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Increased corticospinal excitability during direct observation of self-movement and indirect observation with a mirror box

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Abstract

To explore the effect of mirror box therapy based on the mirror neuron (MN) system of the primary motor cortex (M1), we examined if direct (without a mirror) and indirect (with a mirror) observation of self-movement in healthy subjects induced changes in motor evoked potential (MEP) evoked by transcranial magnetic stimulation (TMS). MEPs were elicited from the first dorsal interosseous (FDI) and the flexor carpi radialis (FCR) muscles. Somatosensory evoked potentials (SEPs) during self-movement observation were also recorded. Both observations of self-movement with and without a mirror increased MEP amplitude. In addition, increase in MEP amplitude was specific to the prime mover muscle involved in the observed movement. The SEPs increased similar to the MEPs during both observations of self-movement with and without a mirror. We conclude that although the MN system can be activated by observing self-movement in a manner similar to that achieved by observing movement of another person, there were no detectable effect on corticospinal excitability that were specific to movements observed with a mirror.

Keywords: Mirror neuron system; Mirror box; Corticospinal excitability; TMS; SEP

The mirror neuron (MN) system is one of the most interesting and provocative findings in the field of neuroscience in the last decade. In monkeys, the MN resides in the left F3 area of monkey's premotor cortex. This area is activated by both, when a monkey observes a particular movement performed by another person and when a monkey imitates a same movement. This F3 area corresponds to area 44 in the left inferior frontal gyrus, i.e., Broca's area, in the human brain [15,21]. Some magnetoencephalography (MEG) studies have reported that the primary motor area (M1) in the left hemisphere is subsequently activated after activation of Broca's area during both movement observation and imitation in human [18,19]. These studies suggest that M1 activates together with Broca's area as the MN system. Thus, transcranial magnetic stimulation (TMS)-induced motor evoked potential (MEP), which reflects changes of M1 excitability, is a useful parameter for assessing activation of the MN system. Some TMS studies have reported that MEP was actually increased during movement observation [6,7,14,22,27,28].

On the other hand, mirror box therapy can reduce phantom arm pain of an amputated arm [20], and also improves arm movements by hemiplegic patients debilitated by strokes [1,25]. The effect of this unique therapy has been proposed to result from both the illusion of kinesthetic sensation and the visual illusion caused by a mirror partition [10,20]. As quoted from a report by Altschler et al. [1], a hemiplegic patient described mirror box therapy as follows: "it looks like my bad arm is moving normally." In this therapy, subjects perform 'mirror symmetric' movements and watch the action of their normal arm reflected in a vertical standing mirror. This action is perceived as the action of the amputated or spastic arm. Although there is a difference between the subject's own arm movement observation in mirror box therapy and another person's movement observation, MN system may be activated in mirror box therapy.

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In the present study, therefore, to explore the possible contribution of the MN system to mirror box therapy, we recorded MEPs from the right first dorsal interosseous (FDI) and the flexor carpi radialis (FCR) muscles during observing left finger or wrist movements without (direct observation) or with a mirror (indirect observation). In addition, to investigate the effects of peripheral afferents inputs, we also examined the change of somatosensory evoked potentials (SEPs) associated with movement observation with or without a mirror.

MEP recordings were carried out on 12 healthy subjects (aged from 19 to 40 years). SEP recordings were carried out on 10 healthy subjects (aged from 22 to 37 years). All their dominant hands were right, judged by interview asking the dominant hand for writing, throwing and using the chopsticks. Subjects gave informed consent and the experiments were performed in accordance with the Declaration of Helsinki. Subjects sat in a relaxed position on a reclining chair, and a custom-built mirror box was placed on a horizontal plate attached to the armrests of a reclining chair in front of subject as shown in Fig. 1A and B. Subjects placed both hands on this mirror box and the position of the right hand was guided by a bar. A vertical standing mirror was set on the mirror box and partially slanted to the left side. The position of the mirror box was carefully adjusted so that the subjects perceived their left arm as their right arm position.

