

# Combining Motor Imagery and Action Observation with Vibratory Stimulation Increases Corticomotor Excitability in Healthy Young Adults

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## Abstract

Vibratory stimulation but also motor imagery and action observation can induce corticomotor modulation, as a bottom-up stimulus and top-down stimuli, respectively. However, it remains unknown whether the combination of motor imagery, action observation, and vibratory stimulation can effectively increase corticomotor excitability. This study aimed to investigate the effect of motor imagery and/or action observation, in the presence or absence of vibratory stimulation, on the corticomotor excitability of healthy young adults. Vibratory stimulation was provided to the palm of the right hand. Action observation consisted in viewing a movie of someone else's finger flexion and extension movements. The imagery condition required the participants to imagine they were moving their fingers while viewing the movie and attempting to move their fingers in accordance with the movie. Eleven right-handed healthy young adults were asked to perform six conditions randomly: 1) vibratory stimulation, imagery, and action observation, 2) vibratory stimulation and action observation, 3) vibratory stimulation and viewing of a blank screen, 4) imagery and action observation, 5) action observation, and 6) viewing of a blank screen. Single-pulse transcranial magnetic stimulation was conducted to assess corticomotor excitability and the peak-to-peak amplitude of the motor evoked potentials. The results showed that vibratory stimulation increases corticospinal excitability. The findings further revealed that performing motor imagery while viewing finger movement is more effective at inducing an augmentation of corticomotor excitability compared to action observation alone. Thus, the combination of motor

imagery, action observation, and vibratory stimulation can effectively augment corticomotor excitability.

## Keywords

Motor Evoked Potential, Transcranial Magnetic Stimulation, Vibratory Stimulation, Motor Imagery, Action Observation

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## 1. Introduction

Sensory inputs can modulate corticospinal excitability [1], which plays an important role in reorganizing the motor cortex during motor learning and motor control [2]. Peripheral somatosensory inputs detect bodily sensations, which are conveyed to the brain via the spinal cord [3]. Proprioceptive inputs from the muscle spindles provide important afferent information for the modulation of corticomotor excitability and enhancement of motor function and performance [1] [4]. The vibratory stimulation to a muscle can be used to provide proprioceptive input to the muscle spindles, which allows exploring the motor response of vibratory stimulation [5]-[13]. The vibration-induced Ia afferents from the muscle spindles project to the spinal cord, which induces involuntary contraction of the vibrated muscle, a phenomenon named tonic vibration reflex (TVR) [14] [15] [16]. While some previous studies reported that vibratory stimulation can also induce a motor response antagonistic to the vibrated muscle, for instance in long loop reflexes [17] [18] [19] [20], it is also involved in an antagonist vibratory response (AVR) [9] [21] [22]. These motor responses are mediated by the primary motor cortex [9]. Therefore, vibratory stimulation can induce excitability of the corticospinal pathway and motor cortex as a bottom-up stimulus.

Additionally, the top-down processing of other sensory information in the absence of peripheral somatosensory input can alter corticomotor excitability. A previous study by Mulder [23] suggested that motor imagery and action observation may contribute to increasing corticomotor excitability when learning a motor performance. Motor imagery refers to a conscious motor representation without any actual movement nor muscle activity [24] [25] [26] [27] [28]. The content of motor imagery can be consciously associated with the intention of a movement [28]. Motor imagery refers to the sensation of a body-centered movement that is internally stimulated without peripheral afferents, resulting in the activation of motor-related areas [29]. The majority of previous studies using functional magnetic resonance images (fMRI) have suggested that motor imagery activated brain regions such as the primary motor cortex [30]-[36], the supplementary motor area [37] [38] [39], the dorsal premotor cortex (PDM) [33] [35] [38], the cerebellum [29] [40] [41] [42], and the parietal cortex [40] [43] [44] [45]. Thus, motor imagery has been utilized to successfully assess the effects of mental stimulation of movement on corticomotor activation [27] [46].

Action observation involves a mirror neuron system which allows for the transformation of visual stimuli into a motor program, contributing to the facilitation of corticomotor excitability [47]-[52]. According to the mirror neuron system, visuomotor neurons are activated when observing or perceiving someone else's action, leading to corticomotor excitability in the observer [48]. In the current study, action observation was performed by viewing a movie of someone else's finger flexion and extension movements, while motor imagery was performed by imagining that the participants were moving their fingers while viewing the same movie as the action observation and attempting to move their fingers in accordance with the movie.

