tDCS combined with kinesthetic motor imagery-based brain computer interface training promotes upper limb function in subacute stroke: A randomized controlled study

Ming Zhang
Department of Mechatronic Engineering, China University of Mining and Technology

Yu Wu
Department of Sports Science, Zhejiang University

Fan Jia
The Affiliated Xuzhou Rehabilitation Hospital of Xuzhou Medical University, Xuzhou Medical University

Ling Gao
The Affiliated Xuzhou Rehabilitation Hospital of Xuzhou Medical University, Xuzhou Medical University

Fengming Chu
The Affiliated Xuzhou Rehabilitation Hospital of Xuzhou Medical University, Xuzhou Medical University

Wei Tang
tangwei@cumt.edu.cn

Department of Mechatronic Engineering, China University of Mining and Technology

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Abstract

Background

Most stroke survivors have upper extremity dysfunction. According to neuroplasticity theory, transcranial direct current stimulation (tDCS) and kinesthetic motor imagery-based brain computer interface (KI-BCI) have the potential to improve the upper extremity function of participants with subacute stroke. However, the efficacy of tDCS combined with KI-BCI in participants with subacute stroke is unknown.

Objective

To investigate whether the combined effect of tDCS and KI-BCI on upper limb function in participants with subacute stroke is more effective than the effects of tDCS or KI-BCI alone.

Methods

We randomized 48 participants into a tDCS group (n = 16), a KI-BCI group (n = 16), and a tDCS-BCI group (n = 16). Participants in the tDCS group received 30 min of tDCS with the anode over M1. The KI-BCI group performed 30 min of KI-BCI training. Participants in the tDCS-BCI group received 15 min of tDCS and 15 min of KI-BCI. All participants received conventional intervention. The treatment cycle consisted of a 1 session each day, 5 days per week for 4 weeks. The Fugl–Meyer Assessment of Upper Extremity (FMA-UE) subscale, Motor Status Scale (MSS), Action Research Arm Test (ARAT), and Modified Barthel Index (MBI) were used to assess upper limb function, and activities of daily living (ADL) before and after the 4-week treatment period. In addition, electroencephalography (EEG) was used to explore potential clinical brain mechanisms.

Results

After four weeks of intervention, the tDCS-BCI group was superior to the tDCS group in terms of the MSS. The FMA-UE, MSS, and MBI scores of the KI-BCI group were superior to those of the tDCS group. There was no difference in the number of quantitative EEGs among the three groups, while the number of quantitative EEGs was greater than before.

Conclusion

TDCS combined with KI-Cl training can improve upper extremity function. However, KI-BCI training alone can improve upper limb function and ADL simultaneously. TDCS could alter the electrical excitatory levels of the cerebral hemispheres.

Trial registry number: ChiCTR2000034730

1 Introduction
Stroke is the second-leading cause of death and the third-leading cause of disability worldwide\textsuperscript{1}. The 80% of survivors have upper extremity motor impairment after acute stroke\textsuperscript{2}, which significantly limits participation in the physical and social environment\textsuperscript{3}. Innate physiological and anatomical plasticity are important processes that underlie substantial gains in motor function after stroke\textsuperscript{4,5}, and the combination of task-specific training and general aerobic exercise is still the gold-standard treatment for poststroke rehabilitation\textsuperscript{6}.

However, it is difficult for some survivors to move the affected limb. As a result, motor imagery (MI), the mental process of imagination of movements, was proposed based on neuroplasticity theory. Specifically, the typical paradigms of MI include visual imagery (VI) and kinesthetic imagery (KI). The former requires subjects to observe the movement of their own body or others in the view of a third person, which induces specific imagery using visualization. The latter can be performed by imagining the somatosensory sensation during body movements from the perspective of the first person according to an external cue\textsuperscript{7,8}. From the perspective of the intensity of activation in the motor cortex, the VI and KI provide a feasible path to realize information perception and cortical activation\textsuperscript{8}.

