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Enhancing Mirror Visual Feedback with Intermittent Theta Burst Stimulation in Healthy Adults

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Abstract:

Background: Excitatory brain stimulation, in the form of intermittent theta burst stimulation (iTBS), combined with mirror visual feedback (MVF), is hypothesized to promote neuroplasticity and motor performance.

Objective: This study aimed to investigate the combined effects of iTBS with mirror training (MT) on the MVF-induced sensorimotor event-related desynchronization (ERD) and the non-dominant hand motor performance in healthy adults.

Methods: Eighteen healthy right-handed subjects were randomly assigned to one of three groups (Group 1: iTBS plus MT, Group 2: iTBS plus sham MT, or Group 3: sham iTBS plus MT). For participants in Groups 1 and 3, motor training was performed for 15 minutes for the right hand over four consecutive days, with MVF superimposing on their inactive left hand behind a mirror. Participants in Group 2 received the same right-hand motor training, but the mirror was covered without MVF. iTBS or sham iTBS was applied daily over the right primary motor cortex prior to the training. Electroencephalography at pre/post-training was recorded while participants performed right-hand movement under mirror and direct view. Motor performance was assessed at baseline and post-training.

Results: Baseline comparisons demonstrated that a shift in sensorimotor ERD towards the right hemisphere was induced by MVF, in mu-1 (8-10 Hz) ($p = 0.002$), mu-2 (10-

12 Hz) (p = 0.004) and beta-1 (12-16 Hz) (p = 0.049) bands. After the training, participants in Group 1 showed a stronger MVF-induced sensorimotor ERD in mu-1 (p = 0.017) and mu-2 (p = 0.009) bands than those in Group 3. No significant interaction effect was noted in motor outcomes.

Conclusions: iTBS appears to prime subjects' brain to be more receptive to MVF. Such intervention might be applied to patients with hemiplegia.

(Words: 280)

Keywords: theta burst stimulation; mirror visual feedback; event-related desynchronization; mirror neuron system

1. Introduction

Observational learning, by means of either video-based action observation (AO) or mirror-based AO (in terms of mirror training [MT]), has been shown to be an effective approach to speed up motor skill acquisition in healthy adults (Bahr et al., 2018) and to facilitate motor skill relearning in patients with hemiplegia (Zhang, Fong, Welage, & Liu, 2018). One of the possible neural correlates underlying the observational learning of motor skills is thought to be the mirror neuron system (MNS). The MNS is defined as a class of neural substrates that discharges during the observation and execution of actions, and was first observed in the ventral premotor cortex (PMv) (Rizzolatti, Fadiga, Gallese, & Fogassi, 1996) and inferior parietal lobule (IPL) (Fogassi et al., 2005) of the macaque monkey. In humans, the activation of various parietal and frontal areas, including the inferior frontal gyrus (IFG), the IPL, and the intraparietal sulcus (IPS), has been consistently reported during AO, as suggested by a meta-analysis of neuroimaging studies (Caspers, Zilles, Laird, & Eickhoff, 2010), which implies a possible homology of macaque MNS in response to AO in humans (i.e., human parietal-frontal MNS).

Mirror visual feedback (MVF) generated during MT seems to be a more effective observational motor learning strategy than video-based AO, since it provides a more vivid sense of body ownership (Bahr et al., 2018). In MT, a mirror apparatus is placed at the midsagittal plane of the participant. Participants are instructed to perform unilateral hand motor training when simultaneously viewing the mirror view of their moving hand from the mirror (Hamzei et al., 2012). Previous evidence has shown that MVF of the moving hand is likely to enhance the transfer effect of unilateral hand motor training on the motor performance of the inactive hand behind the mirror (Zult et al., 2014). The effect of MVF on the motor performance of the untrained hand (i.e., the hand behind the mirror) is likely to be due to the activation of the corresponding sensorimotor cortex through recruiting the MNS (Zult et al., 2014). Sensorimotor mu (i.e., central alpha, 8-12 Hz) event-related desynchronization (ERD) appears to be a marker of instant sensorimotor activation when observing the MVF (Bartur et al., 2015; Lee, Li, & Fan, 2015). Some simultaneous electroencephalography (EEG)-functional magnetic resonance imaging (fMRI) studies have reported the significant correlation between observation-induced mu ERD and blood-oxygen-level dependent (BOLD) responses in various areas across the parietal-frontal MNS and primary sensorimotor cortex (Arnstein et al., 2011; Braadbaart, Williams, & Waiter, 2013; Yin, Liu, & Ding, 2016). These findings indicate that sensorimotor mu ERD may reflect the downstream modulatory activity of the MNS on the sensorimotor cortex (Muthukumaraswamy, Johnson, & McNair, 2004). A similar phenomenon has also been found in studies focusing on the beta band (12-30 Hz) (Hobson & Bishop, 2017).

