute of Health stroke scale ranging from 0 (least affected) to 42 (most affected); FMA: Fugl-Meyer Assessment scale, upper limb section ranging from 0 (most affected) to 66 (least affected); MRC: Medical Research Council Scale for Muscle strenght, upper limbs ranging from 0 (most affected) to 80 (least affected); MAS: Modified Ashworth Scale for spasticity in the upper limb joints ranging from 0 (least affected) to 24 (most affected) .

Patient	Age (y)	Time/Event (m)	Side	Site	Etiology	NIHSS	FMA	MRC	MAS
BCI-1	59	8	Right	cortico-subcortical	ischemic	9	11	45	6
BCI-2	66	3	Right	Cortical	ischemic	9	17	50	0
BCI-3	64	2	Right	Subcortical	ischemic	9	10	46	6
BCI-4	54	2	Right	Subcortical	ischemic	5	49	76	0
BCI-5	70	2	Left	Subcortical	ischemic	11	8	46	6
BCI-6	57	2	Right	Subcortical	ischemic	4	44	72	0
BCI-7	75	2	Left	Subcortical	ischemic	12	31	56	5
BCI-8	52	3	Right	Subcortical	ischemic	7	10	49	3
BCI-9	58	2	Left	cortico-subcortical	ischemic	12	7	44	5
BCI-10	62	2	Left	Subcortical	haemorragic	10	40	67	0
BCI-11	65	1	Left	Cortical	ischemic	6	57	70	0
BCI-12	82	2	Right	Cortical	ischemic	11	20	59	0
BCI-13	62	2	Left	Subcortical	haemorragic	11	15	54	0
BCI-14	72	5	Left	Subcortical	ischemic	10	9	49	3
AVG	***64,1***	***2,7***				***9,0***	***23,4***	***55,9***	***2,4***
CTRL-1	62	3	Left	Subcortical	ischemic	3	54	72	7
CTRL-2	75	3	Left	cortico-subcortical	ischemic	9	44	72	0
CTRL-3	64	2	Left	cortico-subcortical	ischemic	6	37	70	2
CTRL-4	58	3	Left	Subcortical	ischemic	10	21	60	6
CTRL-5	34	2	Left	cortico-subcortical	ischemic	8	9	43	0
CTRL-6	44	1	Left	Subcortical	ischemic	9	5	41	0
CTRL-7	54	3	Left	cortico-subcortical	ischemic	11	4	40	3
CTRL-8	64	5	Right	cortico-subcortical	ischemic	5	13	48	8
CTRL-9	76	4	Right	Subcortical	ischemic	11	11	51	0
CTRL-10	71	1	Right	Subcortical	ischemic	6	59	76	0
CTRL-11	62	2	Left	Subcortical	haemorragic	7	18	55	2
CTRL-12	64	3	Right	Subcortical	haemorragic	13	14	60	8
CTRL-13	47	1	Left	Subcortical	ischemic	8	15	50	4
CTRL-14	58	1	Right	Subcortical	ischemic	4	35	63	0
AVG	***59,5***	***2,4***				***7,9***	***24,2***	***57,2***	***2,9***

Functional Outcome (S3)

Table S3 shows clinical outcome measures in the two groups at pre- and post- assessments. Wilcoxon Matched Pairs Test was performed to analyze pre- to post- evaluations changes in the two groups. The column MCID reports the difference (post - pre assessment) in the upper limb section of FMA; the Minimal Clinically Important Difference was reached if the difference was above 7 points.

Patient	FMA			MRC		MAS		NIHSS	
	PRE	*POST*	*MCID*	*PRE*	*POST*	*PRE*	*POST*	*PRE*	*POST*
BCI-1	11	14	*3*	45	48	6	6	9	8
BCI-2	17	37	*20*	50	71	0	3	9	6
BCI-3	10	18	*8*	46	54	6	5	9	7
BCI-4	49	65	*16*	76	78	0	0	5	2
BCI-5	8	11	*3*	46	46	6	5	11	8
BCI-6	44	62	*18*	72	75	0	0	4	2
BCI-7	31	58	*27*	56	72	5	3	12	5
BCI-8	10	17	*7*	49	55	3	3	7	5
BCI-9	7	11	*4*	44	47	5	7	12	8
BCI-10	40	54	*14*	67	73	0	0	10	5
BCI-11	57	66	*9*	70	77	0	0	6	1
BCI-12	20	47	*27*	59	70	0	2	11	5
BCI-13	15	41	*26*	54	67	0	0	11	5
BCI-14	9	17	*8*	49	53	3	5	10	7
AVG	***23,4***	***37,0***		***55,9***	***63,3***	***2,4***	***2,8***	***9,0***	***5,3***
SD	***17,3***	***21,7***		***11,0***	***12,1***	***2,7***	***2,5***	***2,6***	***2,3***
P value	***0,000982***			***0,001474***		***ns***		***0,000982***	
CTRL-1	54	56	*2*	72	72	7	6	3	3
CTRL-2	13	19	*6*	48	51	8	9	10	8
CTRL-3	37	56	*19*	70	74	2	2	6	4
CTRL-4	44	47	*3*	72	72	0	0	8	9
CTRL-5	21	32	*11*	60	62	6	8	9	8
CTRL-6	9	14	*5*	43	46	0	3	11	7
CTRL-7	5	6	*1*	41	42	0	0	11	10
CTRL-8	4	5	*1*	40	41	3	3	5	5
CTRL-9	11	13	*2*	51	52	0	0	11	10
CTRL-10	59	62	*3*	76	69	0	0	6	5
CTRL-11	18	42	*24*	55	68	2	4	7	4
CTRL-12	14	17	*3*	60	62	8	9	13	10
CTRL-13	15	18	*3*	50	52	4	3	8	7
CTRL-14	35	43	*8*	63	68	0	0	4	4
AVG	***24,2***	***30,7***		***57,2***	***59,4***	***2,9***	***3,4***	***8,0***	***6,7***
SD	***18,2***	***19,9***		***12,2***	***11,7***	***3,2***	***3,4***	***3,0***	***2,5***
P value	***0,000982***			***0,028057***		***ns***		***0,005356***	