According to the principle of Hebbian plasticity, motor recovery after stroke requires not only active motor control in the cortex, but also functional projections of motor demands towards muscle effectors, thereby activating complete cortico-muscular pathways\textsuperscript{9}. A brain-computer interface (BCI) is a communication approach between a user and a computer that does not rely on the normal neural pathways of the brain and muscles\textsuperscript{10}. BCI systems applied for motor neuromodulation purposes are used to induce activity-dependent plasticity by allowing the user to pay close attention to a task requiring the activation or deactivation of specific brain signals\textsuperscript{11,12}. Electroencephalography (EEG) equipment is widely used to record brain signals in BCI systems because it is noninvasive, has high temporal resolution, has the potential for user mobility and has a relatively low cost\textsuperscript{13}. EEG-based BCIs can be classified into two types: evoked and spontaneous\textsuperscript{14}. In evoked systems, external stimulation, such as visual, auditory or sensory stimulation, is needed. The stimuli evoke responses in the brain that are then identified by the BCI system to determine the will of the user\textsuperscript{15}. In spontaneous BCIs, no external stimulation is needed, and control actions are taken based on activity produced as a result of mental activity\textsuperscript{14}. A common example of a spontaneous BCI is an MI-BCI\textsuperscript{16}. VI and KI are widely employed in BCI tasks and have been proven to have beneficial effects. However, KI was proven to be more effective for BCI systems. Marchesotti's research discussed the relationship between BCI aptitude and the adaptability of paradigms\textsuperscript{17}, and suggested that users with high BCI aptitude can obtain a greater decoding accuracy for MI-EEG using the KI paradigm. A similar result was reported in Toriyama's research\textsuperscript{18}, where contrasting experiments between the KI and VI were executed, and the results showed that the event-related desynchronization feature of the KI was more strongly associated with that during motor execution.
Transcranial direct current stimulation (tDCS) is a noninvasive neuroregulatory technique that uses constant low-intensity direct current (1–2 mA) to modulate neuronal activity in the cerebral cortex\textsuperscript{19}. tDCS affects the resting membrane potentials of neurons through the modulation of sodium- and calcium-dependent channels and N-methyl-D-aspartate (NMDA)-receptor activity\textsuperscript{20}. After stroke, the excitability of the injured hemisphere is suppressed, the excitability of the healthy hemisphere is increased, and the affected side of the cortex is over-suppressed, which affects functional recovery. Studies have shown that anode tDCS can depolarize the neuronal potential of the brain to enhance cortical excitability, while the cathode can hyperpolarize the neuronal potential to reduce cortical excitability\textsuperscript{21}. Therefore, purposeful modulation of cortical excitability using tDCS may induce plastic changes within the network of sensorimotor areas of the cortex and improve the upper limb function of participants with subacute stroke. Additionally, tDCS is a relatively inexpensive, simple, and portable technique, with great potential for use in the rehabilitation of stroke.

Accordingly, we hypothesized that the KI-BCI and tDCS could significantly improve upper limb function in participants with subacute stroke. We hypothesized that compared with KI-BCI and tDCS alone, KI-BCI combined with tDCS could have greater benefits. To investigate this question, a three-armed randomized controlled trial that compared KI-BCI, tDCS and combined KI-BCI and tDCS was performed. The objective of this article is two-fold: first, to study the effectiveness of KI-BCI combined with tDCS in participants with subacute stroke; second, to explore the potential clinical brain mechanism of this combination therapy by using EEG.

2 Methods

2.1 Trial design

This study was a randomized controlled study. Participants who are participants with subacute stroke received either 20 sessions of tDCS, KI-BCI or tDCS combined with KI-BCI. This study was conducted under the principles of the Declaration of Helsinki. The Ethics Committee of Xuzhou Rehabilitation Hospital approved the entire protocol and instrumentation (No.XK-LW-20200428-003) and was registered in the Chinese Clinical Trials Registry Platform (identifier:ChiCTR2000034730). All participants signed an informed consent form before the start of the trial.

2.2 Participants

Participants were recruited from Xuzhou Rehabilitation Hospital between July 2020 to December 2023. Those participants were then evaluated for eligibility after being introduced to the study. Inclusion criteria for participants were as follows: (1) first-ever stroke diagnosed by computed tomography or magnetic resonance imaging (chronicity $\geq$ 14 and < 180 days); (2) Mini-Mental State Examination (MMSE)$^{22}$ score $\geq$ 20; and (3) aged between 40 and 80 years. Participants with subacute stroke were excluded if they: (1) history of seizures; (2) other neurological, neuromuscular, orthopedic diseases; (3) a scalp deformity due to surgery; or (4) with medical instability such as heart/respiratory failure, deep
venous thrombosis, acute myocardial infarction, non-compensated diabetes, active liver disease, or/and kidney dysfunction medication of antispastic therapy (any antispastic medicine or botulinum toxin injection). If the following conditions occurred during the treatment process, the participant’s experimental process was terminated: (1) progressive aggravation of the condition; (2) poor compliance; or (3) occurrence of epilepsy, manic episodes or other conditions after treatment.

2.3 Sample size calculation

The sample size was calculated using PASS version 15.0 (NCSS, LLC, Kaysville, Utah, The United States). We determined the sample size by the Fugl-Meyer Assessment for Upper Extremity (FMA-UE)\textsuperscript{23}. The presumed effect size was based on a pilot study of 5 individuals in each group. With type I error of 0.05, and power of 0.90, the estimated sample size was estimated to be 14 participants. Expecting a dropout rate of 20%, we included 16 participants per group.

2.4 Randomization and blinding

We randomly assigned 48 participants with subacute stroke into three groups: tDCS group (n = 16), KI-BCI group (n = 16), and tDCS-BCI group (n = 16). Before recruitment, an unrelated assistant developed a computer-generated random sequence and stored it in sealed, sequentially envelopes labeled with the name of one of the three groups. Due to the way of intervention, physical therapists were not blinded. Evaluators were independent of the intervention and were blinded to the assigned group.