Previous studies have suggested mechanisms by which motor imagery and action observation may have a significant influence on corticomotor excitability as a top-down process, however, it remains unclear whether motor imagery combined with action observation is more effective at modulating corticomotor excitability compared to action observation alone. It can be assumed that adding motor imagery to action observation would augment the effect on corticomotor excitability since motor imagery is considered to be involved in multimodal sensation including visual and spatial perspectives [53] [54] [55]. Moreover, it is unknown whether the combination of vibratory stimulation with motor imagery and action observation is more effective at inducing corticomotor excitability compared to these conditions without vibratory stimulation. Further, previous studies have suggested that vibratory stimulation induces muscle activities, both agonist to and antagonist to the vibrated muscles, such as the TVR, AVR, and the long loop reflexes. However, it is not clear whether the motor response differs between the agonist and antagonist muscles of the vibrated muscles.

Transcranial magnetic stimulation (TMS) is a noninvasive brain stimulation method that has been widely utilized to measure descending corticocortical and corticospinal excitability [56]. It can stimulate a selective brain region of the motor cortex, while the TMS-evoked motor response of the target muscle is commonly measured by the amplitude of motor evoked potential (MEP) [56]. Hence, the modulation of corticomotor excitability by motor imagery, action observation, and vibratory stimulation can be assessed using TMS.

The purpose of this study was to investigate the effect of vibratory stimulation combined with motor imagery and action observation on corticomotor excitability in healthy young adults. The effects of three factors on the MEP amplitudes were examined: 1) vibration (with and without vibratory stimulation), 2) imagery and movie (motor imagery while viewing the movie, only viewing the movie, and viewing a blank screen), and 3) muscle activity (finger extensors and flexors). We hypothesized that: 1) vibratory stimulation would increase the MEP amplitudes compared to conditions without vibration, 2) motor imagery during viewing of the movie would augment the MEP amplitudes compared to only viewing the movie or viewing a blank screen, while the MEP amplitudes would be greater when viewing the movie compared to viewing a blank screen, and 3) there would be a difference in the MEP amplitudes between finger extensors and

flexors.

## 2. Materials and Methods

### 2.1. Participants

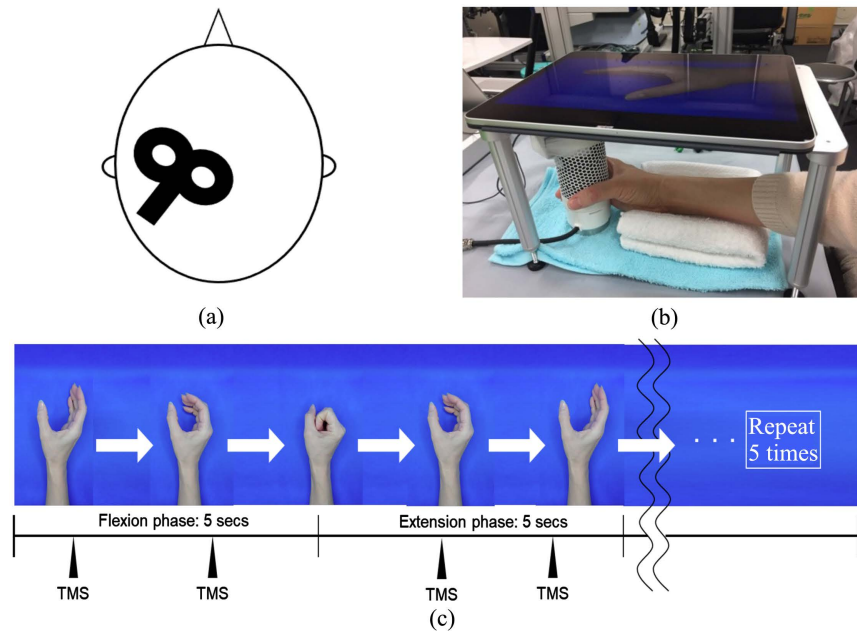
Nineteen right-handed healthy young adults between 20 and 25 years of age and comprising seven females and 12 males participated in this study. The power calculation for the sample size was performed using G \* Power [57] (effect size  $f = 0.3$ ,  $\alpha = 0.05$ , power  $(1 - \beta) = 0.9$ , correlation among repeated measures = 0.5,  $\eta_p^2 = 0.06$ ). The required sample size with an actual power of 0.9 was of 17. Participants were excluded if they had a history of epileptic seizures, musculoskeletal diseases (including the fatigue of an arm), or cardiovascular diseases. Handedness was assessed using the Edinburgh Handedness Inventory [58]. Eight participants were excluded from the analysis due to noise in the collected data. Thus, a total of eleven participants were included for the final analysis (five females and six males; age,  $22.5 \pm 1.56$  years; height,  $165.0 \pm 7.59$  cm; body weight,  $59.5 \pm 11.64$  kg; Body mass index,  $21.7 \pm 3.21$ ). The protocol for this study was approved by the Research Ethics Committee of the National Institute of Information and Communications Technology. All the participants signed a written informed consent form.