MI-based BCI Training (S4)

Table S4 shows lesions side and Brain-Computer Interface (BCI) control features in the BCI group patients.

Patient	Side	Channels	Frequency (Hz)
BCI-1	Right	Cpz, Cp2	14-15
BCI-2	Right	Cz, Cp4, Cp6	14-15
BCI-3	Right	C2, Cp2 e Cp4	20-21
BCI-4	Right	Cp2, Cp4	20-21
BCI-5	Left	C3, Cp3	12-13
BCI-6	Right	C2, Cp2	22-23
BCI-7	Left	C3, C5	16-17
BCI-8	Right	C2, Cp2	18-19
BCI-9	Left	Cpz, Cp1	22-23
BCI-10	Left	C3, Cp3	16-17
BCI-11	Left	C3, C5	14-5 / 16-17
BCI-12	Right	C2, C4	22-23
BCI-13	Left	C3, C5, Cp1	12-13 /22-23
BCI-14	Left	Cp3, Cp1	16-17 / 22-23

TMS results (S5)

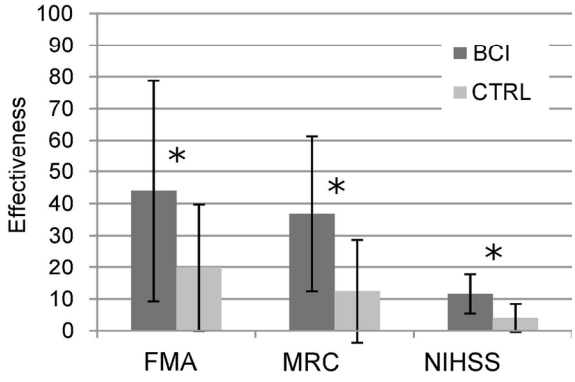
Table S5 shows the results of the TMS analysis. Motor Evoked Potential (MEP) amplitude values from First Dorsal Interosseus (FDI) muscles obtained at rest were compared to those obtained during MI of hand movements for each patient. Given the high variability among individuals for MEP amplitude, data were normalized and amplitude changes occurring during MI tasks were expressed as percentage of amplitudes at rest. MEP amplitude increases (Mean ±SD) are shown in the unaffected FDI muscle during unaffected hand MI (first column)and affected FDI muscle during affected hand MI (second column).

Paired sample T-tests were performed considering all patients together for unaffected and affected hand MI respectively (UH, AH). The same analysis was run for each group separately for unaffected hand MI. The reduced number of patients with recordable MEPs from the affected hand prevented us to run the separate group analysis for AH MI. Between-group analysis was performed for unaffected hand only (results shown in the manuscript) for the same reason.

Task **Muscle**	*UH MI* *Unaffected FDI*	*AH MI* *Affected FDI*
% amplitude increase (all patients)	172.8 ± 122.9	150.7 ± 69.3
paired sample t-test p value (sample size)	*0.0002 (n=23)*	*0.006 (n=9)*
% amplitude increase (BCI)	185.0 ± 144.8	184.0 ± 94.8
paired sample t-test p value (sample size)	*0.008 (n=12)*	*not performed (n=3)*
% amplitude increase (CTRL)	159.5 ± 95.1	134.1 ± 49.3
paired sample t-test p value (sample size)	*0.007 (n=11)*	*not performed (n=6)*