2.5 Interventions

The participants were invited for an initial assessment to verify their eligibility based on the inclusion criteria. Upon providing informed consent, eligible participants were assigned to one of three groups. The treatment protocol is displayed in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>tDCS group</td>
<td>Conventional intervention (30 min/session) + tDCS therapy (30 min/session)</td>
</tr>
<tr>
<td>KI-BCI group</td>
<td>Conventional intervention (30 min/session) + KI-BCI (30 min/session) treatment</td>
</tr>
<tr>
<td>tDCS-BCI group</td>
<td>Conventional intervention (30 min/session) + tDCS therapy (15 min/session) + KI-BCI (15 min/session)</td>
</tr>
</tbody>
</table>

Abbreviations: tDCS transcranial direct current stimulation; KI-BCI kinesthetic imagery-based brain computer interface.

2.5.1 Conventional intervention

All participants received conventional interventions including mobilization, neural facilitation techniques, sit to stand exercise, balancing training and functional electrical stimulation to eliminate potential confounding effects caused by non-use and muscle atrophy. Additionally, participants in Brunnstrom
stage I-III received virtual reality training with an intelligent feedback training system (A2, Guangzhou Yikang). The system could generate 3-dimensional game environments suitable for the participants which were presented on a large display (101.6 cm ×57.7 cm). The weight and range of motion parameters of the machine arm of the system were adjusted according to the participants’ affected arms. After the training begins, the system issues voice prompted to guide participants to perform specific movements displayed on the screen, thus training the participant's ability of shoulder flexion/extension and adduction/abduction on the affected side, the grasping ability of the hand as well as the coordination and control of the joints of the upper limb. Participants with upper limb dysfunction in Brunnstrom stage IV and above were trained with the BioMaster virtual scenario training system (Guangzhou Zhanghe Electrical). Before training, a wireless motion sensor was placed on the participants’ affected upper arms or forearms to evaluate their upper limb movement ability. The system generated personalized virtual reality games based on participants’ conditions, such as vegetable and fruit matching, flying airplanes, and collecting treasures, aimed at improving shoulder mobility. Games such as wiping tables, sawing wood, and cutting vegetables were provided to improve the mobility of the elbow, wrist, and forearm joints. Figure 1 illustrates the state of the participants during virtual reality training. The conventional therapy was performed one session per day, for 30 min per session.

2.5.2 tDCS

ActivaDose®II portable transcranial direct current stimulator (Wogao Medical Equipment Co., Ltd., Nanjing, China) was used to intervene in the central nervous system of participants with subacute stroke. The motor evoked potential module of the Magstim Rapid² repetitive transcranial magnetic stimulator (Magstim, UK) was used to locate M1, and the best stimulation point of M1 was found by adjusting the intensity and direction of the stimulation coil.

In this study, the unilateral stimulation mode of tDCS was used. The anode was positioned over the M1 on the affected hemisphere, while the cathode was placed over the contralateral orbit. The electrode piece was a 5 cm × 5 cm isotonic saline sponge electrode, the total current intensity was 2 mA, and the treatment was performed for 1 session/day, 5 sessions per week for 4 weeks.

2.5.3 MI-BCI

To avoid interference with the EEG signals, the training took place in a dimly lit and quiet environment. The experienced therapist guided participants to relax their body. Visual and auditorial clues were provided by L-B300 EEG Acquisition and Rehabilitation Training System (Zhejiang Mailian Medical Technology Co., Ltd.) to guide participants accomplish MI tasks. The motor imagery content underwent transformations based on the indicated arrows provided by the screen (for simple tasks) and different scenes (for complex tasks, such as the kitchen or bedroom). The BCI will collect electrical signals from the prefrontal motor regions (FP1, FP2, F3, F4, C3, C4, Fz, Cz) (see in Fig. 2). Threshold parameters, including the threshold and mechanical arm speed, were automatically set through time-window and frequency band optimization. The Welch power spectral estimation method was used to calculate the power spectrum energy ratios for each frequency band. According to band power, we calculated the DAR
(delta/alpha power ratio), DTABR (delta + theta)/(alpha + beta) power ratio), and DABR (delta/(alpha + beta) power ratio) and generated motor imagery scores. Higher scores indicated a higher level of concentration in the participant’s imagined movements. When the participant’s score exceeded the predetermined task threshold, the apparatus entered an active-assisted movement mode, where a robotic arm provided assistance to drive the participant’s affected upper limb and complete the movement task. Each training session was performed once per day, with five sessions per week for 4 weeks.

2.6 Outcome measures

Demographic and anthropometric data of the participants were collected at baseline. The primary outcome was FMA-UE. The secondary outcome was pain intensity. Measurements were carried out before and four weeks after the intervention to observe the effects of the three interventions and the trend of changes over different time periods.

2.6.1 Fugl-Meyer Assessment-Upper Extremity (FMA-UE)

The FMA-UE is the most commonly used assessment for measuring post-stroke impairment. It evaluates the motor function of the upper limbs, wrists, and hands. It includes 4 subsections: (1) shoulder-arm, (2) wrist, (3) hand, and (4) coordination and speed designed to measure impairment from proximal to distal and synergistic to isolated voluntary movement. The 33 items are scored on an ordinal scale of 0 (absent), 1 (partial impairment), and 2 (no impairment) that make up a total maximum of 66 points.