### 2.2. Surface Electromyography

Surface electromyography (sEMG; EMG Multi Analysis Progame MaP1038L, Nihonsanteku Co., Ltd., Osaka, Japan; sampling frequency: 2048 Hz) was recorded from the right extensor digitorum communis (EDC) and right flexor digitorum superficialis (FDS). Two bipolar Ag-AgCl surface electrodes (BA-U410 m(A)-015, Nihonsanteku Co., Ltd., Osaka, Japan) were placed over the belly of each muscle [59] [60]. The sEMG signals were amplified (gain 1000), band-pass filtered (10 - 1000 Hz), and recorded using the EMG Multi Analysis Progame MaP1038L software.

### 2.3. Transcranial Magnetic Stimulation

Single-pulse TMS was provided using a Magstim 200 (Magstim Co., London, UK) with a figure-of-eight shaped coil (7-cm internal diameter). The location of the coil was referred to the 10 - 20 International System and the coil was placed at the C3 of the 10 - 20 System Positions over the hand area of the primary motor cortex on the left hemisphere. The determined location was marked by a reference sticker apposed on the head of the participants wearing a cap for adherence. The coil was tangentially held with the handle pointing backward and laterally approximately  $45^\circ$  to the sagittal plane, with an orientation allowing to induce electric current flow in a posterior-anterior direction (**Figure 1(a)**). The optimal position (hot spot) to elicit a reliable MEP was adjusted by determining the resting motor threshold (RMT) in each participant. The RMT was defined as the minimum intensity of TMS required to evoke MEPs larger than  $50 \mu\text{V}$  peak-to-peak amplitude at least five times out of the 10 trials performed at rest [56].



**Figure 1.** Experimental Set-up and Schematic Representation of the Protocol. (a) The coil was tangentially positioned over the participant's left primary motor cortex with the handle pointing backward and laterally, approximately 45° to the sagittal plane, allowing to generate posterior-anterior current flow. (b) Hand intervention device combining vibratory stimulation and visual images. A video screen was placed on a small stand and a vibratory device was set underneath the stand on the table. Participants softly held the vibratory device and received the vibratory stimulation while viewing a movie of someone else's finger movement displayed on the screen. (c) Procedure for the conditions involving viewing of the movie is illustrated. One condition was composed of 50 sec. The movie starts with 5-sec of flexion phase, followed by 5-sec of extension phase, and these movements are repeated five times in each condition. Single-pulse transcranial magnetic stimulation (TMS) was randomly delivered twice within each flexion phase and extension phase of the movie in the conditions involving the movie. In the conditions without the movie, the TMS pulses were delivered in the same manner as in the other conditions. Participants received 20 pulses of TMS in each condition.

#### 2.4. Vibratory Stimulation and Action Observation

Vibratory stimulation was provided to the palm of the right hand using a hand intervention device combining vibratory stimulation and visual images (PLANSTAFF CO., LTD., Tokyo, Japan; **Figure 1(b)**). A vibration frequency of 115 Hz with a low-amplitude of 1 mm was used, considering that previous studies have suggested that a vibratory stimulation with a high-frequency between 80 and 120 Hz and low-amplitude induces strong activation of Ia afferent axons innervating muscle spindles [8] [16] [61]. The vibration device was placed on a table under a small stand holding a video screen (see **Figure 1(b)**).

The action observation was provided on a video screen (approximately 300 mm wide × 200 mm high) (**Figure 1(b)**). A 50-sec movie was depicted on the screen showing someone else's right hand movement. The movie starts with a position of fingers extension, then all fingers are flexed and clenched into the palm for five seconds (*i.e.*, flexion phase), followed by fingers extension toward

the start position for five seconds (*i.e.*, extension phase). These movements are repeated five times (**Figure 1(c)**). The position of the video screen was adjusted to each participant's hand position, making them feel as much as possible that the hand on the screen was their own hand.

