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# A mirror reflection of a hand modulates stimulus-induced 20-Hz activity

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ABSTRACT

Mirror therapy is one of the promising rehabilitation therapeutic interventions but the neural basis of the therapeutic effect remains unknown. It has been reported that the 20-Hz rhythmic activity is induced in the primary motor cortex after median nerve stimulation and the amount of the induced activity is decreased when the primary motor cortex is activated. In the present study, to elucidate the neural mechanisms underlying mirror therapy, we investigated whether the mirror reflection of a hand holding a pencil modulates the stimulus-induced 20-Hz activity. Neuromagnetic brain activities were recorded from 11 healthy right-handed subjects while they were viewing their hand holding a pencil or its mirror reflection. The right median nerve was stimulated and the stimulus-induced 20-Hz activity over the left rolandic cortex dominantly innervating right-hand movements was quantified. The stimulus-induced 20-Hz activity was strongly suppressed when subjects viewed the right hand holding a pencil or the mirror reflection of the left hand looking like the right hand holding a pencil, compared with when subjects viewed the left hand holding a pencil or the mirror reflection of the right hand looking like the left hand holding a pencil. These results suggest that the human left primary motor cortex is strongly activated when the subjects view not only the right hand holding a pencil but also the mirror reflection of the left hand looking like the right hand holding a pencil. This may be one of the neural mechanisms responsible for the therapeutic effect of mirror therapy.

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## Introduction

The neuroscience-based rehabilitation is a potent method to improve therapeutic outcome (Woldag and Hummelsheim, 2002; Sharma et al., 2006). Mirror therapy was first introduced to treat "phantom limb" pain (Ramachandran and Rogers-Ramachandran, 1996), in which amputees could feel to move the phantom limb while watching a mirror reflection of the intact hand movement and experienced pain relief after the treatment. The mirror therapy was also applied to rehabilitation of hemiparesis after stroke (Altschuler et al., 1999; Sathian et al., 2000; Stevens and Stoykov, 2003; Yavuzer et al., 2008), in which patients performed movements of the unimpaired limb while watching its mirror reflection superimposed on the position of the impaired limb and showed a significant recovery of the paretic arm movement. These reports indicate that mirror therapy is one of the promising rehabilitation therapeutic interventions; however, the neural basis of the therapeutic effect remains unknown.

Magnetoencephalographic (MEG) studies have demonstrated that the 20-Hz rhythmic activity is induced in the primary motor cortex after median nerve (MN) stimulation and modulated by various types of movements including actual movement, motor imagery and action observation. It has been shown that the stimulus-induced 20-Hz activity is abolished when subjects execute actual hand movements (Salmelin and Hari, 1994), significantly suppressed when subjects imagine themselves performing the movements (Schnitzler et al., 1997) and also when subjects observe another person performing the similar hand movements (Hari et al., 1998). These authors have reported that the suppression of the stimulus-induced 20-Hz activity indicates the activation of the primary motor cortex (Salmelin and Hari, 1994; Schnitzler et al., 1997; Hari et al., 1998): The strong activation of the primary motor cortex induces the strong suppression of the stimulus-induced 20-Hz activity. Several studies have used it as an indicator of the functional state of the motor cortex (Järveläinen et al., 2004; Ichikawa et al., 2007).

In the present study, to make a beginning for elucidating the neural mechanisms underlying mirror therapy, we examined whether the mirror reflection of a hand modulates the stimulus-induced 20-Hz activity. Because about 90% of humans are right-handed and left-hemisphere dominant for manual skills (Volkmann et al., 1998), we

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focused the 20-Hz activity induced in the left hemisphere after right MN stimulation.

## Materials and methods

### *Subjects*

The experiments were carried out on 11 healthy right-handed subjects (six females, five males; age range, 19-31, mean=25). The study was performed in conformity with the Declaration of Helsinki, and approved by the Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine. All subjects gave informed written consent prior to participation.

### *Experimental paradigm*

The subject was seated comfortably in a magnetically shielded room, with both hands placed in the mirror box in front of the subject (Fig. 1). The mirror box was constructed by attaching a 25 cm by 30 cm mirror inside at an angle of 15–20° lateral to the sagittal plane. The position of the mirror box and the angle of the mirror were carefully adjusted so that the left or right hand looks like the right or left hand, respectively. The right MN was stimulated over the wrist to produce the stimulus-induced 20-Hz activity. The stimuli were 0.3 ms constant-current pulses once every 1.5 s with stimulus intensities below the motor threshold to avoid a twitch of the thumb holding a pencil (2.4–4.7 mA, mean=3.5 mA, 80% of the motor threshold in each subject).

