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 modulates the stimulus-induced 20-Hz activity. Neuroimaging studies 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 was measured. The 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. 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 Hummelshaim, 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; Vauzer 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.

Magnetoecephalographic (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 (Jarveläinen 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 (Volkman et al., 1998), we...
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 experi-
mental 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 right 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 condi-
tions 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 magnet-
ometers. In this study, the data recorded from 204 planar gradi-
ometers 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 measure-
ment, 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.

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 (Sal-
melin 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 (Salmelin 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”
(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. 2. Schematic illustration of the experimental conditions.](image-url)

(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.

![Fig. 3. Representative MEG activities during Rest condition.](image-url)

(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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the rebound of 20-Hz activity was strongly suppressed during the hand holding a pencil. When the subject held a pencil in his left hand, 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 two-way repeated measures ANOVA revealed a significant main effect on the “viewing” factor (Which hand looks like holding a pencil?) and “holding” factor. 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?) and “holding” factor (Which hand holds a pencil?) and no significant interaction between “viewing” and “holding” factors. Additionally, there was no significant interaction between “viewing hand” and “holding hand” factors, 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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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 bene

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