## 2.5. Experimental Protocol

Each participant was comfortably seated in a chair located in front of the hand intervention device and where she/he was able to hold the vibratory device comfortably, so the distance between the chair and table was not fixed for each participant. Also, the participant was asked to wear earplugs in both ears. Electromagnetic wave noise suppression sheets were placed on the table and chair to reduce the noise of the sEMG signal. The tested right forearm of the participant was positioned on the table inside a small stand (**Figure 1(b)**). The participants were asked to hold the vibratory device softly in the palm of their right hand and relax the rest of the body. Also, the participants were instructed not to move their whole body during the data recording as any body movement could cause noise in the sEMG signals. The participants were allowed to familiarize themselves with the vibratory stimulation before data acquisition.

A custom-written MATLAB program (version 2017a, The MathWorks Inc., Natick, Massachusetts, USA) was used to provide single-pulse TMS pulses which were randomly delivered twice within each flexion phase and extension phase of the movie across all conditions using the same intensity as the RMT (**Figure 1(c)**). A total of 20 trials of TMS stimulations were provided in a condition, of which 10 trials represented each flexion phase and extension phase of the movie. The TMS system delivered trigger pulses that synchronized with the sEMG system.

A total of six conditions were performed as follows: 1) Vibratory stimulation, imagery, and viewing the movie ( $V^+I^+M^+$ ) condition where participants were instructed to view the finger movement on the movie and imagine they were moving their fingers while receiving vibratory stimulation, while attempting to move their fingers in accordance with the finger movement displayed on the movie, 2) Vibratory stimulation and viewing the movie ( $V^+I^-M^+$ ) condition where participants were instructed to view the finger movement on the movie while receiving vibratory stimulation, without imagining the finger movement, when just viewing the movie, 3) Vibratory stimulation and viewing the blank screen ( $V^+I^-M^-$ ) condition where participants were instructed to view the blank screen while receiving vibratory stimulation, 4) Imagery and viewing the movie ( $V^-I^+M^+$ ) condition where participants were instructed to view the finger movement on the movie and imagine they were moving their fingers, while attempting to move their fingers in accordance with the finger movement on the movie, 5) Viewing the movie ( $V^-I^-M^+$ ) condition where participants were instructed to view the finger movement on the movie, without imagining the finger movement, when just viewing the movie, and 6) Viewing the blank screen ( $V^-I^-M^-$ ) condition where participants were instructed to view the blank screen (**Table 1**).

**Table 1.** Summary of the six study conditions.

|  | Vibration (V) | Imagery (I) | Movie (M) |
|--|---------------|-------------|-----------|
| V <sup>+</sup> I <sup>+</sup> M <sup>+</sup> | +             | +           | +         |
| V <sup>+</sup> I <sup>-</sup> M <sup>+</sup> | +             | -           | +         |
| V <sup>+</sup> I <sup>-</sup> M <sup>-</sup> | +             | -           | -         |
| V <sup>-</sup> I <sup>+</sup> M <sup>+</sup> | -             | +           | +         |
| V <sup>-</sup> I <sup>-</sup> M <sup>+</sup> | -             | -           | +         |
| V <sup>-</sup> I <sup>-</sup> M <sup>-</sup> | -             | -           | -         |

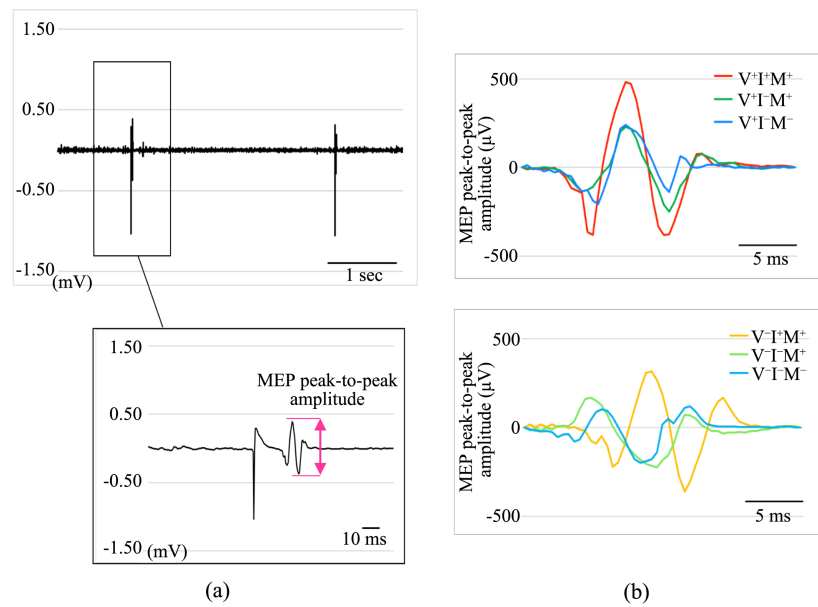
In the V<sup>+</sup>I<sup>-</sup>M<sup>-</sup> and V<sup>-</sup>I<sup>-</sup>M<sup>-</sup> conditions without the action observation, TMS pulses were delivered in the same manner as in the other conditions. The order of conditions was randomly assigned as determined by computer-generated random numbers.