The experiment consisted of a Rest condition and four experimental conditions (Fig. 2):

Rest: The subject rested relaxed without holding anything and looked at a point in the front wall (about 3 m away from the subject).

Right hand: The subject viewed the right hand holding a pencil through a transparent plastic (25 cm×30 cm).

Reflected-Right hand: The subject viewed the right hand holding a pencil reflected in a mirror as the left hand holding a pencil.

Left hand: The subject viewed the left hand holding a pencil through a transparent plastic.

Reflected-Left hand: The subject viewed the left hand holding a pencil reflected in a mirror as the right hand holding a pencil.

In the experimental conditions, the subject held a pencil very softly not to produce any distinct muscle activity by holding a pencil because the muscle activity that is different from that during Rest condition would modulate the stimulus-induced 20-Hz activity in the primary motor cortex (Schnitzler et al., 1997).

Cortical magnetic signals during Rest condition were first recorded to identify a sensor pair showing the strongest reactivity in each subject. Then, cortical magnetic signals were recorded during the four experimental conditions. The order of the four experimental conditions was balanced across subjects. Each condition lasted about 3 min with short intervening pauses and was performed three times.

### *Recording*

Cortical magnetic signals were recorded with a 306-channel whole-head neuromagnetometer (Vectorview; Elekta Neuromag, Finland), which contains 204 planar gradiometers and 102 magnetometers. In this study, the data recorded from 204 planar gradiometers were used for analyses because they provide an optimal signal-to-noise ratio for superficial cortical current sources such as the pericentral mu-rhythm generators (Simões et al., 2004). The recording passband was 0.03–330 Hz and the signals were digitized at 1003 Hz and stored for off-line analysis.

Surface electromyograms (EMGs) were recorded to check the relaxation of subject's hand muscles. Pairs of cup electrodes were placed over the extensor digitorum and flexor digitorum superficialis muscles of both hands. Interelectrode distance was approximately 3 cm. The EMGs were continuously monitored during MEG measurement, and the subject was announced to relax the hands when any different muscle activity from that during Rest condition was observed on the EMGs. Vertical electrooculogram and the markers indicating the delivery of the stimuli were also recorded.

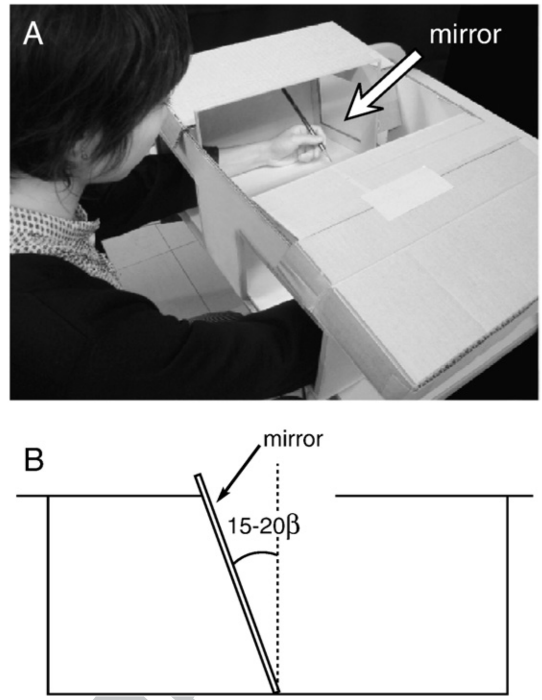


**Fig. 1.** The mirror box. (A) The subject placed both hands in the box and viewed one hand holding a pencil through an opening in the top of the box. The unnecessary visual input of hands was prevented by covering them with sliding boards on the top of the box. In this figure, the subject views the mirror reflection of her right hand looking like her left hand holding a pencil. When the subject views her left hand directly, a transparent plastic is placed instead of the mirror. (B) A cross-section showing the interior of the mirror box.