The primary motor cortex (M1) is the critical region for motor output (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995) and its excitability can be modulated by non-invasive brain stimulation (NIBS) techniques, including repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS). Studies have demonstrated that M1 excitability is essential for MVF-induced behavioral motor changes. Inhibiting the right M1 with NIBS could eliminate the training effect of MT on the improvement of motor performance of the untrained left hand in healthy adults (Nojima et al., 2012), while facilitating the right M1 with NIBS prior to MT seems to yield a greater effect of promoting motor performance of the untrained left hand in healthy adults (Hoff et al., 2015; von Rein et al., 2015).

Combining excitatory M1 stimulation of the right motor cortex with MT may boost motor skill acquisition of the untrained hand in healthy adults. Previous experiments only investigated the acute effect on motor performance of a single-session NIBS combined with MT (Hoff et al., 2015; von Rein et al., 2015). No study to date has examined the cortical activation accompanying the improvement of motor performance. In the present study, we first investigated the condition-difference between the mirror view and the direct view of the hand movement, to explore the pattern of MVF-induced sensorimotor ERD. Sensorimotor ERD in both mu and beta bands has been found to be more prominent over the unilateral hemisphere (i.e., contralateral to the moving hand) during motor preparation of a unilateral hand movement, and will expand bilaterally during movement execution (Espenhahn et al., 2017; Pfurtscheller & Lopes da Silva, 1999). The laterality of bilateral sensorimotor ERD during unilateral hand movement could be mediated by the recognition of the MVF (Bartur et al., 2015; Lee et al., 2015; Rossiter et al., 2015). Therefore, we expected that MVF applied during unilateral right-hand movement could induced a shift in sensorimotor ERD towards the contralateral hemisphere (i.e., right hemisphere, contralateral to the MVF and ipsilateral to the moving hand), compared to unilateral right-hand movement with direct view of the hand movement. Furthermore, we examined whether combining multiple-session excitatory rTMS, in the form of intermittent theta burst stimulation (iTBS) over the right M1, with MT using the right (trained) hand, had a greater facilitating effect on enhancing the lateralization of MVF-induced sensorimotor ERD towards the contralateral hemisphere and the motor performance of the left (untrained) hand in healthy adults than applying iTBS or MVF alone.

2. Methods

2.1 Participants

All participants were healthy adults who consented to participate. Their written informed consent was obtained before their participation. The participants were recruited by convenient sampling from a local university. Recruits who met all of the following criteria were included in the experiment: (1) aged 18 to 30; (2) right-handed according to the Edinburgh handedness inventory (Oldfield, 1971); and (3) normal or corrected-to-normal vision. Participants meeting any of the following criteria were excluded: (1) any contraindication to rTMS, such as a history of epilepsy, metal implant, and pregnancy (Rossi, Hallett, Rossini, & Pascual-Leone, 2011); (2) a history of any neurological or psychiatric disease; (3) sustained upper limb injuries in the past three months; and (4) congenital deformities of the bilateral upper extremities. The present study was approved by the Human Ethics Committee, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University (reference number: HSEARS20180120003).

2.2 Experimental procedure

Participants were randomly allocated into one of three groups: Group 1: iTBS plus MT, Group2: iTBS plus sham MT by using a covered mirror, or Group 3 sham iTBS plus MT, by drawing lots. All participants had to attend two assessment sessions and four consecutive training sessions. Depending on the availability of participants, their training sessions were launched on the same day as or within two days after the baseline assessment. Baseline assessment included an EEG recording, a motor performance assessment and a motor threshold assessment.

2.3 EEG acquisition

The primary outcome was ERD. EEG was captured with a 64-channel cap using a [a]Digital DC EEG Amplifier and Curry 7 (Compumedics Neuroscan, USA). Electrode impedance was kept below 10 kOhm and the signal was sampled at 1024 Hz. The participants were asked to perform an open-and-close hand movement with their right hand in response to an auditory cue every five seconds (Rossiter et al., 2015) and to relax their hand after completing the movement. Movements were performed under two conditions. The first was mirror view of the hand movement: participants performed a unilateral right-hand movement with MVF, which was created by using [b]prism eyeglasses for MT (SHIL, Golden Jubilee G81 4DY, UK). The eyeglasses created a simultaneous mirror view of left-hand movement when participants moved their right hand. The second was direct view of the hand movement: participants performed a unilateral right-hand movement while watching their moving hand directly. The left-hand was hidden by a white box. The order of conditions was randomized by drawing lots (See Fig. 1). A total of 70 movements were collected for each condition. During EEG recording, EMG was recorded via the common extensor and flexor in the bilateral forearms, using two paired Ag/AgCl electrodes (2 cm apart).