After each condition, the participants were asked to report on their subjective feelings as follows: 1) to what extent they felt that the palm of the right hand was opened during vibratory stimulation (Vib AVR sensation) and 2) to what extent they felt that the palm of the right hand was squeezed during vibratory stimulation (Vib TVR sensation). These questions were scored from 1 (not at all) to 10 (very strong) through verbal expression, and each score in each condition was analyzed to assess differences between the conditions. This questionnaire was conducted as a previous study reported that vibratory stimulation might induce TVR and AVR [9]. In the current study, as vibratory stimulation was applied to the palm (*i.e.*, finger flexors), the TVR and AVR were expected to affect the FDS and EDC, respectively. The mean and standard deviation are presented in **Table 2**.

## 2.6. Data Processing and Analysis

The acquired sEMG data were analyzed offline using MATLAB (version 2017a, The MathWorks Inc., Natick, Massachusetts, USA) to calculate the peak-to-peak amplitude of MEP responses from each trial [56]. First, the timing of each TMS trial was detected from the recorded trigger pulses of TMS (**Figure 2(a)**), and the time window from 10 to 35 ms after the trigger was extracted in each trial (**Figure 2(b)**). Due to TMS-induced noise on the recorded sEMG signals, a linear trend was subtracted from the time-series data signal within the time window. Also, some trials in which data within the time window displayed only positive (>0) or negative (<0) signals due to noise were eliminated from the analysis. As the result, eight participants were excluded. Further analysis was conducted in the eleven remaining participants. The peak-to-peak amplitudes of MEP in the time window of each trial were calculated in each condition. The MEP amplitudes of the EDC during the extension phase of the movie and MEP amplitudes of the FDS during the flexion phase of the movie were used for further analysis, considering that Yahagi and Kasai [62] reported that the MEP amplitude of the





**Figure 2.** An Example of Recorded Traces from Extensor Digitorum Communis (EDC) of a Typical Participant. (a) Upper panel represents a part of the recorded original time-series sEMG data obtained from EDC of a subject in the  $V^+I^+M^+$  condition. The enlarged view (lower panel) represents the recorded data for a TMS pulse and the MEP response. MEPs were elicited by single-pulse TMS over the left primary motor cortex. The peak-to-peak amplitude of the MEP is indicated as a red arrow. (b) Examples of MEPs for the same subject in each condition are depicted for the extracted time window of 10 to 35 ms from the TMS trigger pulse of each trial, after linear trend removal processing. The upper panel shows the three conditions with vibratory stimulation ( $V^+I^+M^+$ ,  $V^+I^-M^+$ , and  $V^+I^-M^-$ ), whilst the lower panel shows the three conditions without vibratory stimulation ( $V^-I^+M^+$ ,  $V^-I^-M^+$ , and  $V^-I^-M^-$ ). Abbreviations: EDC, extensor digitorum communis; sEMG, surface electromyography; TMS, transcranial magnetic stimulation; MEP, motor evoked potential.

**Table 2.** Scores of subjective assessment measures.

| Vibration | AVR sensation |             |             | TVR sensation |             |             |
|-----------|---------------|-------------|-------------|---------------|-------------|-------------|
|           | $V^+I^+M^+$   | $V^+I^-M^+$ | $V^+I^-M^-$ | $V^+I^+M^+$   | $V^+I^-M^+$ | $V^+I^-M^-$ |
| Score     | 4.5 ± 3.0     | 3.0 ± 2.8   | 3.1 ± 2.4   | 5.2 ± 2.3     | 3.2 ± 2.3   | 4.4 ± 2.0   |

Notes: Values are mean ± standard deviation. Abbreviations: AVR, antagonist vibratory response; TVR, tendon vibratory response.

wrist palmar flexor muscle is affected by the motor images of palmar flexion. In addition, five trials of response in each condition were shown to be required to reliably assess corticomotor excitability [63] [64]. Thus, the average of the MEPs from five trials in each EDC and FDS during the extension phase and flexion phase of the movie, respectively, were used in each condition for statistical analysis.