2.6.2 Motor Status Scale (MSS)

The MSS measures shoulder, elbow, wrist, and finger movements, which developed based on the FMA and expanded the measurements on fingers and has gone through reliability and validity studies in subacute survivors.

2.6.3 The Modified Barthel Index (MBI)

The MBI is used to evaluate participants’ activities of daily living (ADL) levels, and consists of 10 items: bowel control, bladder control, grooming, bathing, eating, dressing, toileting, climbing stairs, transferring, and walking. Each item is scored from 0 to 15 based on the participant’s ability to complete the task. A higher score indicates a better ability to function independently.

2.6.4 The Action Research Arm Test (ARAT)

The ARAT assesses the fine motor function of the upper limbs by evaluating four fundamental movements: grasp, grip, pinch, and gross movement. It consists of a total of 19 items, with a cumulative score of 57. The higher the score, the better the fine motor function of the upper limb.
2.6.5 Electroencephalography Acquisition, Processing, and Analysis

32-channel (NeuSen.W32, Neuracle, China) EEG signals were collected at a sampling frequency of 1000 Hz. The 32-channel Al-AgCl electrodes were distributed according to the 10–20 system standards as shown in Fig. 3. Before the EEG recordings, preparation procedures including hair cleaning, cap positioning, and impedance checking were conducted to ensure that the electrode-to-scalp impedance was below 5 kΩ. Participants were sat comfortably in a quiet, dim, electrically shielded room. An experienced EEG technician instructed participants to keep their upper extremity still and minimize blink frequency. The recording began after the EEG signals had stabilized.

If there were large artifacts in the range of 0–30 Hz, these EEG signals were discarded. Then, the 5th-order IIR filter in EEGLAB was used to apply low pass filtering with a low frequency of 30 Hz to the remaining EEG signals, where the baseline drift was removed and overflowed. Independent component analysis (ICA) was used to remove artifacts: EEGLAB’s default runica algorithm was used to calculate ICA, and we used EEGLAB’s ‘adjust’ plugin to remove 3 of the 32 independent components, thus removing the ECG and EOG artifacts. EEG 50 Hz power signals were filtered using a fixed-lag Kalman smoother. Fourier transform was used for time-frequency conversion, dividing the frequency range into alpha (8–13 Hz), beta (14–30 Hz), theta (4–7 Hz), and delta (0.5-3 Hz) waves. We selected electrical signals from the frontal motor areas (FP1, FP2, F3, F4, C3, C4, Fz, and Cz) and analyzed them. The DAR, DTABR, and DABR were calculated.

\[
\hat{p}_x(x) = \frac{1}{Q} \sum_{m=0}^{Q} S_q(w) \text{ (Welch)}
\]

\[
DAR = \frac{P_\delta}{P_\alpha}
\]

\[
DTABR = \frac{P_\delta + P_\theta}{P_\alpha + P_\beta}
\]

\[
DABR = \frac{P_\delta}{P_\alpha + P_\beta}
\]

\(\hat{p}_x(x)\) is the power spectrum density (PSD), \(P_\alpha, P_\beta, P_\theta, \) and \(P_\delta\) are the PSD of \(\alpha, \beta, \theta, \) and \(\delta\) bands, respectively.

2.7 Statistical analysis

The measurement data were expressed as mean (SD). The Shapiro-wilk test was performed to test for a normal distribution, and Levene's test was applied to check the equality of variance for all measures. Differences in demographic and clinical variables between groups at baseline were compared using one-way analysis of variance (ANOVA) or Kruskal-Wallis analysis, depending on whether the data distribution
was normal or not. A post-hoc check of the baseline data was performed using Tukey's method to avoid family-wise errors.

A mixed-model ANOVA was used to see the time effect, group effect, and time × group interaction effect between the three groups. Mauchly's test was used to justify the sphericity of the variance-covariance matrix. If the variance-covariance matrix lacked sphericity, Greenhouse-Geisser was used to correct it. Baseline values were added as covariates in the analysis to eliminate the effect of confounding factors on the results. The partial eta squared $\eta_p^2$ was obtained from Greenhouse-Geisser within the subject effect. A significance level of 0.05 was established, and all statistical analyses were completed with SPSS software, version 26 (SPSS Inc, Chicago, Illinois, the United States).