### *Data analysis*

The data analysis was in the same principle as described in our previous study (Ichikawa et al., 2007). MEG epochs from 0.1 s before the onset of stimulus to 1.4 s after the onset of stimulus were collected. Each epoch was inspected visually, and all epochs coinciding with significant EMGs, blinks or eye movements were excluded from the data analysis. The temporal spectral evolution (TSE) method (Salmelin and Hari, 1994; Nagamine et al., 1996) was employed to calculate the average levels of 20-Hz activity as a function of time with respect to MN stimuli. The continuous MEG signals were bandpass-filtered through 18–23 Hz, and then rectified and averaged with respect to the onset of stimulus and smoothed with a 15-Hz low-pass filter. Then the values of root-mean-square of the TSE signals from the gradiometer pair measuring two orthogonal derivatives of the magnetic field at the location were calculated to express the 20-Hz activity levels as TSE curves. Because the MEG signals from planar gradiometers are strongest when the sensors are located just above cortical current sources, the data from the sensor pair showing the strongest TSE response were used to evaluate the stimulus-induced 20-Hz activity levels (Salenius et al., 1997; Schnitzler et al., 1997; Tamura et al., 2005; Ichikawa et al., 2007). The values of root-mean-square of the TSE levels from the two orthogonal gradiometers denoted as pair were also used to express the mean TSE levels in a time window from 0.2 to 0.7 s after stimulation and the mean values were compared among the conditions with a two-way repeated measures ANOVA using "holding"

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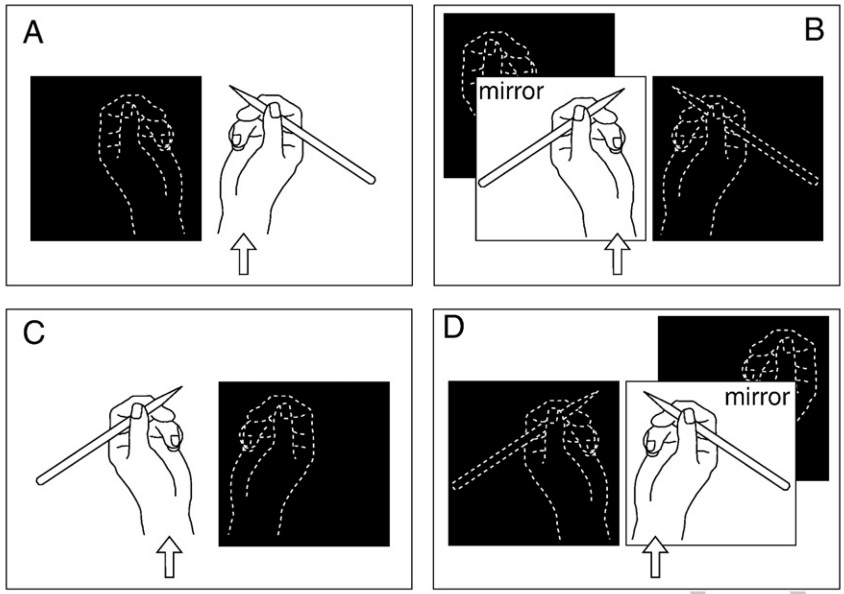


**Fig. 2.** Schematic illustration of the experimental conditions. (A) Right hand: The subject views the right hand holding a pencil through a transparent plastic. (B) Reflected-Right hand: The subject views the mirror reflection of the right hand looking like the left hand holding a pencil. (C) Left hand: The subject views the left hand holding a pencil through a transparent plastic. (D) Reflected-Left hand: The subject views the mirror reflection of the left hand looking like the right hand holding a pencil. The hands covered with sliding boards are shown by white dashed lines on black backgrounds. The arrow in each condition indicates the hand that the subject views.

(Which hand holds a pencil?) and "viewing" (Which hand looks like holding a pencil?) as factors. Significance was set at $p<0.05$.

## Results

Because of an absence of reactive 20-Hz rhythms, two subjects were discarded from the analysis. The present results, therefore, are based on nine subjects (six females, three males; age range, 19–31, mean = 25). The bursts of 20-Hz rhythmic activity were induced after the MN stimulation in nine subjects (Fig. 3A). The 20-Hz activity was quantified by the TSE method. About 100–150 epochs in each condition were averaged with respect to the onset of stimulus. TSE curves showed the distribution of the enhancement of 20-Hz activity after right MN stimulation. The most prominent increase in the stimulus-induced 20-Hz activity was observed over the left rolandic cortex corresponding to the hand area in the primary motor cortex (encircled in Fig. 3B). The 20-Hz activity showed a slight suppression immediately after MN stimulation and then the activity started to increase about 0.2–0.3 s after MN stimulation. The rebound peaked around 0.5 s after MN stimulation and slowly decayed until the next

