Electroencephalogram evidence for the activation of human mirror neuron system during the observation of intransitive shadow and line drawing actions

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Abstract
Previous studies have demonstrated that hand shadows may activate the motor cortex associated with the mirror neuron system in human brain. However, there is no evidence of activity of the human mirror neuron system during the observation of intransitive movements by shadows and line drawings of hands. This study examined the suppression of electroencephalography mu waves (8–13 Hz) induced by observation of stimuli in 18 healthy students. Three stimuli were used: real hand actions, hand shadow actions and actions made by line drawings of hands. The results showed significant desynchronization of the mu rhythm (“mu suppression”) across the sensorimotor cortex (recorded at C3, Cz and C4), the frontal cortex (recorded at F3, Fz and F4) and the central and right posterior parietal cortex (recorded at Pz and P4) under all three conditions. Our experimental findings suggest that the observation of “impoverished hand actions”, such as intransitive movements of shadows and line drawings of hands, is able to activate widespread cortical areas related to the putative human mirror neuron system.

Key Words
neural regeneration; clinical practice; mirror neuron system; action understanding; direct matching hypothesis; mu suppression; event-related desynchronization; mu rhythm; electroencephalogram; impoverished hand actions; grants-supported paper; photographs-containing paper; neuroregeneration

Research Highlights
(1) We investigate for the first time mirror neuron system activity using electroencephalography and intransitive actions by line drawings and shadows of hands as stimuli.
(2) Electroencephalography findings reveal that intransitive movements by sketched fingers activate brain areas where human mirror neurons reside.
(3) There is no significant difference in the degree of mu rhythm desynchronization induced by the three conditions: real hand actions, hand shadow actions and line drawing actions.
(4) The stimulus color, texture and fill do not affect the extent of mirror neuron activation by an action.

Abbreviations
MNS, mirror neuron system; EEG, electroencephalogram
INTRODUCTION

The essential mechanism of the human mirror neuron system (MNS) is to transfer specific sensory information into a motor format to establish a direct match between action perception and the execution of the same action[1-2]. By directly mapping observed actions of others to the observer’s inner representation of the same action, the MNS is thought to facilitate the observer’s understanding of the goals and intentions behind the action[3-4]. A recent transcranial magnetic stimulation study[5] reported an increase in resonant motor activity during observation of a shadow animation representing abduction or adduction of the right index finger, compared with observation of a white background. However, electroencephalography (EEG) evidence of human MNS activity during the observation of intransitive shadow movements is still scarce. Furthermore, no evidence exists of human MNS activity during the observation of movements made by a line drawing of a hand. This EEG study on whether the observation of shadow and line drawing motion induces MNS activity is therefore timely and newsworthy. We used EEG recording to study the MNS response to impoverished stimuli consisting of movements of shadows and line drawings of hands.

EEG has been suggested to be a promising, cost-efficient and non-invasive means of indirectly examining MNS activity in humans. In particular, the relative suppression of the mu rhythm (8–13 Hz), measured with surface electrodes over the sensorimotor cortex (C3, Cz and C4), can be used as an index of mirror neuron activity[6]. Like the “alpha block”, mu wave suppression is considered to reflect an event-related EEG desynchronization caused by an increase in neural activity[7-8]. The mu rhythm is suppressed equally when subjects observe an action as when they perform it; therefore, mu suppression is thought to reflect activity of the human MNS[9]. This approach of measuring desynchronization of mu waves has been employed previously in a great number of studies[10-14].

