Shadows in the mirror

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Mirror neurons are a class of visuo-motor neurons activated by both the execution and passive observation of object-related actions. Evidence for the existence of mirror neurons in the human brain comes in part from transcranial magnetic stimulation studies showing that observation of an action causes subliminal activation of corresponding corticospinal pathways within the motor system. During daylight and lighted conditions movement is nearly always preceded, accompanied, and followed by shadows. Shadows that are cast as someone observes a biological movement could potentially provide information for action recognition. The objective of this study was to assess the mirror system's ability to resonate with shadowed movements. Primary motor cortex excitability was evaluated here by motor-evoked potentials elicited during single-pulse transcranial magnetic stimulation and recorded from two hand muscles as participants observed a prehensile action performed in two illumination conditions: one in which the observed action was fully illuminated and one in which a moving body part was partially shadowed. It will be shown that overall modulation of the primary motor cortex excitability during action observation is significantly lower for the shadowed with respect to the fully illuminated condition. Processing shadows determines a modulation of corticospinal excitability, suggesting that the mirror system is finely tuned to that visual aspect of biological movements. *NeuroReport* 24:63–67 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Studies on monkeys have shown that mirror neurons are cells in the ventral premotor area F5 that discharge when a primate executes or sees a specific action [1,2]. Evidence for the existence of a mirror neurons system in the human brain comes in part from transcranial magnetic stimulation (TMS) studies demonstrating that there is a variation in the amplitude of TMS-induced motor-evoked potentials (MEPs) during action observation [3,4]. These data suggest that the motor system simulates under threshold an observed action in a strictly congruent manner. The muscles involved are the same ones used by the person carrying out an observed action and their activation is temporally and strictly coupled with its dynamics [5]. Similar paradigms have been applied since then to further investigate the nature of corticospinal neural activity induced by the particular intrinsic visual characteristics of an observed action. It has, for example, been demonstrated that motor facilitation contingent upon action observation strictly reflects the temporal dynamics of the kinematics of an observed action [6] and is modulated by the laterality of the observed acting effector [7] as well as by the observer-model posture [8]. But in everyday life there are also environmental factors that might contribute to the perception that a body is moving such as when it is not illuminated uniformly. Kersten *et al.* [9,10] reported, in fact, that three-dimensional aspects of a spatial layout can be perceived in a dramatically different way depending on shadow conditions. It has, moreover, been demonstrated that shadows might have differential effects on the motor system as they provide highly informative cues for action planning [11].

The issue being addressed by this work is how, as far as corticospinal excitability is concerned, the mirror system copes with stimuli which include information regarding shadows. As shadows are processed rather implicitly, they are interesting visual features that might help us to uncover which aspects of an observed action are encoded by the mirror neuron system.

TMS was, thus, applied over the primary motor cortex (M1) while participants observed a model performing a whole hand grasp involving all the fingers of one hand in two lighting conditions: one in which all aspects of the moving hand were uniformly illuminated and one in which some were shadowed. We hypothesized that if the human mirror neuron system processed information on shadows, then M1 excitability should be modulated during action observation across the two conditions.

Methods

Participants

Thirty healthy individuals (21 females and nine males: age = 24 ± 9 years) between 22 and 33 years (mean 27 years) took part in the experiment. All were right handed according to the Standard Handedness Inventory [12],

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had normal or corrected-to-normal visual acuity, and did not have any contraindication to TMS [13,14]. All the participants gave their written informed consent to participate in the study and while they were unaware of its purpose, some aspects about its aims were revealed after the experimental session was concluded. The study protocol was approved by the Ethics Committee of the University of Padova and was carried out in accordance with the principles of the 1964 Declaration of Helsinki. None of the individuals taking part in the experiment experienced discomfort or adverse effects during the experiment.

Experimental stimuli

To create the stimulus material, a model was filmed performing an action in two illumination conditions: (i) in which the model's hand was fully illuminated as she reached and grasped a mug; (ii) in which she carried out the same action but her little finger was in the shadow (Fig. 1). The model was seen naturally grasping the mug with a whole hand grasp (i.e. the opposition of the thumb with the other fingers). An animation effect was obtained by presenting a series of single frames each lasting 33 ms (resolution 720 × 576 pixels, color depth 24 bits, frame rate 30 fps) plus the first and last frames which lasted 500 and 1000 ms, respectively.

