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Immediate effects of visual–motor illusion on resting-state functional connectivity

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ABSTRACT

Visual-motor illusion (VMI) is to evoke a kinesthetic sensation by viewing images of oneself performing physical exercise while the body is at rest. Previous studies demonstrated that VMI activates the motor association brain areas; however, it is unclear whether VMI immediately alters the resting-state functional connectivity (RSFC). This study is aimed to verify whether the VMI induction changed the RSFC using functional near-infrared spectroscopy (fNIRS). The right hands of 13 healthy adults underwent illusion and observation conditions for 20 min each. Before and after each condition, RSFC was measured using fNIRS. After each condition, degree of kinesthetic illusion and a sense of body ownership measured using the Likert scale. Our results indicated that, compared with the observation condition, the degree of kinesthetic illusion and the sense of body ownership were significantly higher after the illusion condition. Compared with the observation condition, RSFC has become a biomarker that shows changes in brain function occurring due to VMI. VMI may be applied to the treatment of patients with stroke or orthopedic diseases.

1. Introduction

Using visual and vibration stimulations, kinesthetic illusion can be evoked. Ramachandran et al. (1996) and Giraux et al. (2003) reported kinesthetic illusion that evoked kinesthetic sensations when viewing an image of one's upper body movements. Recently, Shibata and Kaneko (2019) reported that visual stimuli presented in self-movement images could evoke kinesthetic illusion. Visual-motor illusion (VMI), as described in these studies, can be used to evoke kinesthetic illusion when viewing images of oneself moving. Furthermore, VMI evokes a sense of body ownership when viewing images of one's physical exercise (Kaneko et al., 2015).

Multiple brain activities have been associated with VMI, including

the increased excitability of the primary motor area (M1) (Dilina et al., 2019; Kaneko et al., 2007). Furthermore, VMI activates the premotor cortex (PMC) and the parietal area (Pa) (Kaneko et al., 2015; Sakai et al., 2020). From a clinical perspective, improved motor function is observed when a VMI of finger movement is presented to patients with paralyzed upper limbs from chronic stroke hemiplegia (Kaneko et al., 2016). We previously reported that ankle dorsiflexion function and the walking speed of a stroke hemiplegic patient with ankle dorsiflexion dysfunction were improved by showing the patient a VMI of their ankle joint movement (Sakai et al., 2018). These studies demonstrated that kinesthetic illusion lasting from 5 to 15 min could immediately improve the motor functions of patients with stroke hemiplegia. These motor function improvements are speculated to be associated with increased

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Abbreviations: VMI, Visual-motor illusion; RSFC, Resting-state functional connectivity; fNIRS, Functional near-infrared spectroscopy.

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activities in motor association areas of the brain (Kaneko et al., 2007; Kaneko et al., 2015; Sakai et al., 2020). Thus, previous studies of VMI have reported a change in brain activity in healthy subjects and improved motor function in patients with stroke hemiplegia. However, it is unclear which neural network is involved. Based on these previous results, we hypothesized that the modulation of a neural network would occur in both motor-associated areas and in other brain areas associated with a sense of body ownership. Thus, the neural activities of multiple brain areas may function as a specific neural network during VMI; such neural networks could be tied with the improvement of motor function. Neural networks will be an important biomarker that determines brain function changes due to VMI for patients with stroke and orthopedic diseases. Furthermore, VMI is may be used to treatment for patients with stroke or orthopedic diseases in a clinical trial. Here, we aimed to investigate whether VMI immediately modulated the neural network related to VMI and a sense of body ownership.

For this purpose, we examined the resting-state functional connectivity (RSFC) using functional near-infrared spectroscopy (fNIRS). Many studies exploited RSFC, which is considered to reflect the functional network of the spontaneously active brain at rest using various imaging techniques of the human brain (Biswal et al., 1995). Recently, in response to various interventions (i.e., cognitive tasks and visual stimulation), RSFC changes have been reported. RSFC appears to reflect the preparatory stage of human behaviors and cognitive processes; thus, RSFC changes could result in changes in actual behavior (Greicius et al., 2003; Nail & Smyser, 2018; Shirer et al., 2012).