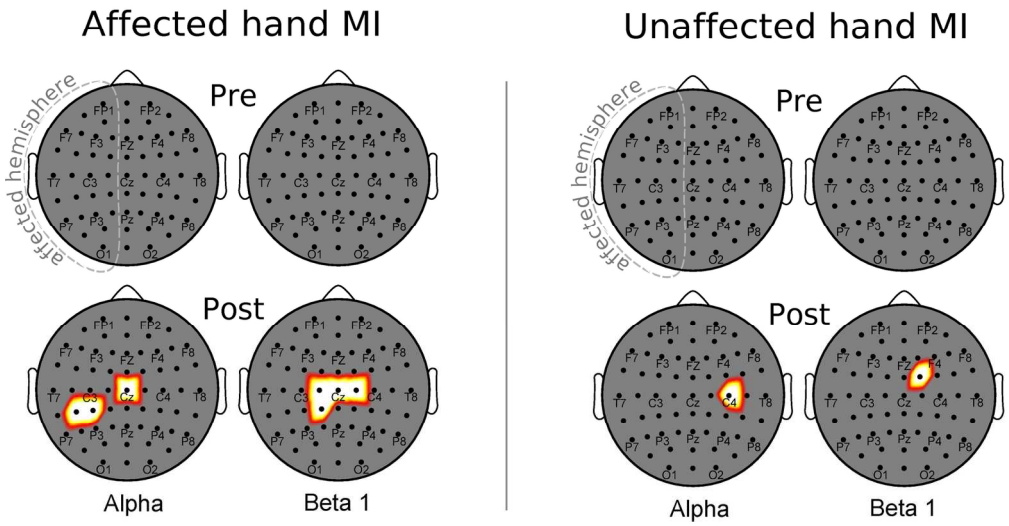
In the prototype setting, the patient is seated with his hands resting on a desk, with an adjustable forearm orthosis that provides support. The hands are covered by a white blanket, on which the cue and feedback for the patients are projected via a custom software program, providing a visual representation of the patient's hands ("virtual hand"). During the session, the therapist is allowed to monitor the patient's mental "activity" continuously through instant BCI feedback, displayed on a dedicated screen: the degree of desynchronization of selected electrodes/frequencies (see Supplementary File S4) determines the vertical velocity of the cursor on the therapist's screen—once the cursor reaches a target in the upper part of the screen, the "virtual hand" performs the imagined movement (feedback to patients in successful trials). The therapist is also allowed to monitor the patient's extent of muscle relaxation based on the EMG signal, recorded from the hand and forearm muscles and displayed on a screen.
160x94mm (300 x 300 DPI)

Figure 2



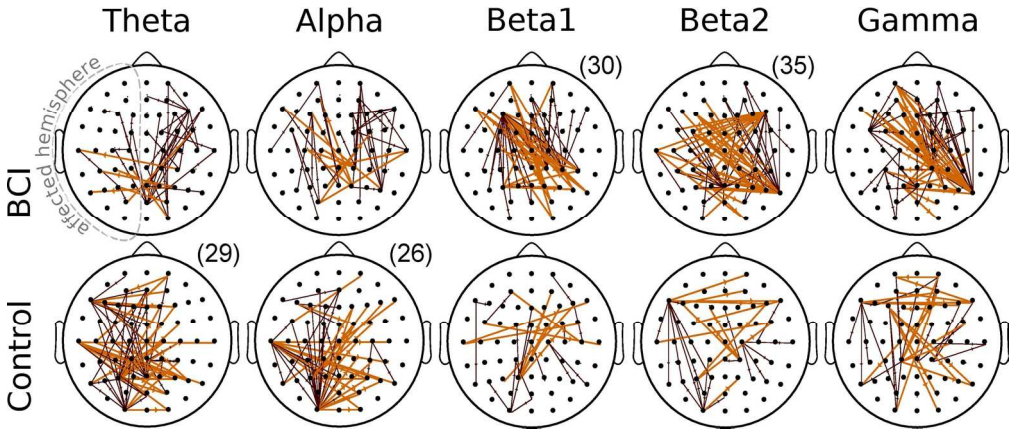
Bar diagram of the effectiveness of clinical outcome measures (FMA, MRC, NIHSS) in the two groups (BCI group, blue; CTRL group red). * denotes significant differences between groups (independent-samples t-test, $p<.05$).
147x185mm (300 x 300 DPI)

Figure 3



Statistical scalp maps associated with tonic grasping movement imagery of the affected (left panel) and unaffected hands (right panel). T-tests were performed to analyze the desynchronization between the BCI and CTRL groups in the PRE (upper row) and POST (lower row) sessions in the alpha and beta1 frequency ranges. The scalp model is seen from above, with the nose pointing toward the upper part of the page, and the affected hemisphere (ah) is shown on the left side of the scalp. The color of each pixel represents the corresponding p-value: gray indicates non significant differences; white-yellow indicates stronger desynchronization ($p<.05$, Bonferroni-corrected) in the BCI group; and black denotes stronger desynchronization ($p<.05$, Bonferroni-corrected) in the CTRL group.
157x155mm (300 x 300 DPI)

Figure 4



Statistical connectivity patterns estimated for the BCI (upper row) and CTRL groups (lower row) in the resting state. The PRE and POST conditions were contrasted to highlight significantly stronger connections in the POST session (one-tailed paired-sample t-test, $p < .05$, FDR-corrected). The scalp model is seen from above, with the nose pointing toward the upper part of the page, and affected hemisphere (ah) is shown on the left side of the scalp. Connections between electrodes are represented by arrows (orange for interhemispheric connections [IHC]; burgundy for others). The number of significantly reinforced IHC is reported in brackets when above the null case.
167x176mm (300 x 300 DPI)