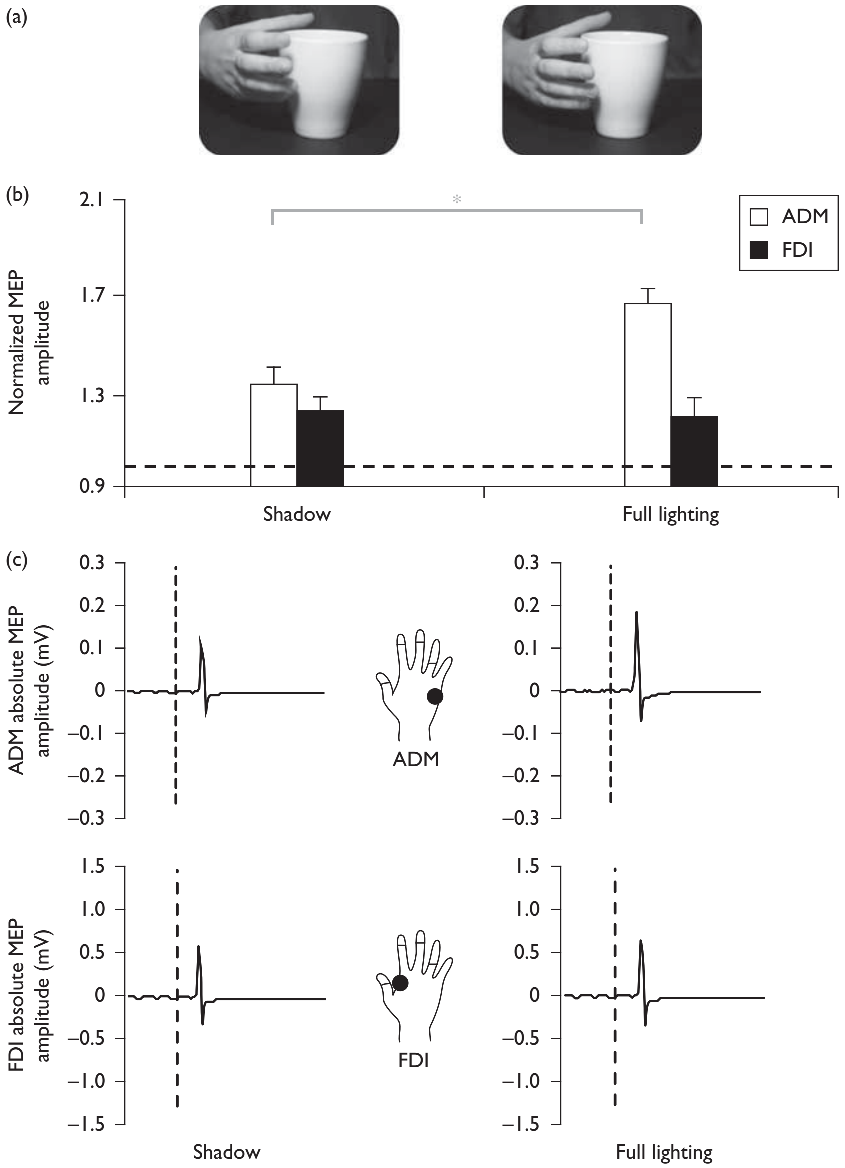
Transcranial magnetic stimulation and motor-evoked potential recording

TMS was delivered using a 70 mm figure-of-eight coil connected to a Magstim 200^2 stimulator (Magstim, Whitland, Dyfed, Wales, UK). The coil was angled 45° relative to the interhemispheric fissure and perpendicularly to the central sulcus with the handle pointing laterally and caudally [15,16]. This orientation induced a posterior–anterior current in the brain which tends to activate corticospinal neurons indirectly through excitatory synaptic inputs [17]. Pulses were delivered over the left M1 corresponding to the hand region. The coil was positioned in correspondence with the optimal scalp position, defined as the position at which the stimulation of a slightly suprathreshold intensity consistently produced the largest MEP from both the abductor digiti minimi (i.e. the muscle serving little finger abduction) and the first dorsal interosseus (i.e. the muscle serving index finger flexion/extension) muscles. The coil was held by a tripod and continuously checked by the experimenters to maintain consistent positioning. The resting motor threshold was determined for each participant as the minimum intensity that induced reliable MEPs ($\geq 50\,\mu V$ peak-to-peak amplitude) in the relaxed muscle in five out of 10 consecutive trials [18]. Stimulation intensity during the recording session was 110% of the resting motor threshold and ranged from 38 to 59% (mean 48.5%) of the maximum stimulator output intensity. MEPs were recorded simultaneously from electrodes placed over the contralateral abductor digiti minimi and first dorsal interosseus muscles. Electromyographic (EMG) recordings were made through pairs of 9 mm diameter Ag-AgCl surface electrodes. The active electrodes were placed over the belly of the right abductor digiti minimi and first dorsal interosseus muscles and the reference electrodes over the ipsilateral proximal interphalangeal joint (belly–tendon montage). The electrodes were connected to an isolated portable ExG input box linked to the main EMG amplifier for signal transmission through twin fiber optic cable (Professional BrainAmp ExG MR; Brain Products, Munich, Germany). The ground was placed over the participant's left wrist and connected to the common input of the ExG input box. The raw myographic signals were bandpass filtered (20 Hz to 1 kHz), amplified before being digitized (5 kHz sampling rate), and stored on a computer for off-line analysis. To prevent contamination of MEP measurements by background EMG activity, trials in which any EMG activity greater than 100 μV was present in the 100 ms window preceding the TMS pulse were discarded. EMG data were collected for 200 ms after the TMS pulse.