Several imaging studies (Klein et al., 2016; Niu and He, 2014; Pourmotabbed et al., 2020; Sasai et al., 2011; Zhao et al., 2016) measured RSFC using various devices such as fMRI, fNIRS electroencephalography (EEG) or magnetoencephalography (MEG). RSFC is often measured using fMRI (Nail and Smyser, 2018), which has the advantage of measuring the whole brain (i.e., basal ganglia, insula, and cerebellum). However, fMRI requires subjects to maintain a strictly static position, and it poses more risks than EEG or fNIRS. Thus, EEG and fNIRS provide a much safer experimental environment compared to fMRI (Niu and He, 2014). EEG has a high temporal resolution; however, the disadvantages of EEG include its requirement for wet electrodes and its vulnerability to motion artifacts (i. e., eye movements, blinks) (Hong and Yaqub, 2019). fNIRS is noninvasive, safe, and inexpensive; it can be used to evaluate RSFC more safely than fMRI or MEG. Moreover, fNIRS settings are easier to operate than those of EEG. However, similar to EEG, fNIRS contains motion artifacts (Naseer and Hong, 2015), and the fNIRS signal is affected by external lights (Hong et al., 2020). Nevertheless, with these points considered, fNIRS is the best approach for clarifying the effects of VMI on the RSFC because fNIRS has fewer positional limitations for the limbs and fewer contraindications compared with fMRI and MEG. Furthermore, VMI is best implemented in a sitting posture. For an optimal adaptation of VMI in patients with stroke or orthopedic diseases, fNIRS was selected as the imaging method in this study because of its highly useful and practically applicable advantages.

2. Materials and methods

2.1. Subjects

We recruited 13 healthy subjects (mean age, 22.2 ± 3.8 years; 8 females; Edinburgh Handedness Inventory score for the right hand, 83.07 ± 11.9). All subjects were right-hand dominant and had no history of neurological or orthopedic disease. Subjects had no visual impairment, and none of the participants had specific skills such as playing the piano or guitar. The study's purpose was explained to the subjects, and written informed consent was obtained from each subject in compliance with the Declaration of Helsinki. This study was conducted with the approval of the Institutional Ethics Committee of Tokyo Metropolitan University.

2.2. Methods

Subjects performed the illusion condition with VMI and then the observation condition. Each condition was performed for 20 min on the right upper limb and implemented with a washout period of 1 month. Before and after each condition, RSFC was evaluated. After each condition, we measured to what extent each condition evoked a kinesthetic illusion and the degree of sense of body ownership during each condition (Fig. 1).

2.3. Conditions

The subjects were seated in a chair with their upper limb placed on a table, maintained in the resting position. The illusion condition comprised a video of the right-hand finger performing an extension and flexion movement. We showed a video of finger extension and flexion movement and performed with a 3-s cycle on the subject's left side. The video was obtained using a tablet in advance of the movement of the left finger (iPad Pro, Apple, Cupertino, CA, USA). To allow the first-person perspective, the individual in the video was seated during filming. The recording was then transposed, rotated, and horizontally flipped, using a video reversal software, to permit subjects to view finger movement as if it was performed on the subject's right side (Fig. 2) (Sakai et al., 2018; Sakai et al., 2020). The hand being measured was positioned to overlap with the image of the finger movement shown in the video. During the observation condition, the same moving image presented as the illusion condition was used. The observation condition was performed with the subject's body positioned in front of the video such that the illusion was not easily induced, and the video was observed (Fig. 2; Shibata and Kaneko, 2019).