### 2.7. Statistical Analysis

A three-way repeated measures analysis of variance (RM ANOVA) was per-

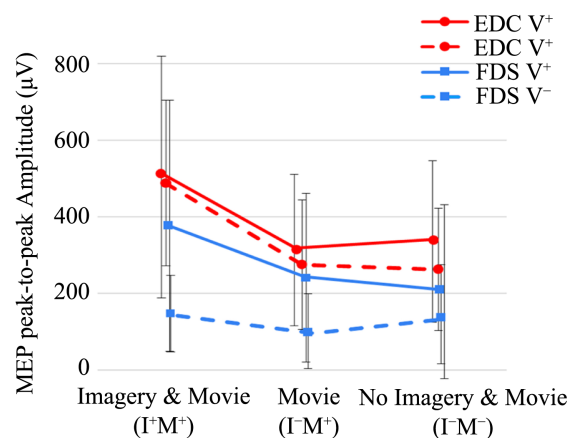


formed using IBM SPSS Statistics version 26 software to determine the effect of three factors on the peak-to-peak amplitudes of MEPs: 1) vibration ( $V^+$ ,  $V^-$ ; 2); 2) imagery and movie ( $I^+M^+$ ,  $I^-M^+$ ,  $I^-M^-$ ; 3); 3) muscle activity (EDC, FDS; 2). The post-hoc test was adjusted using the Bonferroni test ( $p < 0.05$ ). To assess the scores of subjective feelings, a two-way RM ANOVA was performed to evaluate the effect of two factors (imagery and movie ( $I^+M^+$ ,  $I^-M^+$ ,  $I^-M^-$ ; 3) and sensation (AVR and TVR; 2)) on the scores of the Vib AVR sensation and Vib TVR sensation.

### 3. Results

All the participants completed the data collection, however, as described above, some recorded data contained TMS-induced noise. Thus, eight participants were excluded and the remaining eleven participants were included for further analysis.

A three-way RM ANOVA showed a significant main effect of the vibration ( $F(1, 10) = 11.52$ ,  $p = 0.007$ ,  $\eta_p^2 = 0.54$ , observed power = 0.86), imagery and movie ( $F(1.23, 12.28) = 12.31$ ,  $p = 0.003$ ,  $\eta_p^2 = 0.55$ , observed power = 0.93), and muscle activity ( $F(1, 10) = 5.5$ ,  $p = 0.041$ ,  $\eta_p^2 = 0.36$ , observed power = 0.53) on the peak-to-peak amplitudes of the MEPs. The results indicated that the MEP amplitudes were higher in the  $V^+$  conditions compared to the  $V^-$  conditions. Also, the MEP amplitudes in the EDC were greater than those in the FDS. The post-hoc test for the factor of the imagery and movie revealed that the MEP amplitudes in the  $I^+M^+$  conditions were significantly higher than those in the  $I^-M^+$  conditions ( $p = 0.015$ ) and higher than those in the  $I^-M^-$  conditions ( $p = 0.012$ ; **Figure 3**). No interaction was found for the vibration \* imagery and movie, vibration \* muscle activity, and imagery and movie \* muscle activity.



**Figure 3.** Results of Motor Evoked Potential (MEP) peak-to-peak Amplitude. The mean of the peak-to-peak amplitude of MEPs across participants for EDC (red line) and FDS (blue line) with and without vibratory stimulation (solid line and dotted line, respectively) is represented in the conditions Imagery and movie ( $I^+M^+$ ), Movie ( $I^-M^+$ ), and No imagery and movie ( $I^-M^-$ ). Each error bar indicates the standard deviation of the mean. Abbreviations: MEP, motor evoked potential; EDC, extensor digitorum communis; FDS, flexor digitorum superficialis.

In terms For the scores of the Vib AVR sensation and Vib TVR sensation, a two-way RM ANOVA showed a significant main effect of the imagery and movie (F (2, 20) = 7.79,  $p = 0.003$ ,  $\eta_p^2 = 0.44$ , observed power = 0.92), but not the sensation (*i.e.*, AVR and TVR) on the scores. The Bonferroni post-hoc test indicated that the scores in the I<sup>+</sup>M<sup>+</sup> conditions were higher than those in the I<sup>-</sup>M<sup>+</sup> conditions only ( $p = 0.001$ ).