2.4 Motor performance assessment

The second outcomes were motor performance in four assessments of the hand motor skills of the left hand, including the nine-hole peg test (NHPT) (Oxford Grice et al., 2003), the Minnesota dexterity test (MDT) (Lafayette Instruments, 1969), the Purdue Pegboard Test (PPT) (Buddenberg & Davis, 2000), and the two-ball rotation task (Nojima et al., 2012). These tests were used in previous studies to evaluate the effect of rTMS or MT in improving motor performance (Alexeeva & Calancie, 2016; Jelic, Milanovic, & Filipovic, 2015; Nojima et al., 2012; Platz, Adler-Wiebe, Roschka, & Lotze, 2018). Three subtests of PPT, including left hand placing, bilateral hands placing, and assembly task, and three unimanual subtests of MDT, including the placing test, the displacing test, and the one-hand turning and placing test, were used as separate motor outcomes. NHPT, MDT, and PPT were assessed twice and their averaged results were used in the final analysis. The two-ball rotation task was assessed with reference to Nojima et al. (2012). For all unimanual tests, only the left hand was tested. The participants were encouraged to complete the assessments quickly and were free to ask for a break if they felt tired. We calculated the time to complete the given tasks of NHPT (Oxford Grice et al., 2003) and MDT (Lafayette Instruments, 1969), the number of pins inserted by left/bilateral hands during 30-second as well as the number of assemblies made during 60-second for PPT (Buddenberg & Davis, 2000), and the number of two-ball rotation during 30-second for the two-ball rotation task (Nojima et al., 2012). They were used as the motor outcomes in further analysis.

2.5 Motor threshold assessment

The stimulation site for iTBS was the right M1. The optimal position was defined as the coil position eliciting the largest motor evoked potential (MEP), with the coil rotated 45° from the sagittal plane. The stimulation position was maintained by a [c]neuro-navigation system (Localite, Bonn, Germany). The positioning of the coil was done by means of Vicra optical tracking using Localite TMS Navigator based on a data set of a standard head. The resting motor threshold (RMT) is defined as the minimum intensity over the hotspot that elicits an MEP of no less than 50 μv in five out of ten trials over the contralateral first dorsal interosseous (FDI). The active motor threshold (AMT) is defined as the minimum intensity over the hotspot that can elicit an MEP of no less than 200 μv in five out of ten trials during a slight voluntary contraction (20% of the maximum) of the contralateral FDI (Huang et al., 2005). MEPs were visualized and measured via the MEP monitor with an inter-trial interval of at least five seconds.

2.6 Intermittent theta burst stimulation session

Daily serial sessions of iTBS were delivered by [d]MagPro stimulators (MagVenture, Denmark) with a figure-of-eight coil (C-B60) over the right M1 on four consecutive days. We followed previous studies using four-day MT for healthy adults (Hamzei et al., 2012; Lappchen et al., 2015) and the iTBS protocol proposed by Huang et al. (2005) (i.e., 20 trains of ten bursts given at eight-second intervals, with a total of 600 pulses, 190 seconds per session). The stimulation intensity of iTBS was set at 80% of individual AMT. Sham stimulation was delivered using the same coil at only 20% of individual AMT. All participants were told that iTBS was delivered in a subthreshold intensity which could not induce significant limb movement or somatosensory perception. The participants were asked to complete a questionnaire regarding the side effects of TBS upon completion of each stimulation session (Rossi et al., 2011).

2.7 Mirror training session

Immediately after each TBS session, participants took a right-hand motor training session with a mirror or a covered mirror. The participants in Groups 1 and 3 were instructed to watch the MVF in the mirror when executing the motor training, with their left hand motionless behind the mirror. The participants in Group 2 were asked to perform the same motor training with a covered mirror (i.e., a non-transparent board) and to watch their moving hand directly (see Fig.2). The purpose of using the Group 2 (i.e., sham MT) was to control the transfer effect of right-hand motor training on the performance of the left hand (Walz et al., 2015). The motor training included several hand dexterity tasks modified from the NHPT, MDT, PPT, and two-ball rotation task. The tasks included picking up, placing and displacing pegs; making assembly with a pin, a washer and a collar, placing, displacing and turning plastic disks and in-hand rotation of two wooden balls. Each session of motor training lasted around 15 minutes.