2.4. Assessments

The assessments performed included RSFC, the degree of kinesthetic illusion, and the degree of sense of body ownership. RSFC was analyzed by fNIRS (Shimadzu Co., Ltd, Kyoto, Japan) using continuous wave (CW type). The wavelengths of fNIRS were 780, 805, and 850 nm. fNIRS measures oxygenated hemoglobin (oxy-Hb) and deoxygenated hemoglobin (deoxy-Hb) based on the modified Beer-Lambert law (Cope and Delpy, 1988). The oxy-Hb signal is more sensitive compared to the deoxy-Hb signal and is used (Yang et al., 2019). The probe position was set to 40 channels in total using 25 probes (13 sources, 12 detectors) of 5 \times 5 (Fig. 3). The probe distance was 30 mm, and the sampling rate was 24 Hz. For the full head holder, the positions of probes were determined to cover the top from the front using the 10–20 system, centered on the central zone. RSFC was obtained by measuring oxy-Hb values for 10 min. The subjects sat in a comfortable chair and their heads secured to avoid shaking. We instructed the subjects to examine a point of regard without thinking. To obtain stable data, the subjects rested before the RSFC measurement at least 2 min.

A seven-point Likert scale was used to evaluate the degrees of



Fig. 1. Protocol and measurement timing. Resting-state was measured before and after each condition. Task period was performed under illusion and observation conditions. After each condition, kinesthetic illusion and sense of body ownership were measured using a seven-point Likert scale.



Fig. 2. Set up of each condition. The subjects were seated in a chair with their upper limb placed on the table and maintained in a resting position. The illusion condition involved observing an image of their own finger movement. The side being measured was positioned such that the hand overlapped with the image of the finger movement on the video. Under the observation condition, the hand was positioned in front of the image, which made it more difficult for the illusion to be induced while the image was observed.



Fig. 3. Region of interest using functional near-infrared spectroscopy. The probe position was set to 40 channels in total, using 25 probes (13 sources, 12 detectors) of 5×5 . The probe distance is 3 cm. As a result of NIRS-SPM using a 3D digitizer, the regions of interest were the dorsolateral prefrontal cortex (DLPFC, channels 1–4), frontal eye field (FEF, channels 5–9), premotor cortex (PMC, channels 10–22), primary motor area (M1, channels 23–27), somatosensory area (Sa, channels 28–31), and parietal area (Pa, channels 32–40).

kinesthetic illusion and sense of body ownership. The degree of kinesthetic illusion was evaluated using the phrase "I feel my hand is moving," with the seven-point Likert scale arranged as follows: -3, "I completely disagree; 0, "I neither agree nor disagree"; and +3, "I very much agree" (Shibata and Kaneko, 2019). The degree of sense of body ownership was evaluated using the statement "The hand of the image feels like a part of my body," with responses on the seven-point Likert scale indicating -3, "I completely disagree"; 0, "I neither agree nor disagree"; and +3, "I very much agree" (Arizono et al., 2016).

2.5. Analyses

The Oxy-Hb data removed data that contained low signal-to-noise ratio at several channels due to failures in source and detector placement (Niu et al., 2012). However, the Oxy-Hb data that include visually clear motion artifacts was nothing. RSFCs were obtained by applying an oxy-Hb value to the 0.009–0.08 Hz band-pass filter (Hu et al., 2020; Niu et al., 2012). The Oxy-Hb data of all channels were averaged in 10 min. Then, the Pearson correlation coefficient r value of all channel pairs was calculated. The r values were converted into Z scores using Fisher's r-to-z transformation. A matrix map of Z scores of the before and after conditions were illustrated using MATLAB (MATLAB R2019; The Math Works Inc., Natick, MA, USA). In addition, changed in RSFCs were illustrated all channels distribution map.