### 3 Results

#### 3.1 Participants characteristics

The flowchart for participants is presented in Fig. 4. We screened 82 participants for eligibility, and 34 were excluded. Therefore, 48 participants were enrolled, assessed at baseline, and randomly assigned into 3 groups. The participants’ baseline demographic characteristics are shown in Table 2. No significant differences were observed between the groups regarding age, gender, stroke type, affected side, and time from onset (Table 2). In addition, there were no significant differences between the two groups in terms of clinical outcomes and brain function at baseline.
Table 2
Baseline demographic and clinical characteristics of the patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>tDCS (N = 14)</th>
<th>KI-BCI (N = 15)</th>
<th>tDCS-BCI (N = 14)</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>60.79 ± 11.48</td>
<td>56.13 ± 8.63</td>
<td>54.93 ± 8.73</td>
<td>1.437</td>
<td>0.250</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.968</td>
<td>0.389</td>
</tr>
<tr>
<td>Male</td>
<td>9 (64.3)</td>
<td>8 (57.1)</td>
<td>10 (71.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>5 (35.7)</td>
<td>7 (42.9)</td>
<td>4 (28.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke type, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.712</td>
<td>0.497</td>
</tr>
<tr>
<td>Ischemic</td>
<td>10 (71.4)</td>
<td>10 (66.7)</td>
<td>12 (85.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>4 (28.6)</td>
<td>5 (33.3)</td>
<td>2 (14.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affected side, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.144</td>
<td>0.866</td>
</tr>
<tr>
<td>Left</td>
<td>5 (35.7)</td>
<td>4 (26.7)</td>
<td>4 (28.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>9 (64.3)</td>
<td>11 (73.3)</td>
<td>10 (71.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time from the onset, days</td>
<td>39.36 ± 12.67</td>
<td>44.07 ± 22.63</td>
<td>38.00 ± 26.76</td>
<td>0.319</td>
<td>0.728</td>
</tr>
<tr>
<td>MMSE</td>
<td>27.64 ± 0.63</td>
<td>27.33 ± 0.62</td>
<td>27.71 ± 0.83</td>
<td>1.237</td>
<td>0.301</td>
</tr>
<tr>
<td>FMA-UE</td>
<td>22.29 ± 1.75</td>
<td>22.00 ± 0.49</td>
<td>22.57 ± 3.26</td>
<td>0.029</td>
<td>0.972</td>
</tr>
<tr>
<td>MSS</td>
<td>36.71 ± 0.79</td>
<td>37.14 ± 0.65</td>
<td>38.21 ± 1.04</td>
<td>0.918</td>
<td>0.408</td>
</tr>
<tr>
<td>ARAT</td>
<td>15.07 ± 2.22</td>
<td>15.93 ± 2.33</td>
<td>12.93 ± 2.65</td>
<td>0.450</td>
<td>0.641</td>
</tr>
<tr>
<td>MBI</td>
<td>35.21 ± 1.84</td>
<td>33.86 ± 0.85</td>
<td>34.79 ± 1.66</td>
<td>0.297</td>
<td>0.745</td>
</tr>
<tr>
<td>DTBAR</td>
<td>5.32 ± 1.37</td>
<td>3.67 ± 1.01</td>
<td>4.85 ± 1.41</td>
<td>0.496</td>
<td>0.613</td>
</tr>
<tr>
<td>DAR</td>
<td>4.79 ± 1.44</td>
<td>3.39 ± 1.04</td>
<td>4.42 ± 1.42</td>
<td>0.410</td>
<td>0.666</td>
</tr>
<tr>
<td>DABR</td>
<td>4.05 ± 1.23</td>
<td>2.75 ± 0.82</td>
<td>3.74 ± 1.24</td>
<td>0.476</td>
<td>0.625</td>
</tr>
</tbody>
</table>

Abbreviations: tDCS transcranial direct current stimulation; KI-BCI kinesthetic imagery-based brain computer interface; MMSE Mini-Mental State Examination; FMA-UE Fugl-Meyer Assessment-Upper Extremity; MSS Motor Status Scale; ARAT Action Research Arm Test; MBI Modified Barthel Index; DTABR δ + θ/α + β power ratio; DAR δ/α power ratio; DABR δ/β power ratio.

3.2 Clinical outcomes

3.2.1 FMA-UE
There were significant time effects (F = 291.500, P < 0.001, η² = 0.471) and interaction effects (F = 11.596, P = 0.001, η² = 0.471) for FMA-UE. Between group comparisons showed that the FMA-UE of the KI-BCI group was significantly greater than the tDCS group (P = 0.003) (Table 3).