A magnetic stimulator (M200, Magstim Co. Ltd., UK) and a figure-of-eight coil (outside diameter of each loop was 9.5 cm) were used to deliver the electromagnetic stimuli. The coil was placed tangentially to the scalp with the handle pointing backward and rotated approximately 30° away from the mid-sagittal line. The coil was held above a suitable spot of left M1 to simultaneously evoke MEPs in both FDI and FCR muscles in the resting condition and great care was taken to maintain the position of the coil relative to the scalp. MEPs were amplified with a band pass of 5 Hz–3 kHz. All amplification procedures were controlled by a signal processor (7S12, NEC San-ei Co. Ltd., Japan). Analog outputs from the signal processor were digitized at a sampling rate of 4 kHz and saved in a computer for off-line analysis (MacLab system, AD Instruments Pty. Ltd., Australia). This system automatically adopts the appropriate sampling rate depending on the sweep time. Stimulus intensity was adjusted to approximately 20% greater than the stimulus required to evoke a MEP with amplitude of 50 μV from FDI muscle.

In the control condition, MEPs were recorded while subjects gazed at a small round mark (1 cm diameter) on a mirror box

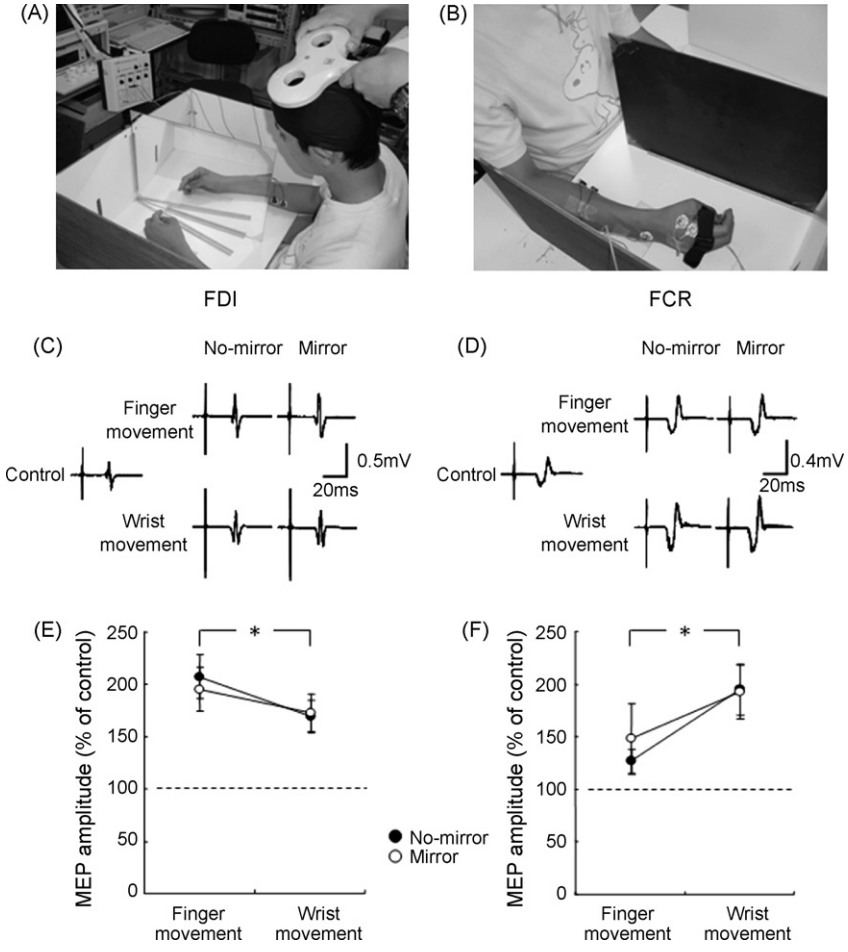

Fig. 1. (A) and (B) show the both sides of view in mirror box experiment, respectively. (C) and (D) show the superimposed wave forms (three trials) obtained from a single subject for FDI and FCR in each condition, respectively. (E) and (F), the means and standard errors obtained from the pooled data for FDI and FCR muscles in each movement condition ($n = 12$), respectively. The horizontal dotted lines indicate the control level in the FDI and FCR muscle, respectively. Asterisks indicate statistical significance between each condition, (*)$p < 0.05$.