All channels were referenced to 10–20 system landmarks (nasion, inion, right and left preauricular points) and recorded using a 3D digitizer (3 SPACE®, FASTRAK®, Polhemus Co., Ltd., Colchester, VT, USA) to determine which brain regions corresponded to the positions of each channel. All channels then converted these coordinates into the locations of 40 channels based on an estimated Montreal neurological institute space (MNI) using NIRS-statistical parametric mapping (NIRS-SPM) (Tsuzuki et al., 2007; 2014). NIRS-SPM transforms the functional image to MNI space using probabilistic registration in reference to 3D digitized data of all channels and landmark positions using the 10–20 system (Tsuzuki and Dan, 2014; Yamazaki et al., 2020). The results demonstrated that the regions of interest (ROI) were the dorsolateral prefrontal cortex (DLPFC, channels 1–4), frontal eye field (FEF, channels 5–9), PMC (channels 10–22), M1 (channels 23–27), somatosensory area (Sa, channels 28–31), and Pa (channels 32–40).

2.6. Statistical analyses

For RSFC, a paired *t*-test was used to compare differences between before and after each condition for ROI. Moreover, multiple tests were controlled using the false-discovery rate (FDR, p < 0.05) (Benjamini and Hochberg, 1995).

Moreover, we used a Wilcoxon signed-rank test to evaluate the degrees of kinesthetic illusion and sense of body ownership with a significance level of $\rm p < 0.05$.

Furthermore, we analyzed relationships between RSFCs that changed significantly before and after each condition and differed between the conditions and degrees of kinesthetic illusion and sense of body ownership using Spearman's correlation coefficient with a significance level of p < 0.05.

3. Results

Based on the seven-point Likert scale, the degree of kinesthetic illusion was + 1 (+1 to + 1) under the illusion condition, which was significantly higher than the value of - 1 (-2 to - 1) under the observation condition (p = 0.008; Fig. 4). The degree of sense of body ownership was + 1 (+1 to + 2) under the illusion condition, which was significantly higher than the value of - 2 (-2 to - 1) under the observation condition (p = 0.027; Fig. 4).

Fig. 5 shows a heat map demonstrating the RSFCs for each condition. After the illusion condition, the RSFC increased between the left DLPFC and the left PMC (channels 1–15), between the right PMC and the right Pa (channels 21–35), between the left and right M1 and right Sa (channels 25–31) compared with before the illusion condition (p < 0.05, FDR corrected; Table 1, Fig. 6A). After the observation condition, the RSFCs between the left M1 and right Pa significantly increased compared with before the observation condition (p < 0.05, FDR corrected; Table 1, Fig. 6B).

Compared with after the observation condition, RSFCs after the illusion condition significantly increased between the left PMC and left Pa (channels 16–33) and between different conditions of the left Pa



Fig. 4. Degrees of kinesthetic illusion and sense of body ownership. A seven-point Likert scale was used to evaluate the kinesthetic illusion and sense of body ownership. The subjects felt a stronger illusion during the illusion condition compared with the observation condition (p = 0.008) and felt a stronger sense of body ownership during the illusion condition compared with the observation condition (p = 0.027).



Fig. 5. Heat map of each condition. A matrix map of Z scores was the resting-state functional connectivity before and after each condition. The matrix map was all channels pairs. Color bars indicate Z scores. X and y axes represent the regions of interest were the dorsolateral prefrontal cortex (DLPFC, channels 1–4), frontal eye field (FEF, channels 5–9), premotor cortex (PMC, channels 10–22), primary motor area (M1, channels 23–27), somatosensory area (Sa, channels 28–31), and parietal area (Pa, channels 32–40).

(channels 33–37; p < 0.05, FDR corrected; Table 2, Fig. 6A). Compared with after the illusion condition, RSFCs after the observation condition were not significantly increased (p > 0.05, FDR corrected; Fig. 6B).

A significant correlation was reported between the degree of kinesthetic illusion and RSFC between the left PMC and the left Pa (channels 16–33) after the illusion condition (r = 0.586, p = 0.032; Fig. 7). No Table 1

Results of resting-state functional connectivity before and after conditions.