## 4. Discussion

### 4.1. Effect of Vibratory Stimulation

The findings showed that the peak-to-peak amplitudes of MEPs increased in the conditions with vibratory stimulation compared to the conditions without vibratory stimulation, irrespective of the conditions of imagery or action observation only, and EDC or FDS. These findings are consistent with the findings from previous studies [11] [65] [66] [67] which demonstrated that muscle tendon vibration augmented MEP amplitudes. By contrast, other studies have reported that the increase of MEP amplitudes after vibratory stimulation occurred only in the vibrated antagonistic muscle [13] or only in the vibrated muscle [10]. There are several plausible mechanisms by which vibratory stimulation could augment MEP amplitudes. First, it has been suggested that the modulation of cortico-spinal excitability was induced by proprioceptive inputs generated by the vibratory stimulation. The vibration-induced Ia afferents innervating to muscle spindles caused an activation of the central nervous system, resulting in increased corticomotor excitability. The underlying mechanism may involve a decrease of motor threshold, an increase of intracortical facilitation or decrease of intracortical inhibition of the vibrated muscle [16]. Second, the long loop reflexes displayed as an automatic motor response to somatosensory stimulation might influence the excitability of the motor cortex. The long loop reflexes have suggested that proprioceptive inputs generated by vibratory stimulation can induce descending corticospinal motor response mediated by the primary motor cortex [17] [18] [19] [20]. Third, muscle vibration can elicit TVR, defined as an involuntary contraction of the vibrated muscle, and AVR, referring to the contraction of muscles antagonist to the vibrated muscles, presumably induced by top-down cortical modulation [5] [9]. Therefore, these findings suggest that vibratory stimulation might augment the excitability in the corticomotor and corticospinal circuits mediated by the primary motor cortex.

### 4.2. Effect of Imagery and Action Observation

The remarkable feature of our findings is that the peak-to-peak amplitudes of MEPs were significantly increased in the I<sup>+</sup>M<sup>+</sup> conditions compared to the I<sup>-</sup>M<sup>+</sup> and I<sup>-</sup>M<sup>-</sup> conditions, regardless of the vibratory stimulation and muscle activity conditions. These findings contrast with our hypothesis, which expected step-wise changes between the three conditions, with the highest value to be observed in the I<sup>+</sup>M<sup>+</sup> conditions and the lowest value in the I<sup>-</sup>M<sup>-</sup> conditions. Our assump-

tions were based on previous studies suggesting that imagining voluntary movement can facilitate the excitability of the contralateral primary motor cortex, by conveying signals to the spinal cord level via descending pathway, thus resulting in increased MEP amplitudes due to a modulation of corticospinal excitability [28] [68] [69] [70]. In addition, motor imagery involves multimodal sensation including visual and spatial perspectives, which could have an impact on the central processing [53] [54] [55]. For the influence of action observation on the corticomotor excitability, it was assumed that the visuomotor neurons are activated when observing or perceiving someone else's action, facilitating corticomotor excitability of the observer through the mirror neuron system [47]-[52]. Additionally, using a visuomotor reaction time task, another study suggested that visual input can elicit the excitability of cortico-cortical connections from the visual cortex to the primary motor cortex, resulting in the modulation of corticospinal excitability [71]. Thus, we expected that motor imagery in addition to action observation would increase more corticomotor excitability compared to action observation alone, and there would be stepwise changes between the three conditions as described above. However, our results instead only showed a significant influence of motor imagery and action observation on the MEP amplitudes. A plausible explanation for these apparently discrepant results could be a lack of corresponding hand movement between the movie and the actual participant's hand or a lack of feeling that the hand on the movie was as the participant's own hand, which might have caused insufficient induction of the mirror neuron system. In general, the mirror neurons are activated when viewing someone else performing the same or similar acts or when interacting with the observer's hand and an object [49] [51]. The task used in the current study was not identical to the hand movement of the movie, which might have resulted in an insufficient induction of corticomotor excitability. Nevertheless, the findings of the current study revealed a significant impact of motor imagery during the action observation on corticomotor excitability. Moreover, based on the participants' subjective measures, feelings about their palms being opened and closed during the vibratory stimulation appeared more important in the V<sup>+</sup>I<sup>+</sup>M<sup>+</sup> condition compared to the V<sup>+</sup>I<sup>-</sup>M<sup>+</sup> condition. These findings emphasize a substantial influence of performing motor imagery while viewing the hand movement of the movie compared to action observation only on the corticomotor excitability. Furthermore, despite the fact that the results of the current study did not clearly show that the V<sup>+</sup>I<sup>+</sup>M<sup>+</sup> condition had a significantly different influence on MEP amplitudes compared to other conditions, the effect of motor imagery and action observation combined with vibratory stimulation might have a greater impact on the augmentation of corticomotor excitability in order to enhance motor performance.