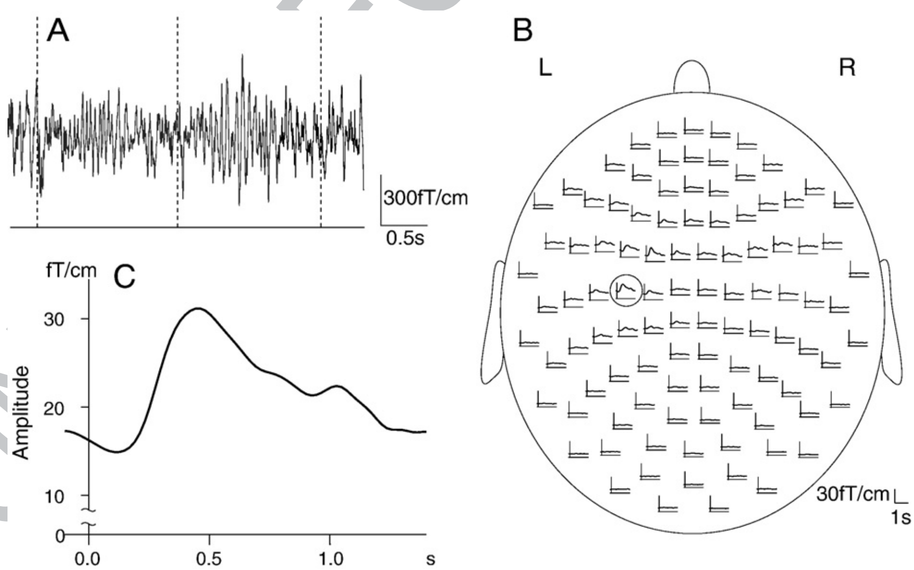


**Fig. 3.** Representative MEG activities during Rest condition. (A) Signals were recorded from a channel over the left rolandic cortex and were bandpass-filtered through 1–100 Hz. Vertical dashed lines indicate right MN stimuli delivered once every 1.5 s. Note that about 20-Hz rhythmic activities are prominently induced after the stimuli. (B) TSE curves showing the distribution of the 20-Hz activity induced after right MN stimulation. The curves show the values of root-mean-square of the TSE signals from the gradiometer pair measuring two orthogonal derivatives of the magnetic field at the location from 0.1 s before the onset of the stimuli to 1.4 s after the stimuli. Vertical lines indicate the onset of the right MN stimuli. The head is viewed from the top. Note that increases in the stimulus-induced 20-Hz activity levels are predominantly observed over the left rolandic cortex. (C) The TSE curve obtained from the most reactive gradiometer pair (encircled in B). Ordinates, 20-Hz activity levels (fT/cm); abscissas, time before and after the onset of right MN stimulation. Note that the TSE curve shows a prominent rebound of 20-Hz activity after a slight suppression period.

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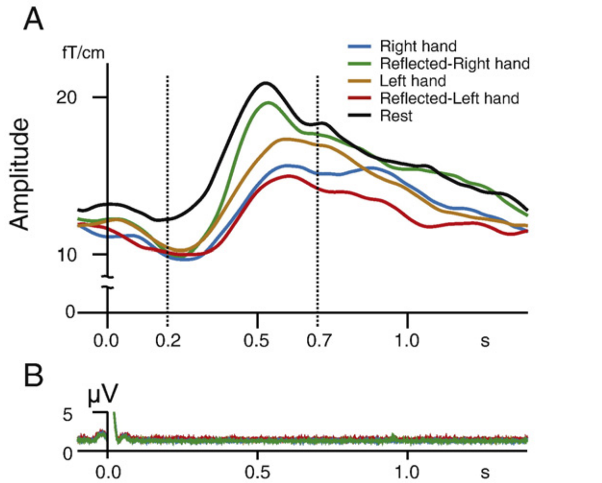


**Fig. 4.** Representative effects of different experimental conditions on the 20-Hz activity quantified with TSE method and EMG. (A) The TSE curves were obtained from the most reactive gradiometer pair. Blue, green, orange, red and black lines indicate TSE curves obtained during Right hand condition, Reflected-Right hand condition, Left hand condition, Reflected-Left hand condition, and Rest condition, respectively. Ordinates, 20-Hz activity levels (fT/cm); abscissas, time before and after the onset of right MN stimulation. (B) Rectified and averaged EMG of the right extensor digitorum muscle recorded simultaneously with the cortical magnetic signals.

stimuli (Fig. 3C). The distribution and time course of the stimulus-induced 20-Hz activity during Rest condition were substantially consistent with the previous observations (Salmelin et al., 1995; Schnitzler et al., 1997; Ichikawa et al., 2007).