Condition	Channels	Region	Before	After	P value
Illusion condition Illusion condition Illusion condition Observation condition	Ch1-15 Ch21-35 Ch25-31 Ch23-40	Lt DLPFC - Lt PMC Rt PMC - Rt Pa Lt Rt M1 - Rt Sa Lt M1 - Rt Pa	$\begin{array}{c} -0.162 \pm \\ 0.060 \\ -0.210 \pm \\ 0.075 \\ -0.170 \pm \\ 0.068 \\ -0.231 \pm \\ 0.037 \end{array}$	$\begin{array}{c} -0.117 \pm \\ 0.036 \\ -0.138 \pm \\ 0.059 \\ -0.093 \pm \\ 0.125 \\ -0.159 \pm \\ 0.062 \end{array}$	p < 0.05 p < 0.05 p < 0.05 p < 0.05

Ch: Channles, Left: Lt, Right: Rt, Dorsolateral prefrontal cortex: DLPFC, Premotor cortex: PMC, Primary motor area: M1, Somatosensory area: Sa, Parietal area: Pa, P values: False discovery rate corrected.

significant correlation was reported between the degrees of kinesthetic illusion and other RSFCs or between the sense of body ownership and any RSFCs (p > 0.05).

4. Discussion

This study confirmed that VMI immediately altered RSFCs measured by fNIRS. The illusion condition evoked higher degrees of kinesthetic illusion and a sense of body ownership compared to the observation condition. After the illusion condition, the RSFC increased between the left DLPFC and the left PMC, between the right PMC and the right Pa, and between the left and right M1 and right Sa compared with before the illusion condition. Compared with after the observation condition, RSFCs after the illusion condition significantly increased between the left PMC and left Pa and between conditions in the left Pa. Furthermore, a significant correlation was reported between the degree of kinesthetic illusion and RSFC between the left PMC and left Pa after the illusion condition.

4.1. Sense of body ownership regarding VMI

During the illusion condition, the kinesthetic illusion was induced by viewing an image of a finger movement designed to overlay the actual upper limb, which was maintained throughout the task. Consequently, compared with the observation condition, the illusion condition induced a stronger sense of body ownership. During the illusion condition, the more robust sense of body ownership is possibly associated with the spatial match between the viewed finger movement-image and the actual upper limb position. Previous rubber hand illusion studies provided important implications for our research in that a sense of body ownership is felt when the somatosensory input is consistent with visual stimulation (Botvinick and Cohen, 1998; Tsakiris, 2010). During the rubber hand illusion, the actual and rubber hands must be spatially positioned close to each other. Recently, a sense of body ownership was reported to occur, even without somatosensory input, if the actual hand and virtual body are spatially close to each other (Slater et al., 2009; Tieri et al., 2015). Thus, our results suggest that visuospatial compatibility between the observed virtual hand and observer's actual hand may trigger the perception of a kinesthetic illusion (i.e., the sense of body ownership). Note that placing the virtual finger movements just

Table 2

Results of resting-state	functional	connectivity	after	conditions.
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Channels	Region	Illusion condition	Observation condition	P value
Ch16-33	Lt PMC - Lt Pa	-0.159 ± 0.082	-0.241 ± 0.075	p < 0.05
Ch33-37	Lt Pa - Lt Pa	-0.028 ± 0.104	-0.118 ± 0.079	p < 0.05

Ch: Channels, Left: Lt, Right: Rt, Dorsolateral prefrontal cortex: DLPFC, Premotor cortex: PMC, Primary motor area: M1, Somatosensory area: Sa, Parietal area: Pa, P values: False discovery rate corrected.



Fig. 7. Correlation analysis between resting-state functional connectivity changes and the degree of kinesthetic illusion. A significant correlation was reported between the degree of kinesthetic illusion and the resting-state func-

tional connectivity between the left premotor (PMC) and the left parietal area

(Pa, channel 16–33), after the illusion condition (r = 0.586, p = 0.032).