The question of what can serve as a visual prerequisite for activation of the MNS is currently controversial. It is widely accepted that activation of the human MNS not only occurs in response to biological actions[15-16], but also in response to actions likely to evoke associations to biological actions[17]. According to the Direct Matching Hypothesis[18-19], human MNS activation is not constrained by the sensory properties of the input, but by the observer’s motor repertoire. Consistent with this, there is evidence from functional MRI, EEG and transcranial magnetic stimulation studies that the human MNS is activated by biological actions conveyed by point-light[20-21] and shadow display[5]. In addition, a recent EEG study[22] showed that human MNS activity, estimated by EEG mu suppression, could be activated by static, ambiguous stimuli such as the Rorschach test cards. The authors proposed that a strong internal representation of a “feeling of movement” may be sufficient to trigger the MNS. However, another recent functional MRI study[23] found that the implied motions of static line drawings of human bodies in highly unstable postures, such as bodies depicted in the Japanese Hokusai Manga cartoons, did not activate any mirror neuron-related areas (e.g., premotor and related areas). It is therefore not clear whether the human MNS can be triggered by impoverished action-related stimuli. Also, it is one thing to show the EEG evidence for that human MNS activity is activated by observation of shadow and line drawing action.

In this study, we assessed whether the human MNS responds to intransitive hand actions under different visual conditions: a video of a real hand movement, a shadow representation of the same action, and a line drawing of the same action. In addition to these stimuli, a baseline condition was presented that consisted of black circles moving in a similar way. We recorded EEG signals from participants during presentation of the stimuli, while they focused on counting the number of times the movement temporarily ceased. We used desynchronization of the mu rhythm as an index of MNS activity. No previous studies have demonstrated that the human MNS is activated by a stimulus as simple as an intransitive shadow movement or a line drawing depiction of hand action.

RESULTS

Quantitative analysis of subjects and behavioral results

Eighteen graduate students were selected and included in the final analysis. All subjects performed with 100% accuracy on the continuous performance task (an attention action-monitoring task) in all conditions (the shadow condition, the line drawing condition and the real hand condition). Therefore, we can infer that any differences found in mu suppression were not due to differences in monitoring of the stimuli.
**Power in the mu frequency range**

The power spectrum of the mu rhythm at scalp locations over the sensorimotor cortex (C3, Cz and C4) of one representative subject is shown in Figure 1. The mu rhythm power was maximal in the baseline condition (C3, Cz and C4), and decreased during the observation of actions performed by the real hand, the shadow hand, and the line drawing of a hand.

![Power spectrum of the mu rhythm](image)

**Mu suppression relative to baseline condition**

Mu suppression was analyzed using three-way repeated measures analysis of variance with condition (shadow, line drawing, real hand), moving object (thumb, forefinger, little finger) and electrode site (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1 and O2) as factors. A significant main effect was found for electrode site \( F(4.301, 73.113)=2.877, P < 0.05 \) with no other significant main effects or interactions. Hence, the data from the three moving objects (thumb, forefinger, little finger) were collapsed for the analysis of mu suppression. Greenhouse-Geisser correction was applied to the degrees of freedom and the corrected probability values were reported.

We further examined whether observation of intransitive hand actions had a significant effect on mu suppression. As in previous studies\[11, 21, 24\], two-tailed \( t \)-tests were performed to examine whether each condition (shadow, line drawing, real hand) suppressed the mu rhythm relative to the baseline. As shown in Figure 2, there was a significant suppression in the 8–13 Hz band at F3, Fz, F4, C3, Cz, C4 and Pz in the shadow condition, line drawing condition and real hand condition, compared with the baseline. However, there was no significant suppression in the 8–13 Hz band at P3, O1 and O2 in any of the experimental conditions. Furthermore, \( t \)-tests showed that there was no significant difference in the power in the 8–13 Hz frequency band over the occipital cortex (O1 and O2), indicating that the mu suppression observed at frontal, central and right parietal scalp electrodes was not mediated by posterior alpha activity.

![Schematics indicating the scalp locations that displayed significant 8–13 Hz suppression by the shadow, line drawing and real hand action stimuli.](image)

**DISCUSSION**

The goal of this study was to investigate whether the MNS-related response in humans, indicated by mu suppression, was elicited by observation of impoverished actions (abstract representations of intransitive hand actions, performed by a hand shadow and a line drawing of a hand) and whether this differed from the response to...
real hand actions. The results showed that there were no differences in mu activity at frontal, central and parietal electrodes for real hand, shadow or line drawing actions, and that there was significant suppression of mu activity relative to the baseline in all cases. In contrast, there was no evidence of suppression of mu activity over occipital electrodes. These results are in agreement with a recent transcranial magnetic stimulation study\[5\] that used hand shadow actions as stimuli, as well as previous EEG studies by Calmels et al\[25-26\] that showed that observation of intransitive finger actions caused widespread activity across the motor cortex related to the putative human MNS. Our results go beyond these studies, however, in demonstrating mu suppression also with line drawing stimuli.