### 4.3. Effect of Agonist and Antagonist Muscles

When comparing MEP amplitudes between EDC and FDS, MEP amplitudes in EDC were found to be greater than those in FDS regardless of the vibration con-

ditions and imagery or action observation conditions. Previous studies have provided inconsistent results. For instance, Suzuki *et al.*, [65] compared how different proprioceptive and visual inputs affect the corticomotor excitability of the wrist dorsi- and palmar-flexors. They demonstrated that the MEP amplitudes of the vibrated wrist dorsiflexors, contrary to the wrist palmarflexors, increased when viewing a video clip compared to viewing a static screen of the video clip, which was proposed to reflect the occurrence of TVR. Likewise, other studies reported identical effects of vibration where the MEP amplitudes increased only in the vibrated muscles [11] [12] [72] [73] [74]. Opposed findings were previously shown by Forner-Cordero *et al.*, [13] who examined whether muscle tendon vibration to wrist palmarflexors induced MEP amplitudes of the wrist dorsiflexor and palmarflexor muscles. They showed that the MEP amplitude of wrist dorsiflexors representing the vibrated antagonistic muscles significantly increased compared to the vibrated wrist palmarflexors. This phenomenon was considered as an AVR which could be caused by a top-down modulation from the primary motor cortex [9] [75]. Overall, the findings on the activation of the agonist and antagonistic muscles from previous studies have remained controversial and seemingly dependent on methodology, including the particular stimuli or tasks used. For the results of the current study, they appeared comparable to the phenomenon of AVR. However, it may be difficult to provide a rational explanation and interpretation for our findings for the MEP amplitudes in the agonist and antagonistic muscles. Thus, a more rigorous methodology is needed to identify the mechanisms underlying the relationship between agonist and antagonist muscles.

In terms of the results of subjective feelings for the Vib AVR and Vib TVR scores, this questionnaire was performed as supplemental analysis since a previous study suggested that vibratory stimulation might induce AVR and TVR [9] and there might be the identical results to the MEP amplitudes in the EDC and FDS (*i.e.*, The vibratory stimulation was applied to the palm (*i.e.*, finger flexors) in this study so the TVR and AVR were anticipated to affect the FDS and EDC, respectively). The results of the current study indicated that the scores in the I<sup>+</sup>M<sup>+</sup> conditions were higher than those in the I<sup>-</sup>M<sup>+</sup> conditions, regardless of the EDC or FDS. These results did not show similarity between the MEP amplitudes in the EDC and FDS and the subjective scores in the Vib AVR and Vib TVR scores, respectively. The potential causation of difference between them is that the previous study [9] examined the sEMG signals during vibratory stimulation, not MEP amplitudes. Also, the previous study utilized kinesthetic illusion during vibratory stimulation which was not our aim in this study. The different methodology might cause different results.

The clinical implication from the findings of this study is that motor imagery and action observation may be utilized with vibratory stimulation to facilitate hand function as a therapeutic approach especially in individuals suffering from central nervous system disorders. Our findings together reveal that performing

motor imagery of a hand movement while viewing a hand movement in a movie during the vibration had a substantial effect on the corticomotor excitability of finger muscles. In individuals with severe hand paresis or unable to actively perform finger movements, due to neurological diseases such as poststroke, brain injury, or cervical spinal cord injury, using a combination of motor imagery and action observation with vibratory stimulation could help augment corticomotor excitability, which could ultimately contribute to an enhancement of impaired hand function in neurorehabilitation [76].

#### **4.4. Limitations**

The sample size was small since some data were excluded from the analysis due to noise. Also, the TMS mapping system allowing to identify the hot spot and center of gravity of the EDC and FDS was not used in this study. In addition, the optimal time for providing the stimulations of vibratory stimulation, motor imagery, and action observation in order to maximize corticomotor excitability in the same conditions was not determined. In addition, this study was not designed to examine the  $2 \times 2$  full design of  $I^+M^+$ ,  $I^+M^-$ ,  $I^-M^+$ , and  $I^-M^-$  conditions so the interaction between the  $I^+$  and  $M^+$  conditions could not be evaluated. Therefore, future studies need to be performed to address these issues with the current methodology.

#### **4.5. Conclusion**

The findings of the current study emphasize that performing motor imagery while viewing a movie of finger flexion and extension movements is more effective at inducing corticomotor excitability compared to action observation alone. In addition, vibratory stimulation significantly increased corticospinal circuit excitability probably via the primary motor cortex. Taken together, these findings suggest that the effect of motor imagery and action observation combined with vibratory stimulation may have a greater impact on the augmentation of corticomotor excitability. Therefore, this approach could be utilized to modulate corticomotor excitability which may contribute to the improvement of finger motor function.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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