2.8 EEG analysis

EEG Preprocessing: Signals captured were processed offline using EEGLab (Delorme & Makeig, 2004) and custom-made Matlab scripts. Raw EEG signals were band-pass filtered between 1 and 80 Hz and then down-sampled at 250 Hz. Additionally, a 50 Hz notch filter was applied. Data were referenced to bilateral mastoid electrodes. Signals with significant movement artifacts and long-term eye closure were rejected during the visual inspection. Then EEG was segmented into 3000-ms epochs (pre-stimulus -1000 ms and post-stimulus 2000 ms, with 0 as the right-hand movement onset point). Movement onset was guided by EMG captured during EEG recording. Raw EMG signals were low-pass filtered at 10 Hz and rectified. The EMG onset point was identified as the first deviation > mean plus three standard deviations beyond the baseline for ten continuous time points (Hodges & Bui, 1996). The results of automatic EMG onset detection were reviewed visually, and incorrectly detected markers were corrected manually. Unexpected muscle contraction, including left-hand movement or movement during the baseline period, was marked and corresponding EEG segments were rejected from the data analysis. Eye movement artifacts were corrected using an independent component analysis algorithm (Delorme & Makeig, 2004). Typical components reflecting blinking and horizontal eye movement were rejected.

EEG time-frequency analysis: Clean epochs were analyzed in the time-frequency domain. The event-related spectral perturbation (ERSP) method with the *newtimef* function of EEGLAB (Makeig, 1993) was used to calculate the power of ERD. The common movement phase was set at 0 to 700 ms, which was guided by the EMG across participants. The ERSP power was baseline corrected using the pre-stimulus interval from -600 ms to -100ms, and then the power was averaged across all trials and converted to log power. Averaged ERSP powers at left (C3) and right (C4) hemispheres during the common movement phase were extracted. Laterality index (LI) was calculated by the following formula (Rossiter et al., 2015) in order to explore the patterns of sensorimotor ERD when participants were executing unilateral hand movement under different visual feedback conditions:

$$\text{Laterality index} = \frac{(\text{C3 ERD power}) - (\text{C4 ERD power})}{(\text{C3 ERD power}) + (\text{C4 ERD power})}$$

Brain response to MVF (i.e., MVF-induced sensorimotor ERD) was defined as the difference in LI between mirror view and direct view.

$$\text{Brain response to MVF} = \text{LI (Mirror view)} - \text{LI (Direct view)}$$

A more negative value indicated more activation towards the right hemisphere during the mirror view condition. Averaged powers at the fixed frequency band and the time interval collected from all 60 channels were computed to construct the EEG topographies by the *topoplot* function of EEGLAB (Delorme and Makeig, 2004). Mu-1 (8-10 Hz), mu-2 (10-12 Hz), beta-1 (12-16 Hz), and beta-2 (16-30 Hz) were investigated separately.

2.9 Statistical analysis

Statistical analysis was performed by SPSS version 23.0. Due to the violation of normal distribution of some variables, Wilcoxon signed-rank tests were performed to compare the difference in LI between mirror view and direct view at baseline. A mixed-effects model with random intercepts and slopes was used to detect any significant difference in the rate of change in motor performance and LI among the three groups, because of its superior capacity to analyze repeated measures data (Gueorguieva & Krystal, 2004). Group effects, time effects, and group by time interaction effects were included as fixed effects, and the random intercept and random slope of change in the dependent variables over time were included as random effects. A negative slope represented the decrease of the variable over time while a positive slope represented the opposite. Between-group differences were investigated by interaction effects and the magnitude was represented by the difference in slopes between two curves. Maximum likelihood estimation was chosen as the estimation method, and the covariance structure was assumed to be unstructured. As our research aimed to compare the combination of iTBS and MT with the single intervention, two comparisons were yielded (Group 1 *vs*. Group 2 and Group 1 *vs*. Group 3). Therefore, the level of significance was set at $p < 0.025$ after Bonferroni adjustment (0.05/2; n = number of comparisons) for the comparison of interaction effects (Perneger, 1998). Within-group differences were examined by separated Wilcoxon signed-rank tests at a significance level of $p < 0.05$. Hedges' g was calculated to determine the effect size of the change scores of motor outcomes between groups due to the small sample size of the present study (Hedges, 1981).