### Table 3
Comparison of clinical outcomes in the tDCS group, MI-BCI group and tDCS-BCI group.

<table>
<thead>
<tr>
<th>Variable/group</th>
<th>Pre-intervention</th>
<th>Post-intervention</th>
<th>P-value time</th>
<th>P-value group</th>
<th>P-value (Time*Group)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FMA-UE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tDCS</td>
<td>22.29 ± 1.75</td>
<td>33.93 ± 1.64a</td>
<td>391.500</td>
<td>0.516</td>
<td>11.596</td>
</tr>
<tr>
<td>KI-BCI</td>
<td>22.00 ± 0.49</td>
<td>40.50 ± 0.86ab</td>
<td>(0.001)*</td>
<td>(0.603)</td>
<td>(0.001)*</td>
</tr>
<tr>
<td>tDCS-BCI</td>
<td>22.57 ± 3.26</td>
<td>36.50 ± 4.07a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MSS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tDCS</td>
<td>36.71 ± 0.79</td>
<td>51.86 ± 1.36a</td>
<td>560.419</td>
<td>6.609</td>
<td>8.445</td>
</tr>
<tr>
<td>KI-BCI</td>
<td>37.14 ± 0.65</td>
<td>58.57 ± 0.98ab</td>
<td>(0.001)*</td>
<td>(0.005)*</td>
<td>(0.001)*</td>
</tr>
<tr>
<td>tDCS-BCI</td>
<td>38.21 ± 1.04</td>
<td>57.93 ± 1.23ab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ARAT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tDCS</td>
<td>15.07 ± 2.22</td>
<td>19.71 ± 2.09a</td>
<td>93.450</td>
<td>0.568</td>
<td>10.995</td>
</tr>
<tr>
<td>KI-BCI</td>
<td>15.93 ± 2.33</td>
<td>26.50 ± 2.94a</td>
<td>(0.001)*</td>
<td>(0.573)</td>
<td>(0.001)*</td>
</tr>
<tr>
<td>tDCS-BCI</td>
<td>12.93 ± 2.65</td>
<td>25.93 ± 3.81a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MBI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tDCS</td>
<td>35.21 ± 1.84</td>
<td>55.00 ± 2.14a</td>
<td>761.997</td>
<td>1.089</td>
<td>11.024</td>
</tr>
<tr>
<td>KI-BCI</td>
<td>33.86 ± 0.85</td>
<td>62.86 ± 0.90ab</td>
<td>(0.001)*</td>
<td>(0.334)</td>
<td>(0.001)*</td>
</tr>
<tr>
<td>tDCS-BCI</td>
<td>34.79 ± 1.66</td>
<td>59.79 ± 2.08a</td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations: tDCS transcranial direct current stimulation; KI-BCI kinesthetic imagery-based brain computer interface; FMA-UE Fugl-Meyer Assessment-Upper Extremity; MSS Motor Status Scale; ARAT Action Research Arm Test; MBI Modified Barthel Index.

Symbols: aDifference of baseline (P < 0.05); bDifference of tDCS (P < 0.05); *Significant effect (P ≤ 0.05).

### 3.2.2 MSS
There were significant time effects ($F = 560.419, P < 0.001, \eta_p^2 = 0.977$), group effects ($F = 6.609, P = 0.005, \eta_p^2 = 0.337$) and interaction effects ($F = 8.445, P = 0.001, \eta_p^2 = 0.394$). Between group comparisons showed that the MSS score ($P = 0.003$) of the KI-BCI group ($P = 0.003$) and the tDCS-BCI group ($P = 0.011$) were superior to the tDCS group (Table 3).

### 3.3.3 ARAT

There were significant time effects ($F = 93.450, P < 0.001, \eta_p^2 = 0.878$) and interaction effects ($F = 10.995, P < 0.001, \eta_p^2 = 0.458$) for the ARAT. After 4 weeks intervention, there was no statistical difference among the three groups (Table 3).

### 3.3.4 MBI

There were significant time effects ($F = 761.997, P < 0.001, \eta_p^2 = 0.983$) and interaction effects ($F = 24.398, P < 0.001, \eta_p^2 = 0.803$) for the ARAT. After 4 weeks intervention, the MBI of KI-BCI group was superior to the tDCS group ($P = 0.005$) (Table 3).

### 3.3.5 QEEG

After 4 weeks of intervention, there was no statistical difference in time, group, and interaction (time × group) effect for the DTABR, DAR, and DABR among the three groups. However, the DABR ($P = 0.024$), DAR ($P = 0.022$), and DTABR ($P = 0.023$) of the tDCS group were significantly higher than those before the intervention. There was no significant difference in DABR between the KI-BCI group and the tDCS-BCI group after the intervention (Table 4).
Table 4
Comparison of QEEG outcomes in the tDCS group, MI-BCI group and tDCS-BCI group.

<table>
<thead>
<tr>
<th>Variable/group</th>
<th>Pre-intervention</th>
<th>Post-intervention</th>
<th>Main effect (Time) F(p)</th>
<th>Main effect (Group) F(p)</th>
<th>Interaction effect (Time*Group) F(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTABR</td>
<td></td>
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<tr>
<td>tDCS</td>
<td>5.32 ± 1.37</td>
<td>2.35 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.064 (0.805)</td>
<td>0.583 (0.565)</td>
<td>2.918 (0.072)</td>
</tr>
<tr>
<td>KI-BCI</td>
<td>3.67 ± 1.01</td>
<td>6.13 ± 2.58</td>
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<tr>
<td>tDCS-BCI</td>
<td>4.85 ± 1.41</td>
<td>6.41 ± 2.18</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>DAR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tDCS</td>
<td>4.79 ± 1.44</td>
<td>1.78 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.003 (0.960)</td>
<td>0.391 (0.681)</td>
<td>1.732 (0.197)</td>
</tr>
<tr>
<td>KI-BCI</td>
<td>3.39 ± 1.04</td>
<td>5.30 ± 3.10</td>
<td></td>
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</tr>
<tr>
<td>tDCS-BCI</td>
<td>4.42 ± 1.42</td>
<td>5.70 ± 2.04</td>
<td></td>
<td></td>
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<tr>
<td>DABR</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>tDCS</td>
<td>4.05 ± 1.23</td>
<td>1.55 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.008 (0.930)</td>
<td>0.421 (0.661)</td>
<td>1.839 (0.179)</td>
</tr>
<tr>
<td>KI-BCI</td>
<td>2.75 ± 0.82</td>
<td>4.35 ± 2.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tDCS-BCI</td>
<td>3.74 ± 1.24</td>
<td>4.92 ± 1.78</td>
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</tr>
</tbody>
</table>