approximately 0.5 m in front of them. These MEP amplitudes in FDI and FCR muscles were adopted as the references in the subsequent experimental conditions. In the experimental conditions, subjects moved their left hand in one of two movements, repeated extension and flexion of index finger (finger movement), or repeated extension and flexion of wrist (wrist movement). These movements were performed while either directly watching the left hand movement (no-mirror) or indirectly watching the left hand movement with a mirror (mirror) as a right hand movement. In the 'no-mirror condition', the mirror was removed from the mirror box. Subjects performed all movements in step with the rhythmic sounds of a metronome set at 60 strokes/min.

Twenty MEPs were recorded and averaged in each condition and peak-to-peak amplitude was measured. During the movement task, TMS was delivered at interval of 5.1 or 6.1 s to avoid synchronization with the movement rhythm dictated by the metronome. This procedure provided observation for the entire movement rather than solely the movement phase. Throughout the experiments, subjects were instructed not to move their right hands and to maintain mental and physical relaxations. Background EMG activities of FDI and FCR muscles were carefully monitored in each MEP recording. If any background EMG activities were detected, the associated MEPs were omitted from the analysis. Subjects were also instructed to concentrate on observing their left hand movement in both 'no-mirror' and 'mirror' conditions.

For SEP recordings, a bar-type paired electrode was employed to stimulate the right median nerve at the wrist. Rectangular electrical pulses, 0.3 ms in duration, were applied at 2 Hz with an isolator. The intensity of these pulses was adjusted to 50% greater than the motor threshold for right thenar muscle and this intensity stimulates both spindle and cutaneous afferents. Small M-waves were monitored to ensure the stimulus consistency. SEPs were recorded with a pair of cup electrodes placed over the scalp at Cpc (middle position between C3 and P3) according to the 10–20 system and referenced to the linked earlobes with a ground electrode on the arm. EEG recordings were amplified with a band pass of 0.5 Hz–1.5 kHz. Procedure of amplification and recording conditions for M-wave were identical to the MEP recordings as described above. Five hundred stimuli were delivered and the averaged waveforms of both SEP and M-wave were recorded, In addition, the peak-to-peak amplitude of the N20 component was measured for each condition. SEPs were recorded in three conditions. In the control condition, subjects gazed at a small round mark (1 cm diameter) on a mirror box approximately 0.5 m in front of them. In the 'no-mirror' condition, subjects directly observed handgrip movements of their left hand, i.e., repetitive palm open and grip. In the 'mirror' condition, subjects observed these same movements with a mirror. Subjects performed these repetitive handgrip movements in step to the rhythm of a metronome set at 60 strokes/min.

Regarding MEP recordings, at first MEP amplitude during each 'movement (finger or wrist) condition' and 'mirror (no-mirror or mirror) condition' were all normalized to control levels in each muscle. Thus, all results of MEPs were expressed as ratios of the control MEP amplitudes for each subject, then grand mean ratio with standard error from pooled data were calculated. Using two-way ('movement' and 'mirror' conditions) ANOVAs with repeated measures on both factors, these MEP data were analyzed, respectively in each muscle. Differences between the control level and the MEP amplitude in each condition were analyzed by paired *t*-tests. Regarding SEP recordings, the N20 amplitudes were normalized by each individual control level in each condition, a paired-*t* test was then performed in order to investigate the difference in the normalized N20 between 'no-mirror' and 'mirror' conditions. Probability less than 5% was recognized as statistically significant.