Reduction of the stimulus-induced 20-Hz activity was observed during the four experimental conditions (Fig. 4A). When the subject held a pencil in his right hand, the rebound of 20-Hz activity was strongly suppressed during the Right hand condition (blue curve) in which the subject viewed his right hand holding a pencil through a transparent plastic whereas it was slightly suppressed during the Reflected-Right hand condition (green curve) in which the subject viewed the mirror reflection of the right hand looking like his left hand holding a pencil. When the subject held a pencil in his left hand, the rebound of 20-Hz activity was strongly suppressed during the Reflected-Left hand condition (red curve) in which the subject viewed the mirror reflection of his left hand looking like his right hand holding a pencil whereas it was slightly suppressed during the Left hand condition (orange curve) in which the subject viewed his left hand holding a pencil through a transparent plastic. EMG activities during the four experimental conditions were marginal (activities of the right finger extensors were shown in Fig. 4B) and the levels of activities were not different from those observed during Rest condition.

The mean values of the nine subject's TSE levels in a time window from 0.2 to 0.7 s after MN stimulation were 15.2±6.8 fT/cm (±SD) in Right hand condition, 15.4±6.2 fT/cm in Reflected-Right hand condition, 16.0±6.8 fT/cm in Left hand condition, 14.6±5.7 fT/cm in Reflected-Left hand condition, and 17.4±7.1 fT/cm in Rest condition, respectively. The mean values of TSE levels were analyzed on "viewing" and "holding" factors. A substantial difference in TSE levels was shown on the "viewing" factor (Fig. 5A) whereas it was not shown on the "holding" factor (Fig. 5B). The statistical analyses using a two-way repeated measures ANOVA revealed a significant main effect on the "viewing" factor (Which hand looks like holding a pencil?) ($F_{1,8}=7.176$, $p=0.028$), but did not show a significant main effect on the "holding" factor (Which hand holds a pencil?) ($F_{1,8}=0.005$, $p=0.946$). Additionally, there was no significant interaction between "viewing" and "holding" factors ($F_{1,8}=1.532$, $p=0.251$), indicating that the difference on the "viewing" factor was not affected by the "holding" factor. These results mean that irrespective of holding a pencil in the left hand or in the right hand, the stimulus-induced 20-Hz activity was strongly suppressed in the left hemisphere when the subjects viewed the hand as their right hand holding a pencil, compared with when the subjects viewed the hand as their left hand holding a pencil.

## Discussion

We demonstrated that the stimulus-induced 20-Hz activity was strongly suppressed in the left hemisphere when the subjects viewed their right hand holding a pencil or the mirror reflection of the left hand looking like their right hand holding a pencil, irrespective of holding a pencil in the left hand or in the right hand. Järveläinen et al. (2004) have shown that the suppression of the stimulus-induced 20-Hz activity varies even when the subject observes two similar movements. The stimulus-induced 20-Hz activity was strongly suppressed when the subject observed another person placing small objects with chopsticks from one dish to another whereas it was

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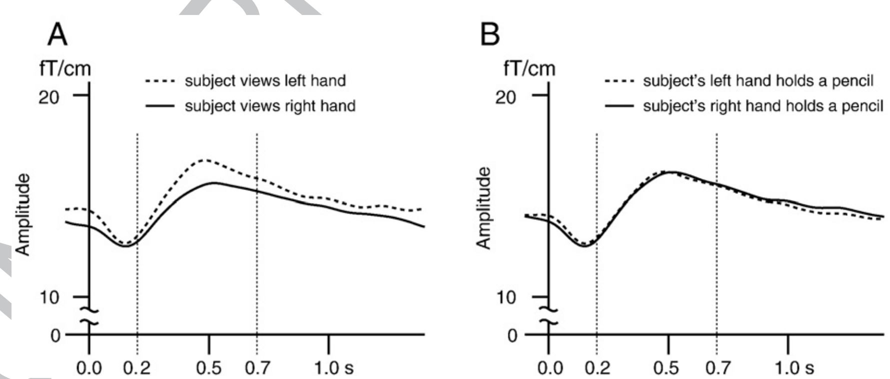