Fig. 6. Increased resting-state functional connectivity in the illusion or observation condition. The blue lines indicate the increased resting-state functional connectivity before and after the illusion condition (A) or the observation condition (B). The red line indicates the increased resting-state connectivity after the illusion condition compared with after the observation condition. P < 0.05 (controlled false discovery rate). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Illusion condition

Observation condition

over the actual hand may enable observers to feel their finger movements, unlike horizontally placing the fingers as during the observation condition.

4.2. Resting-state functional connectivity during VMI

Previous studies demonstrated that VMI activated the M1, PMC, and Pa (Kaneko et al., 2007; Kaneko et al., 2015; Sakai et al., 2020), which agrees with our results of increased RSFCs between the M1, PMC, and Pa, as well as other brain regions after the illusion condition compared with before the illusion condition. Moreover, the functional connection between the DLPFC and PMC is activated during motor execution (Kim et al., 2018). Previous studies reported that VMI increased excitability in the M1 (Dilina et al., 2019; Kaneko et al., 2007). Furthermore, DLPFC is associated with motor control (Miller and Cohen, 2001). Previous studies suggested that VMI increases the RSFC of brain regions associated with motor execution, which may underlie functional motor improvements.

The RSFC after the illusion condition among frontal-parietal network brain regions increased compared with those after the observation condition. Naito et al. (2016) reported that the frontal-parietal network, which connects the PMC, Sa, and Pa, includes the brain regions involved in the illusion. In this study, a significant correlation was observed between the degree of kinesthetic illusion and RSFC between the left PMC and left Pa after the illusion condition. As per the frontal-parietal network suggested by Naito et al. (2016), after the illusion condition, the strength of the connection between the left PMC and left Pa correlated with the degree of kinesthetic illusion.

Furthermore, brain areas related to a sense of body ownership was reported the frontal-parietal network (Ehrsson et al., 2004; Ehrsson et al., 2005; Gentile et al., 2011; Kanayama et al., 2009; Zeller et al., 2011). Moreover, functional connectivity of a sense of body ownership was reported between the left DLPFC and the left PMC (Arizono et al., 2016). The PMC and Pa were reported crucial in multisensory integration and area of convergence of visual and proprioceptive (Ehrsson et al., 2004; Ehrsson et al., 2005; Kanayama et al., 2009; Zeller et al., 2011). Moreover, the frontal cortex are involved in the sense of body ownership and self-location (Esslen et al., 2008; Lenggenhager et al., 2011). In particular, the frontal cortex plays a role in convergence of multisensory information and control of action (Miller and Cohen, 2001). In our study, the RSFC increased between the left DLPFC and the left PMC, between the right PMC and the right Pa, between the left PMC and left Pa, and between the left Pa. Our results was in agreement with previous studies reporting on networks related to a sense of body ownership (Arizono et al., 2016; Ehrsson et al., 2004; Ehrsson et al., 2005; Esslen et al., 2008; Gentile et al., 2011, Lenggenhager et al., 2011; Kanayama et al., 2009; Zeller et al., 2011). Based on these, our results suggest that RSFC reflects change in the sense of body ownership by the VMI.

The illusion condition in this study method evoked a sense of body ownership. It increased the strength of the RSFC related to a sense of body ownership, which suggests that the inducement of VMI may apply to the treatment of patients with stroke or orthopedic disease. Furthermore, the RSFC suggest become a biomarker that shows changes in brain function occurring due to VMI.

4.3. Limitations

The primary limitations of our study are small sample size and that we did not measure brain activity during the conditions. Furthermore, not all ROIs could be measured because of the small number of fNIRS probes.

5. Conclusion

This study examined whether VMI immediately alters RSFC using fNIRS. We reported that VMI induces a sense of body ownership and

alters the RSFCs related to motor execution and a sense of body ownership. These important results suggest that VMI may be applied to the treatment of patients with stroke or orthopedic diseases. Furthermore, the RSFC suggest become a biomarker that shows changes in brain function occurring due to VMI.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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