Fig. 1C and D provide the superimposed MEPs (three trials) obtained from the FDI and FCR of a single subject for each condition, respectively. Amplitudes of these MEPs were the almost same to the mean amplitude in each condition of this subject. Fig. 1E and F present the means and standard errors obtained from the pooled data ($n = 12$) for MEPs of FDI and FCR, respectively. The horizontal dotted line indicates the control level, and the values are the ratios of MEP amplitude normalized by the control level. In both muscles, ANOVAs showed significant main effects for the 'movement condition' (FDI: $F_{1,22} = 6.82$, $p = 0.016$; FCR: $F_{1,22} = 6.522$, $p = 0.018$), but did not show significant main effects for the 'mirror condition' (FDI: $F_{1,22} = 0.021$, $p = 0.886$; FCR: $F_{1,22} = 0.118$, $p = 0.735$). Additionally, there was no significant interaction between 'movement condition' and 'mirror condition' in both muscles (FDI: $F_{1,22} = 0.290$, $p = 0.596$; FCR: $F_{1,22} = 0.291$, $p = 0.595$). This indicated that the difference in the 'movement condition' was not affected by the 'mirror condition'. In other words, irrespective of direct observation of the left hand movement or observation of it through the mirror, MEPs in the right hand were selectively increased when the homonymous muscle in the left hand was activated as a prime mover. MEP amplitudes in each condition were significantly increased from the control level (FDI: finger movement, $t = -6.249$, $p < 0.0001$, wrist movement, $t = -5.506$, $p < 0.0001$; FCR: finger movement, $t = -2.092$, $p = 0.048$, wrist movement, $t = -5.436$, $p < 0.0001$).

Fig. 2A presents averaged waveforms of both SEP and M-wave for each condition obtained from a single subject. The N20 component is indicated by the shaded gray box. Amplitudes of M-waves in the thenar muscle were indistinguishable in these three conditions, indicating consistent stimulus intensity provided for the three conditions. Fig. 2B provides the means and standard errors obtained from the pooled data ($n = 10$) for N20 amplitude. The horizontal dotted line indicates the control level, and the values are the ratios of SEP amplitude normalized by the control level. Compared to the control level, N20 amplitudes were significantly larger than the control level in each condition (no-mirror: $t = -2.618$, $p = 0.028$; mirror: $t = -2.574$, $p = 0.030$), and there was no significant difference in the facilitation of SEPs between no-mirror and mirror conditions ($t = 0.921$, $p = 0.382$). Therefore, this indicated no significant facilitatory effect on somatosensory inputs of observation of the left hand movement through the mirror.

The findings in the present study are as follows: (1) MEP amplitudes in both FDI and FCR muscles were increased in the 'movement condition', (2) a marked increase in MEP amplitude was shown in the specific muscle in the right hand that was

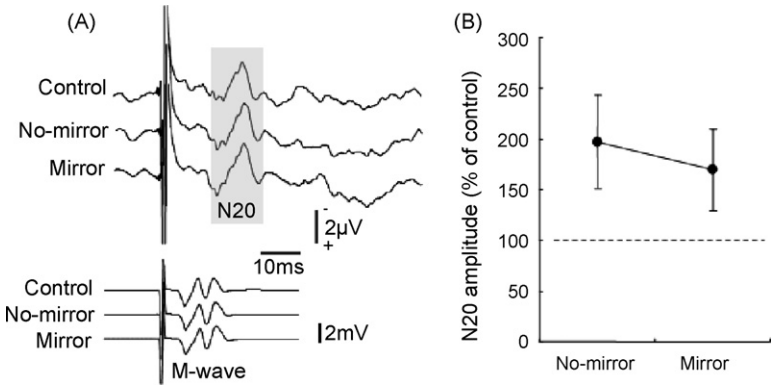

Fig. 2. (A) The upper set of traces are a representative example of averaged SEP waveforms in 'control', 'no-mirror' and 'mirror' conditions. The N20 components are indicated by gray area shading. The lower set of traces is a representative example of averaged M-waves from the thenar muscle in these conditions. These records were obtained from a single subject. (B) Mean and standard errors obtained from pooled data of the N20 amplitude ($n = 10$) in 'no-mirror' and 'mirror' conditions. The horizontal dotted line indicates the control level. Asterisks indicate statistical significance between each condition, (*)$p < 0.05$.