Previous studies\[27-28\] have revealed that the visual perception of finger action activates a widespread network of brain areas, especially frontal and parietal regions. Our results showed a significant suppression of 8–13 Hz signal power at F3, Fz, F4, Pz and P4 in all the experimental conditions compared with the baseline. There was no significant difference in mu suppression at C3, Cz and C4 between line drawing and real hand actions, even though the line drawing stimulus lacked any “filler” information about the physical appearance of a human hand. These data are consistent with the Direct Matching Hypothesis\[18-19\], which holds that the human MNS can be driven by abstract representations of hand actions, and that the activation of the human MNS is not constrained by the sensory properties (size, shape, color, texture and filler) or by the sensory channels (visual or auditory), but relies on the observer's motor repertoire. This hypothesis is reinforced by our observation that mu suppression was seen in the conditions of hand shadow and line drawing actions.

The data contrast with a previous fMRI study\[29\] and a recent transcranial magnetic stimulation study\[30\] failed to find a preferential activation in mirror neuron areas during the observation of intransitive hand actions. The difference in the design of the baseline condition may account for these discrepancies. Enitcott et al\[30\] and Jonas et al\[29\] used a static hand as the baseline measure. Other studies show that the observation of a static hand or object can activate the human MNS\[31-33\], and this may make it difficult to observe additional activity in the other conditions. Here, we used a more appropriate baseline involving a circle moving in a non-biological manner. This baseline conveys motion (as do the action stimuli) but not in a manner that should activate the human MNS. Differences relative to the baseline can then be observed.

In conclusion, we found that mu rhythm suppression occurred across the sensorimotor cortex, the frontal cortex, and the central and right posterior parietal cortex during the observation of intransitive hand actions made by hand shadows, line drawings of hands, and real hands. This study further substantiates that observation of impoverished action stimuli, such as intransitive actions of hand-like shadows and line drawings, activates a widespread perceptual-motor system related to the putative human MNS.

SUBJECTS AND METHODS

Design
An observational study with randomized stimuli.

Time and setting
The experiments were performed at the Department of Computer Science and Technology, Tongji University, China, from June to July 2011.

Subjects
Eighteen healthy graduate students at Tongji University in China (two female, 16 male; average age 25 years; range 22–29 years) were recruited by signs posted on campus. All participants were right-handed, had normal or corrected-to-normal vision, and were naïve with respect to the purpose of the experiments. The experimental protocol was in accordance with the Regulations for the Administration of Medical Institutions, formulated by the State Council of China\[34\]. All participants provided written informed consent before experiments began.

Methods
Stimuli
The stimuli used in our four experiments are as follows:

1. Hand shadow actions (shadow condition). In this condition, three different actions were made by the shadow of a right hand. The actions involved the rhythmic (1 Hz) movement of the thumb, forefinger or little finger between 0° (adducted) and approximately 40° (abducted). It was easy to recognize the movement of the hand-like shadow (Figure 3A).

2. Line drawing actions (line drawing condition). In this condition, a line drawing of a right hand made the same movements as described for the shadow condition.
(3) Real hand actions (real hand condition). In this condition, the actions were performed by a real human hand (Figure 3C).

(4) Bidirectional movements of two or three black circles on a white background (baseline condition). In this condition, the movement of the circles mimicked the trajectories of the static and moving finger tips. As shown in Figure 3, the movement D-D1-D was visually equivalent to the trajectory of A-A1-A; the movement D-D2-D was visually equivalent to the trajectory of B-B2-B; and the movement D-D3-D was visually equivalent to the trajectory of C-C3-C. Importantly, these circle movements were not perceived as animate.