Procedure

Each participant was tested during a single experimental session lasting ∼40 min. Testing was carried out in a sound-attenuated Faraday room. The participants were seated in a comfortable armchair and their head was positioned on a fixed head rest so that the eye–screen distance was 80 cm. The participant's right arm was positioned on a full-arm support, whereas his/her left arm remained relaxed with the hand resting on the legs. Each participant was instructed to keep their hands in a prone position and as still and relaxed as possible. The task was to pay attention to the visual stimuli presented on a 19 inch monitor (resolution 1280 × 1024 pixels, refresh frequency 75 Hz, background luminance of 0.5 cd/m^2) set at eye level. The participants were instructed to passively watch the video-clips and to avoid making any movements. To keep the participants fully attentive to what was being shown, they were told that they would be questioned at the end of the session about the visual stimuli presented. Twenty trials were presented for each of the two types of video-clips, for a total of 40 trials. The order of presentation of the trials was randomized across participants. Before the video presentation, the baseline corticospinal excitability was assessed by acquiring 20 MEPs while the participants passively watched a white fixation cross on a black background on the computer screen. Twenty more MEPs were recorded at the end of the experimental session. By comparing the MEP amplitudes for the two baseline series we were able to check for any corticospinal excitability change related to TMS *per se*. The average amplitude of the two series was utilized to set each participant's individual baseline for the data normalization process. TMS-induced MEPs were acquired from the right abductor digiti minimi and

Fig. 1



(a) Frames extracted from the two video-clips serving as stimuli for this experiment. (b) Histograms of normalized motor-evoked potential (MEP) amplitudes across conditions (shadow, full-lighting) for abductor digiti minimi (ADM) and first dorsal interosseus (FDI) muscles following observation of a whole hand grasp. Scale bars represent the SE of means. The horizontal dotted line indicates the MEP baseline. (c) Typical MEP recordings from the abductor digiti minimi and the first dorsal interosseus muscles for one participant across conditions (shadowed, full-lighting) following observation of a whole hand grasp.

the right first dorsal interosseus muscles. Each video presentation was followed by a 10-s rest interval. During the first 5 s of the rest period, a message reminding the participants to keep their hands still and fully relaxed appeared on the screen. A fixation cross was presented for the remaining 5 s. Twenty MEPs per muscle per condition were acquired at the time the model's hand contacted the object for a total of 80 MEPs per participant. Stimuli presentation and the timing of TMS stimulation were managed by E-Prime V2.0 Software (Psychology Software Tools Inc., Pittsburgh, Pennsylvania, USA) running on a PC. All the participants watched two experimental conditions: a 'full-lighting' condition in which they observed video-clips in which a model performed a whole hand grasp to handle a mug in a fully illuminated context and a 'shadow' condition in which they saw the same scene but illuminated differently with shadows cast on the model's little finger.