Abbreviations: tDCS transcranial direct current stimulation; KI-BCI kinesthetic imagery-based brain computer interface; DTABR δ + θ/α + β power ratio; DAR δ/α power ratio; DABR δ/β power ratio.

Symbols: <sup>a</sup>Difference of baseline (<i>P</i> < 0.05).

4 Discussion

This randomized controlled study investigated whether the combined effect of tDCS and KI-BCI on upper limb function in participants with subacute stroke is more effective than the effect of tDCS or KI-BCI alone. Our results showed that tDCS combined with KI-BCI training significantly improved upper extremity function (include fingers) compared with tDCS alone. There was no statistically difference between the KI-BCI combined with tDCS or use KI-BCI alone. In contrast, participants with subacute stroke who received KI-BCI training alone showed better performance in upper limb function (including and excluding fingers) and ADL than those who received tDCS alone. In terms of neurophysiological indicators, there were no significant differences among the three groups after intervention, but the participants who received tDCS treatment showed better quantitative electroencephalography (QEEG) indicators compared to before treatment.
Physical rehabilitation programs, especially when delivered as soon as possible after the onset of stroke, can be highly efficacious. Notwithstanding, the rate of improvement in functional ability regained through physical rehabilitation tended to peak after a few months post-stroke and eventually tapered. Therefore, this study focused on participants with first-ever subacute stroke to minimize the influence of disease duration on rehabilitation outcomes. Recovery after stroke implies reorganization of the cortex to compensate for the lesioned area. This is possible through neuroplasticity mechanisms, whereby the brain learns and reorganizes itself to compensate for lost functions. In this context, interventions that increase or prolong neuroplasticity have been the target of recent investigations.

Neuroplasticity refers to the brain's ability to undergo functional and structural changes in response to external or internal stimuli from the environment or organs in the body. Synaptic plasticity is recognized as a fundamental aspect of neuroplasticity. Long-term potentiation (LTP) is a primary manifestation of synaptic plasticity. LTP changes promote structural changes and are likely to lead to alterations in the interconnectivity between neurons by activating formerly silent synapses or creating new ones. This structural plasticity, represented by modifications in connectivity patterns, synaptic numbers, as well as changes in branching patterns of axons and dendrites, is similar to LTP, which is believed to result from modifications at the dendritic level involving glutamatergic receptors such as the NMDA receptor, as well as an increased release of BDNF. Furthermore, the normal brain achieves and maintains functional matching and balance between the two hemispheres through reciprocal interhemispheric inhibition. After stroke, the affected hemisphere shows reduced excitability, while the unaffected hemisphere exhibits excessive inhibition towards the affected hemisphere. Anodal stimulation in tDCS typically increases neuronal excitability at the site of stimulation. In this study, anodal stimulation was specifically targeted at the M1 region of the affected hemisphere, leading to a significant decrease in QEEG values compared to the pre-stimulation measurements in the tDCS group. Rizzo et al. employed anodal tDCS stimulation on the affected primary motor cortex, effectively rectifying pathological interhemispheric inhibition and leading to improvements in upper limb motor function in participants. However, the cortical plasticity outcomes of non-invasive brain stimulation vary extensively both between and within individuals. Despite the tDCS group showing less improvement compared to the other two groups, there was still a significant enhancement in upper limb function compared to the pre-treatment condition.

Links between induced cortical excitability changes and alterations of motor behavior or learning are hard to establish and there is a need for clear mechanistic bridges between the pre-conditioning of different circuits through brain stimulation and motor function and learning. BCI can establish an alternative non-muscular channel between the participant’s brain activity and a computer, providing neurofeedback in a closed-loop. Non-invasive approaches (e.g., EEG, MEG, fMRI) have been commonly used in BCIs for stroke rehabilitation. These approaches involve both recording brain activity and controlling an external actuator, such as a robotic arm, for motor rehabilitation. In essence, BCIs either
help participants to learn to volitionally produce specific movement-related brain patterns, or they target brain structures and pathways thought to play an essential role in motor learning and motor control\textsuperscript{47}.