3. Results

3.1 Characteristics of participants

A sample of 18 participants were recruited in the present study. Table 1 summarizes their characteristics. The data of two participants were removed from data analysis, due to significant noise in the baseline EEG data of one and in the post-training EEG data of the other. Therefore, baseline comparison of EEG was performed on 17 cases and pre-post comparison of EEG was conducted on 16 cases (Group 1 = 5 cases *vs.* Group 2 = 6 cases *vs.* Group 3 = 5 cases). The motor outcomes of all participants were used in final analysis.

3.2 Neurophysiological manifestation of MVF

The topography of ERD power for each frequency band during mirror view and direct view is shown in Fig. 3. The data distribution and probability density of LI under mirror view and direct view is shown in Fig. 4. Wilcoxon signed-rank tests showed significant between-condition differences of LI in mu-1 (8-10 Hz) ($Z = -3.12$, $p = 0.002$), mu-2 (10-12 Hz) ($Z = -2.91$, $p = 0.004$), and beta-1 (12-16 Hz) ($Z = -1.97$, $p = 0.049$) bands. No significant between-condition difference was noted in the beta-2 (16-30 Hz) band

(Z = -0.17, p = 0.87).

3.3 Change in brain responses to MVF after intervention

Table 2 and Fig. 5 show the results of the LI difference across the three groups. Regarding the mu-1 band, participants in Group 1 and Group 2 showed a larger response to MVF (i.e. a more negative LI difference between mirror view and direct view) after intervention (p = 0.043 and 0.046, respectively) than at the baseline. Within-group difference was not significant among the participants in Group 3 (Z = -0.67, p = 0.500). A mixed-effects model showed that the MVF-induced sensorimotor ERD of participants in Group 1 decreased faster than in Group 3 (Group 3 *vs.* Group 1, β = 0.23; standard error [SE] = 0.06, p = 0.017), but decreased at a similar rate to that in Group 2 (Group 2 *vs.* Group 1, difference in slope = 0.11; SE = 0.08, p = 0.210);

Regarding the mu-2 band, a mixed-effects model also showed that the MVF-induced sensorimotor ERD of participants in Group 1 decreased faster than in Group 3 (Group 3 *vs.* Group 1, β = 0.26; SE = 0.09, p = 0.009); however, within-group differences did not reach a significant level in all groups (all $p > 0.05$). Regarding the beta band, either within-group difference or between-group interaction was insignificant.

3.4 Change in motor outcomes after intervention

Table 3 and Fig. 6 show the motor outcomes across the three groups. A mixed-effects model showed that between-group interactions were not significant in all motor outcomes after Bonferroni corrections (all $p > 0.025$). However, in PPT, there was a trend indicating that the left-hand motor performance of participants in Group 1 improved faster than in Group 3 (Group 3 *vs*. Group 1, difference in slope = -1.42; SE = 0.63, $p = 0.038$). The effect size (Group 1 *vs*. Group 3) was large (Hedges' $g = 1.14$).

4. Discussion

This study demonstrates that: (1) MVF induced a shift of sensorimotor ERD towards the contralateral hemisphere in mu-1, mu-2, and beta-1 bands, in contrast to the direct view condition; (2) the combination of iTBS with MT induced a higher response to MVF, denoted by a larger difference in the LI of mu ERD between mirror view and direct view, in contrast to sham iTBS with MT. However, the change in response to MVF in Group 1 (iTBS with MT) was similar to that in Group 2 (iTBS with sham MT); and (3) no significant between-group difference in motor outcomes was noted.