**Procedure**

The four different stimuli were presented in a random order across subjects. Subjects observed the stimuli on a color monitor (Lenovo, Beijing, China) at a viewing distance of 1 000 mm. The stimuli were presented continuously within an 80 × 80 mm window centered on the screen for 80 seconds (24 frames per second) with a 2-minute rest period between blocks. The stimuli subtended approximately 4.6° of visual angle against a uniform white background. There was no abrupt change in the location of the hand actions in the transition from the final frame to the first frame of the animation, so the natural flow of movements was preserved. Thus, the subjects saw the same pattern (hand, shadow of a hand or line drawing of a hand) in the first frame and the final frame in each 1-second animation.

Subjects were explicitly instructed to pay attention to the stimuli and to limit their eye and head movements. To ensure that subjects were paying continuous attention to the stimuli, they were asked to engage in a counting task. In each 80-second period of stimulus presentation, the animation would stop moving for 1–2 seconds between four and six times, and the subjects were asked to count the number of times the stimuli stopped and report it at the end of each block.

**EEG recordings**

The EEG was recorded by 11 Ag/AgCl electrodes from F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1 and O2, using the international 10-20 method of electrode placement. The signal power of the mu rhythm at three overlapping electrodes over the motor cortex is shown in Figure 1. Horizontal electrooculogram recording electrodes were positioned at the outer canthi of both eyes and vertical electrooculogram recording electrodes were placed approximately 2 cm above and below the left eye. Electrodes placed behind each ear (mastoid bones) were electronically linked and served as the reference electrode. All channels were amplified using the SynAmps (Neuroscan, Texas, USA) (bandpass: 0.05–30 Hz, sampling rate: 1 000 Hz). The impedances of all electrodes were measured and confirmed to be less than 10 kΩ both before and after testing. Once the electrodes were in place, subjects comfortably sat in an acoustically and electromagnetically shielded testing chamber.

**EEG data processing**

To reduce muscle artifacts in the EEG signal, the subjects were instructed to assume a comfortable position and to avoid movements. Ocular artifacts in the continuous EEG data for each subject were removed offline prior to analysis by the automatic application of a voltage threshold procedure provided in the Neuroscan 4.3 software. EEG oscillations in the mu frequency band (8–13 Hz) recorded over occipital cortex have been reported to be affected by states of expectancy and awareness[35] so recordings from C3, Cz and C4 may be
contaminated by this posterior activity. Therefore, as in previous EEG studies[14, 36-37], the first and last 10 seconds of data in each block were removed in all subjects, to eliminate this possible contamination. A 1-minute segment of data following the initial 10 seconds was obtained for each condition. EEG data were analyzed only if the data were sufficiently “clean”, with no subject movement or eye blink artifacts. For each clean segment, the integrated power in the 8–13 Hz range was calculated using a Fast Fourier Transform. Data were partitioned into epochs of 1 second from the start of the segment, and Fast Fourier Transform was performed on the segmented data (1,024 points). A cosine window was used to minimize artifacts resulting from data splicing.

**Computation of mu suppression**
The mu suppression was calculated as the ratio of the power in the experimental conditions (shadow action, line drawing action and real hand action) to the power in the baseline condition. As is common in this type of study[22, 38-39], a ratio was used to control for variability in the absolute mu power as a result of individual differences such as scalp thickness and electrode placement and impedance. A log transformation was applied to each ratio because of the non-normal distribution of the ratios. Thus, negative and positive log ratios are indicative of mu suppression and enhancement, respectively.

**Statistical analysis**
The data were analyzed with SPSS 17.0 software (SPSS, Chicago, IL, USA). Mu suppression was analyzed using three-way repeated measures analysis of variance with the following factors: condition (shadow, line drawing, real hand), moving object (thumb, forefinger, little finger) and electrode site (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1 and O2). A Greenhouse-Geisser correction was applied to the degrees of freedom and the corrected probability values were reported. Two-tailed t-tests were used to test whether each condition (shadow, line drawing, real hand) suppressed the mu rhythm relative to the baseline. A value of \( P < 0.05 \) was considered statistically significant.

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