mainly activated as a prime mover in the observed movement by left hand, (3) there was no significant difference between direct (no-mirror) and indirect (mirror) observations on MEP amplitudes, (4) The amplitude of the N20 component of the SEPs were increased during movement observation and (5) similar to the MEPs, there was no significant difference between direct (no-mirror) and indirect (mirror) observations on SEP amplitudes.

Some previous studies have demonstrated that corticospinal excitability of the resting hand muscle is facilitated by contralateral homonymous muscle's isometric contraction more than 50% [17] or 37.5% [26] of maximum voluntary contraction. In the present study, subject performed the phasic finger or wrist movements without load. Under these conditions, contraction levels of the FDI or FCR were relatively low. Additionally, the timing of TMS was not synchronized with specific phases of muscle contractions. Thus, the MEP facilitation we observed is unlikely to be caused by voluntary muscle contraction accompanying contralateral finger or wrist phasic movement.

In preceding studies, MEP amplitude was increased during observation of another person's movement and this increase was specific to the muscle involved in the observed movement [7,27,28]. These studies attributed these results to MN system. In the present study, MEP amplitude was increased during observation of self-movement regardless of whether the observation was direct or indirect. The increase in MEP amplitude was specific to the muscle involved in the observed movement, consistent with the previous reports. Recently, Fourkas et al. [8] have reported that the MEP from FDI muscle was increased during motor imagery and suggested that the MN system could be activated for matching imagined actions with an inner visuomotor template. One hypothesis is that motor imagery of self-movement activates the MN system in a manner similar to observation of movement by others. In an fMRI study [3], activity was observed in Broca's area during motor imagery of self-movement of the finger. These reports provide a suitable explanation for the movement-dependent increase in MEP amplitude during observation of a subject's own hand movement in the present study.

The present results with SEP recordings support this hypothesis. The amplitude of the N20 component of SEP significantly increased during observations of self-movement in both the 'no-mirror' and 'mirror' conditions (Fig. 2). In addition to M1, primary somatosensory area contributes to the MN system [2,4,16]. MEG of primary somatosensory cortex during median nerve stimulation has revealed greater activity during observation of another's hand movement. Thus, the human cortical somatosensory network is closely connected to the MN system and may be providing information that is necessary for preserving the sense of self during action observation. Our SEP recordings demonstrate a significant increase in amplitude during observation of self-hand movement. This result supports the idea that the MN system can be activated.

However, the effect of the mirror box was not confirmed in the present study. There was no difference in MEP amplitude between the 'no-mirror' and 'mirror' conditions. In addition, the amplitude of the N20 component of SEPs did not differ between the 'no-mirror' and 'mirror' conditions. Previous studies have demonstrated sensorimotor reorganization occurred after upper limb amputation, especially in traumatic amputees with phantom limb pain [5,9,11–13,23,24]. These studies provide a theoretical base for mirror box therapy for amputees to treat their phantom limb pain in amputees. Indeed, mirror box therapy drastically reduces phantom limb pain in the traumatic amputees and improves movement of the spastic side of hemiplegic patients [1,20]. In the present study, we studied healthy subjects. Some subjects reported that they experienced unusual sensation such as movement of their unmoved right hand behind the mirror. But, SEP did not significantly change in the mirror box conditions. We conclude that the somatosensory afferents that contributed to the kinesthetic sensations were not enhanced under our mirror box condition and that mirror box therapy lacks the potential to increase M1 excitability in healthy subjects. However, the MN system was activated during self-movement observation in both "mirror" and "no-mirror" conditions in a manner similar to that associated with observation of another person's movement.

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