The neurophysiological manifestation of MVF in our study was broadly in line with previous reports. Lee et al. (2015) showed that the MVF of hand movement could enhance the magnitude of mu ERD (8-12 Hz) over the contralateral sensorimotor cortex in healthy individuals, which is similar to our findings. However, MVF applied during unilateral hand movement did not significantly influence mu-2 ERD (10-12 Hz) in the study of Bartur et al. (2015). It has been reported that mu-2 ERD (10-11 Hz) is accompanied by hand motor imagery in healthy adults (Yi et al., 2014). We note that participants in the study of Bartur et al. (2015) were required to imagine bilateral movement, which may diminish the interhemispheric difference of mu-2 ERD when receiving MVF. Some studies have also observed the effect of MVF on modulating beta ERD (Espenhahn et al., 2017; Pfurtscheller & Lopes da Silva, 1999). Bartur et al. (2015) showed that the interhemispheric asymmetry of beta-1 ERD (12-20 Hz) during unilateral hand movement was reduced by MVF in healthy individuals, similar to our findings regarding the beta-1 band (12-16 Hz). In this study, MVF did not induce a shift in beta-2 (16-30 Hz) ERD towards the contralateral hemisphere in healthy adults. This finding is comparable to a previous magnetoencephalography (MEG) study (Rossiter et al., 2015) which reported that no significant change in beta ERD (15-30 Hz) was induced by MVF in healthy individuals when performing bilateral hand movement. However, Rossiter et al. (2015) found that MVF could significantly reduce the interhemispheric asymmetricity of beta ERD (15-30 Hz) during bilateral hand movement in patients with stroke. This implies that the neurophysiological effect of

MVF in healthy adults might be different from that in stroke survivors with abnormal interhemispheric inhibition caused by brain lesions (Rossiter, Boudrias, & Ward, 2014).

In this study, brain response to MVF, as measured by sensorimotor mu ERD, was enhanced in participants who received iTBS with MT, in contrast to participants who received sham iTBS with MT. However, we did not observe significant differences in MVF-induced beta ERD in the three groups. Moreover, the participants who received four-day iTBS with MT tended to present better motor performance in their untrained hand in PPT than those who received sham iTBS with MT. A previous study showed that applying single-session iTBS alone may facilitate the motor performance of the non-dominant hand in PPT in healthy adults (Jelic et al., 2015), which is comparable to our findings. Caution should be taken in interpreting this result, as the interaction effect was deemed insignificant after Bonferroni corrections and only one motor outcome showed this trend. To determine whether the neurophysiological effect can be translated into behavioral changes, further replications are necessary. We observe no difference in the motor outcomes and MVF-induced sensorimotor ERD between participants who received iTBS with MT and those who received iTBS with sham MT, which may be caused by different reasons – the intensity of the four-session MT might not be enough to induce a training effect, and that the transfer effect of unilateral hand movement without a mirror might also contribute to the change of bilateral sensorimotor neuroplasticity (Walz et al., 2015).

This study has demonstrated that the combination of iTBS and MT enhances the brain's response to MVF and tends to facilitate the motor performance of the untrained hand in PPT, in contrast to sham iTBS with MT. We could not conclude that the same effect would appear in the stroke population. However, the present study with healthy individuals calls for the investigation of the effects of the combined intervention in facilitating the reacquisition of lost motor skills in patients with stroke. The sensorimotor ERD induced by MVF is likely to be an index of sensorimotor activation along with the functional improvement in patients with stroke.

4.1 Limitations

Certain limitations of the present study should be noted when interpreting the results. Firstly, this study has a small convenience sample and further investigation with a larger sample is necessary to replicate the findings. Because of this, we have not carried out a power calculation for this study. Secondly, we did not perform dimension reduction or used a composite score for motor outcomes, but allowed multiply testing without adopting a more stringent level of significance using Bonferroni method with regard to the exploratory nature of the present study; however, there was still not any significant between-group difference, indicating that the neurophysiological effect may be hard to generalize to behavioral change in healthy subjects. Thirdly, the LI of the bilateral ERD were small with large deviation. A previous experiment has suggested that ERD could index the instant sensorimotor activation in response to MVF, but its detection of brain activation lateralization may be less precise than other neuroimaging tools (Lee et al., 2015). Lastly, we used the MVF-induced sensorimotor ERD as an index to evaluate the instant sensorimotor activation induced by MVF, but we did not evaluate the MEP, which would provide additional information about corticomotor excitability in resting state. Lastly, we only explored the effect of a relatively short-term (4-session) training. A previous study using a longer training duration of 15-session of MT found that MVF enhanced the transfer effect of unilateral hand training, but the results showed that there was an increase in the interhemispheric inhibition (from the trained to the untrained M1) (Zult et al., 2016). Therefore, the combined effect of both interventions on sensorimotor plasticity and motor performance, when they are applied in long-term, awaits for further investigation.

4.2 Summary

Our study shows that MVF is likely to activate the contralateral sensorimotor cortex (i.e., contralateral to MVF and ipsilateral to the moving hand), and that iTBS appears to have a priming effect on the subject's brain, making it more receptive to MVF. However, this neurophysiological effect was not generalized to the motor performance in healthy adults. Further research will be carried out to investigate the clinical efficacy of combining both interventions in the stroke population.