BCIs for the induction of plasticity may be designed in two ways. The first encompasses an instruction to the user on how to produce specific types of brain signals. The second involves pairing specific, naturally occurring brain states with external stimuli\textsuperscript{45}. Our study selected the latter paradigm in which subjects need to produce MI in order to activate M1 circuits in a task-related manner in order to enhance or promote sensorimotor rhythms coordinating the firing activity of large populations of neurons within a certain area\textsuperscript{48}. According to João D et al.\textsuperscript{49}, the activation of brain areas involved in action preparation during MI aligns with the involvement of the premotor cortex in neuroplastic changes associated with the recovery of function after a stroke\textsuperscript{50}. Specifically, the KI we used in this study could induce a cognitive substitution wherein subjects perceive the virtual body's movement as their own. Notably, more evidence suggested that KI shared a common neural substrate for brain activity ae real movement\textsuperscript{51}. Miyawaki et al.'s findings\textsuperscript{52} suggested that the impact of KI on upper limb motor function was mediated indirectly through its influence on spasticity. In this study, the attention scores of participants during motor imagery tasks were displayed in real-time on a screen, providing them with visual feedback of their brain activity. This neurofeedback intervention aimed to enhance the participants' attention, potentially leading to improvements in their motor abilities\textsuperscript{53}. Additionally, motor imagery can be considered as a cognitive task, while training involving active movements or active-passive movements (driven by a robotic arm) can be viewed as motor tasks. Therefore, the KI-BCI treatment protocol can also be seen as a dual-task paradigm of cognitive-motor integration. Studies have confirmed that cognitive-motor dual-task training is effective in promoting the recovery of both cognitive and motor functions\textsuperscript{54,55}.

Based on the potential mechanism of tDCS and KI-BCI, we hypothesized that combining tDCS-induced synaptic plasticity with the activation of the motor cortex through KI-BCI could enhance the impact of each individual treatment on neuralplasticity. However, contrary to our hypothesis, participants who received both tDCS and KI-BCI treatments did not show significantly better upper limb function, which aligns with the findings of previous studies\textsuperscript{56}. We speculate that this may be attributed to the need for a longer duration of treatment to achieve functional plasticity following tDCS-induced synaptic plasticity, which was not achieved in this study.

There are a few limitations in this study. First, this study had a relatively small sample size. Second, the number of EEG channels collected in this study was limited, which may restrict the precise localization of brain activity, signal interpretation, and detailed analysis, potentially reducing the statistical power of the study. Third, this study did not extract time-domain features from the EEG signals nor analyze the specific frequency band changes, which may have resulted in some changes going unnoticed.

## 5 Conclusion
In conclusion, tDCS combined with KI-BCI training can improve the upper extremity function (including fingers). While KI-BCI training alone can improve upper limb function and ADL simultaneously. Although the effect of tDCS in improving upper limb function is not significant compared to the two approaches mentioned above, it can alter the electrical excitatory levels of the cerebral hemispheres. Future studies could investigate the parameters of tDCS such as treatment duration to figure out the better paradigm of tDCS.

**Abbreviations**

MI: motor imagery; VI: visual imagery; KI: kinesthetic imagery; BCI: brain-computer interface; EEG: electroencephalography; tDCS: transcranial direct current stimulation; NMDA: N-methyl-D-aspartate; MMSE: Mini-Mental State Examination; FMA-UE: Fugl-Meyer Assessment for Upper Extremity; ADL: activities of daily living; MSS: Motor Status Scale; MBI: Modified Barthel Index; ARAT: Action Research Arm Test; ICA: independent component analysis; QEEG: quantitative electroencephalography; LTP: long-term potentiation.

**Declarations**

**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the first or corresponding author upon reasonable request.

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**Authors' information**

1. Department of Mechatronic Engineering, China University of Mining and Technology, Jiangsu, China
2. The Affiliated Xuzhou Rehabilitation Hospital of Xuzhou Medical University, Xuzhou Medical University, Jiangsu, China
3. Department of Sports Science, Zhejiang University, Hangzhou, China

**Author contributions**
All authors contributed to the study. ZM defined the study protocol and were responsible for patient recruitment and screening. WY and JF performed the data analysis and wrote the manuscript. GL and CFM coordinated the data collection and conducted the therapy sessions. TW helped with the study design and clinical implementation. All authors read and approved the final manuscript.

**Corresponding author**

Correspondence to Tang Wei.

**Ethics Declarations**

The Ethics Committee of Xuzhou Rehabilitation Hospital approved the entire protocol and instrumentation (No.XK-LW-20200428-003) and was registered in the Chinese Clinical Trials Registry Platform (identifier:ChiCTR2000034730). All participants signed an informed consent form before the start of the trial.

**Consent to participants**

All the patients signed the written informed consent for publication before enrolment.

**Competing interests**

The authors declare that there is no conflict of interest.

**References**


23. Chan NH, Ng SSM. Psychometric properties of the Chinese version of the Arm Activity Measure in people with chronic stroke. Front Neurol. 2023;14:1248589.


Figures
Figure 1

The state of patients during virtual reality training.
Figure 2

Photograph of the BCI-robot training system. The whole system includes an EEG amplifier collecting real-time EEG, a PC processing EEG signal providing visual and auditory feedback, and a triggered-robot hand supporting sensory and movement feedback.
Electroencephalography (EEG) Recording

Figure 3

The montage of real-time EEG electrodes.

Figure 4

Flow chart for participant selection and assignment.