**Fig. 5.** Grand average TSE curves of the stimulus-induced 20-Hz activity across all subjects on "viewing" and "holding" factors. The TSE curves were obtained from the most reactive gradiometer pair. (A) "viewing" factor (Which hand looks like holding a pencil?). A dashed line shows the average of TSE curves that were obtained during the Left hand condition in which the subjects viewed their left hand holding a pencil and during the Reflected-Right hand condition in which the subjects viewed the mirror reflection of their right hand looking like their left hand holding a pencil. A solid line shows the average of TSE curves that were obtained during the Right hand condition in which the subjects viewed their right hand holding a pencil and during the Reflected-Left hand condition in which the subjects viewed the mirror reflection of their left hand looking like their right hand holding a pencil. (B) "holding" factor (Which hand holds a pencil?). A dashed line shows the average of TSE curves that were obtained during the Left hand condition in which the subjects viewed their left hand holding a pencil and during the Reflected-Left hand condition in which the subjects viewed the mirror reflection of their left hand looking like their right hand holding a pencil. A solid line shows the average of TSE curves that were obtained during the Right hand condition in which the subjects viewed their right hand holding a pencil and during the Reflected-Right hand condition in which the subjects viewed the mirror reflection of their right hand looking like their left hand holding a pencil. Ordinates, 20-Hz activity levels (fT/cm); abscissas, time before and after the onset of right MN stimulation.

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weakly suppressed when the subject observed another person doing similar movements without touching or moving the objects. This suggests that the stimulus-induced 20-Hz activity is strongly suppressed during observation of meaningful movements. Moreover, Ichikawa et al. (2007) have also shown that the stimulus-induced 20-Hz activity is modulated by the way to present a hand. The stimulus-induced 20-Hz activity was strongly suppressed when the subject observed another person's hand movements presented in the same direction as the subject's hand whereas it was weakly suppressed when the subject observed the similar hand movements presented in the opposite direction to the subject's hand. This may imply that the stimulus-induced 20-Hz activity is strongly suppressed when the movements could be easily taken in relation to the subject's own movements. In the present study, visual input of the right hand holding a pencil or of the mirror reflection of the left hand looking like the right hand holding a pencil would be meaningful and be closely related to the subject's own movements in the left hemisphere dominantly innervating right-hand movements. This may be one of the reasons why the stimulus-induced 20-Hz activity was strongly suppressed in the left hemisphere when the subjects viewed their right hand holding a pencil or the mirror reflection of the left hand looking like their right hand holding a pencil, compared with when the subjects viewed their left hand holding a pencil or the mirror reflection of the right hand looking like their left hand holding a pencil.

The suppression of the stimulus-induced 20-Hz activity has been reported to indicate activation of the primary motor cortex in early MEG studies (Salmelin and Hari, 1994; Schnitzler et al., 1997; Hari et al., 1998): The stimulus-induced 20-Hz activity is completely suppressed during execution of actual movements and partially suppressed during motor imagery or observation of movements. In the present study, the stimulus-induced 20-Hz activity was strongly suppressed when the subjects viewed their right hand holding a pencil or the mirror reflection of the left hand looking like their right hand holding a pencil, compared with when the subjects viewed their left hand holding a pencil or the mirror reflection of the right hand looking like their left hand holding a pencil. This result suggests that not only visual input of the right hand holding a pencil but also visual input of the mirror reflection of the left hand looking like the right hand holding a pencil strongly activates the left primary motor cortex. This effect must be beneficial in rehabilitation therapy. For example, the exercises in training of the impaired right hand while the subject is viewing movements of the unimpaired left hand reflected in a mirror as the right hand moving could strongly activate the patient's left dominant hemisphere innervating the impaired right hand. This may be one of the neural mechanisms responsible for the therapeutic effect of mirror therapy.

The effect of somatosensory input (“holding”) was weaker than that of the visual input (“viewing”). In the present study, the somatosensory input did not contain the proprioceptive input induced by hand movements. The effect of visual input may increase when other inputs (motor and somatosensory) are weak. The mirror therapy would be of great use for neurological patients with motor and somatosensory deficits.

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