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Equipment

a. SymAmps2 amplifier and Curry 7, Neuroscan, Charlotte, NC, USA

b. Prism eyeglasses for mirror therapy, SHIL, Golden Jubilee G81 4DY, UK

c. MagPro X100 and MagOption rTMS stimulator with Coil C-B60 Butterfly, Standard, MagVenture, Denmark

d. Localite TMS Navigator, Localite, Germany

Conflicts of interest

The authors declare no conflict of interest.

Contributors

JZ and KF designed the study and performed the experiment and data analysis. JZ wrote the first version of the manuscript, which both authors revised, and KF approved the final manuscript.

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Table 1. Characteristics of participants

	Group 1 (n = 6)	Group 2 (n = 6)	Group 3 (n = 6)
Age (years, mean ± SD)	25.30 ± 2.00	26.50 ± 2.17	26.33 ± 2.25
Gender (female/male)	3/3	2/4	4/2
Educational level			
Master student	3	3	4
Doctorate student	3	3	2

Table 2. Results of laterality index differences across three groups

		Descriptive data Mean (SE)		Within-group differences	Between-group differences			
		Baseline	**Post**	***Z*** ***p***	**Comparisons**	**Difference in slope** ***β***	**SE**	***p***
LI differences of mu-1	Group 1	-0.08 (0.04)	-0.26 (0.07)	-2.02 0.043*	Group 2 *vs*. Group 1	0.11	0.08	0.210
	Group 2	-0.06 (0.03)	-0.13 (0.03)	-2.00 0.046*	Group 3 *vs*. Group 1	0.23	0.06	0.017**
	Group 3	-0.09 (0.04)	-0.04 (0.08)	-0.67 0.500				
LI differences of mu-2	Group 1	-0.12 (0.06)	-0.28 (0.06)	-1.21 0.225	Group 2 *vs*. Group 1	0.10	0.09	0.247
	Group 2	-0.06 (0.04)	-0.12 (0.03)	-0.94 0.345	Group 3 *vs*. Group 1	0.26	0.09	0.009**
	Group 3	-0.11 (0.06)	-0.01 (0.05)	-1.75 0.08				
LI differences of beta-1	Group 1	-0.19 (0.11)	-0.16 (0.07)	-0.67 0.500	Group 2 *vs*. Group 1	-0.20	0.14	0.173
	Group 2	0.03 (0.06)	-0.15 (0.07)	-0.73 0.463	Group 3 *vs*. Group 1	0.13	0.15	0.396

	Group 3	-0.23 (0.13)	-0.07 (0.08)	-1.21 0.225				
LI differences of beta-2	Group 1	-0.11 (0.10)	-0.02 (0.07)	-0.94 0.345	Group 2 *vs*. Group 1	-0.13	0.15	0.420
	Group 2	0.06 (0.06)	0.01 (0.07)	-0.52 0.600	Group 3 *vs*. Group 1	-0.13	0.15	0.365
	Group 3	0.02 (0.13)	-0.02 (0.12)	-0.41 0.686				

* $p < 0.05$; ** $p < 0.025$

Abbreviation: LI: laterality index

Table 3. Results of motor outcomes across three groups

		Descriptive data Mean (SE)		Within-group differences	Between-group differences			
		Baseline	Post	*Z* *p*	Comparisons	Difference in slope *β*	SE	*p*
NHPT-left hand	Group 1	20.29 (0.85)	18.80 (0.75)	-2.20 0.028*	Group 2 *vs.* Group 1	-0.45	0.58	0.442
	Group 2	19.93 (0.52)	17.99 (0.76)	-2.20 0.028*	Group 3 *vs.* Group 1	-0.07	0.58	0.909
	Group 3	18.51 (0.44)	16.95 (0.57)	-2.01 0.028*				
PPT-left hand	Group 1	14.33 (0.79)	16.25 (0.48)	-2.20 0.027*	Group 2 *vs.* Group 1	-0.75	0.63	0.250
	Group 2	14.75 (0.62)	15.92 (0.61)	-1.75 0.080	Group 3 *vs.* Group 1	-1.42	0.63	0.038*
	Group 3	15.00 (0.58)	15.50 (0.53)	-0.65 0.516				
PPT-bilateral hands	Group 1	11.83 (0.67)	12.83 (0.57)	-1.90 0.057	Group 2 *vs.* Group 1	0.17	0.51	0.749
	Group 2	11.92 (0.64)	13.08 (0.47)	-2.04 0.041*	Group 3 *vs.* Group 1	-0.17	0.51	0.749

	Group 3	12.50 (0.56)	13.33 (0.57)	-1.38 0.167				
PPT-assembly	Group 1	40.92 (1.74)	41.92 (1.60)	-0.84 0.400	Group 2 *vs.* Group 1	0.25	1.09	0.821
	Group 2	41.83 (2.51)	43.08 (2.48)	-1.16 0.246	Group 3 *vs.* Group 1	1.00	1.09	0.372
	Group 3	42.33 (1.72)	44.33 (1.44)	-2.20 0.027*				
MDT (Placing) -left hand	Group 1	67.75 (2.63)	65.38 (2.44)	-1.36 0.173	Group 2 *vs.* Group 1	-0.90	2.14	0.681
	Group 2	65.96 (2.48)	62.70 (2.73)	-1.78 0.075	Group 3 *vs.* Group 1	0.31	2.14	0.886
	Group 3	67.20 (2.39)	65.15 (1.22)	-1.15 0.249				
MDT (Displacing) -left hand	Group 1	49.51 (2.73)	49.04 (2.49)	-0.11 0.917	Group 2 *vs.* Group 1	-3.06	2.36	0.210
	Group 2	48.85 (2.24)	45.32 (1.65)	-2.20 0.028*	Group 3 *vs.* Group 1	0.08	2.36	0.974
	Group 3	49.87 (1.63)	49.47 (2.01)	-0.11 0.917				
MDT (One-hand turning and placing) -left hand	Group 1	77.01 (2.86)	73.69 (3.40)	-1.36 0.173	Group 2 *vs.* Group 1	-4.89	2.85	0.104
	Group 2	81.19 (4.47)	72.97 (4.01)	-2.20 0.028*	Group 3 *vs.* Group 1	-1.86	2.85	0.523

	Group 3	79.17 (2.83)	73.98 (1.79)	-1.36 0.173				
Two-ball rotation -left hand	Group 1	15.67 (1.67)	18.33 (1.52)	-2.22 0.026*	Group 2 *vs.* Group 1	-1.50	0.81	0.081
	Group 2	20.83 (2.57)	22.00 (2.74)	-1.52 0.129	Group 3 *vs.* Group 1	0.00	0.81	0.999
	Group 3	16.33 (2.03)	19.00 (2.27)	-0.21 0.039*				

* $p < 0.05$; ** $p < 0.025$

Abbreviation: NHPT: Nine-hole peg test; PPT: Purdue pegboard test; MDT: Minnesota dexterity test

Figure legends

The color should be used for Figure 1 and Figure 3-6 in print

Fig. 1. Demonstration of the EEG experiment design. (A) Mirror view condition: participants performed right hand open-and-close movement when receiving mirror visual feedback created by prism glasses; (B) Direct view condition: participants performed right hand open-and-close movement when directly viewing the moving hand and (C) EEG experimental paradigm for both conditions: Participants performed the movement in response to an auditory cue every five seconds.

Fig. 2. Demonstration of mirror training and sham mirror training.

Fig. 3. Topography of ERD powers: (A) Mu-1 band (8-10 Hz); (B) Mu-2 band (10-12 Hz); (C) Beta-1 band (12-16 Hz) and (D) Beta-2 band (16-30 Hz). Participants moved their right hand under mirror view and direct view during EEG recording.

Fig. 4. Data distribution and probability density of laterality index under mirror view and direct view: (A) Mu-1 band (8-10 Hz); (B) Mu-2 band (10-12 Hz); (C) Beta-1 band (12-16 Hz) and (D) Beta-2 band (16-30 Hz).

Fig. 5. Results of laterality index differences: (A) Mu-1 band (8-10 Hz); (B) Mu-2 band (10-12 Hz); (C) Beta-1 band (12-16 Hz) and (D) Beta-2 band (16-30 Hz). The significant interaction effect between Group 1 and Group 3 is represented by '**' ($p <$

0.025)

Fig. 6. Results of motor outcomes for the untrained hand: (A) NHPT-left hand; (B) PPT-left hand; (C) PPT-bilateral hands; (D) PPT-assembly; (E) MDT (placing)-left hand; (F) MDT (displacing)-left hand; (G) MDT (one hand turning and placing)-left hand and (H) Two-ball rotation-left hand. Abbreviation: NHPT: Nine-hole peg test; PPT: Purdue pegboard test; MDT: Minnesota dexterity test. The significant interaction effect between Group 1 and Group 3 is represented by '*' ($p < 0.05$)