Data analysis

For each condition, peak-to-peak amplitudes of the MEPs recorded from both the abductor digiti minimi and first dorsal interosseus muscles were measured and averaged at each time point. MEP amplitudes deviating more than 2 SD from the mean for each type of action and trial contaminated by muscular preactivation were excluded as outliers ($<2\%$). A paired sample *t*-test (two-tailed) was used to compare the amplitude of MEPs recorded from the abductor digiti minimi and first dorsal interosseus muscles during the two series of baseline trials at the beginning and at end of the experimental session. Ratios were then computed using the participants' individual mean amplitude of MEPs recorded during the two fixation periods as baseline (MEP ratio = MEP obtained/ MEP baseline). A mixed-design analysis of variance was conducted on the MEP ratios with 'illumination' (shadow, full-lighting) as a within-subjects factor and 'muscle' (first dorsal interosseus, abductor digiti minimi) as between-subjects factors. Sphericity of the data was verified before performing statistical analysis (Mauchly's test, $P > 0.05$). Post-hoc pairwise comparisons were carried out using *t*-tests and the Bonferroni correction for multiple comparisons was applied. The comparisons between normalized MEP amplitude and baseline were performed using one-sample *t*-tests.

Results

The mean raw MEP amplitudes recorded during the two baseline blocks at the beginning and end of the experimental session were not significantly different in the abductor digiti minimi (328.85 vs. 341.79 μV, respectively; $t_{29} = -0.61$, $P = 0.55$) or the first dorsal interosseus (798.29 vs. 764.96 μV, respectively; $t_{29} = 0.82$, $P = 0.42$) muscles. This suggests that TMS *per se* did not induce any changes in corticospinal excitability during our experimental procedure. The mean MEP ratios from the abductor digiti minimi and the first dorsal interosseus muscles for each illumination condition (shadow, full-lighting) are outlined in Fig. 1. The mixed-design analysis of variance on the normalized MEP amplitudes showed a significant main effect of illumination [$F_{(1,58)} = 8.78$, $P < 0.05$, $\eta^2_p = 0.13$] and a significant 'muscle by illumination' interaction [$F_{(1,58)} = 8.59$, $P < 0.05$, $\eta^2_p = 0.13$]. As expected, post-hoc contrasts revealed that there were no differences in MEP amplitudes for the first dorsal interosseus muscle depending on illumination (Fig. 1). MEPs activity was significantly lower for the shadow with respect to the full-lighting condition for the abductor digiti minimi muscle (Fig. 1). Furthermore, for the full-lighting condition there was a greater involvement of the abductor digiti minimi muscle with respect to the first dorsal interosseus ($P < 0.05$). This latter effect is usually noticed following the observation of whole hand prehensile actions [19]. Notably, MEP amplitudes for both the abductor digiti minimi and first dorsal interosseus muscles were significantly higher than the baseline ones ($P_s < 0.05$) even for the shadow condition.

Discussion

Shadows and their effect on human performance have been studied with respect to both the perceptual [20] and the motor domains [11]. What remains to be seen is to what extent motor resonance is modulated by shadows as far as corticospinal excitability is concerned. The present study takes research on this subject a step further by providing evidence that MEPs activity from the abductor digiti minimi muscle is specifically modulated by shadows. A visual scene containing shadows seems then to contain a potentially useful item when a stimulus is being encoded.

It is generally thought that the mirror system in humans is composed by at least three groups of areas, namely (i) the superior temporal sulcus, (ii) the ventral precentral cortex and the inferior frontal gyrus, and (iii) the posterior parietal cortex [21]. What is relevant with regard to the present study is that the mirror system receives complex visual information from the superior temporal sulcus which is involved in the perception of biological motion [22,23] as well as in processing shadows [24]. Brain-injured patients with lesions involving the right temporal lobe are notably unable to process shadows to optimize performance [24].

Taken together, these data seem to suggest that the visual information channeled from the superior temporal sulcus component of the mirror system to the motor centers contains data regarding specific visual features – such as shadows – characterizing the action being observed. Our finding that MEPs are altered following M1 stimulation in response to shadows seems to support the hypothesis that the superior temporal sulcus does indeed process that information channeling it to the other components of the system which is modulated accordingly.

Ida *et al.* [25] recently hypothesized that showing a digital human model performing a tennis serve using complicated pictorial information would enhance the discrimination performance of an observer. The results obtained refuted this hypothesis as a simplified model (stick-figure) evoked higher discrimination accuracy in the perception of the motion speed than a complicated one. Simple motor representations facilitate finer judgments to a greater extent than complex, accurate ones. Similar results were obtained by a study concerning shadow motions which proved to be sufficient to activate motor areas but only if a biological movement was involved [26]. In the same way, it could be hypothesized that motor resonant responses are lower when stimuli are only partially visible (shadowed) and higher when they are well illuminated, as the former represent a complicated model, whereas the latter a simplified one.

An alternative explanation for the findings presented here is that the lower corticospinal excitability described was simply because of impaired vision of the moving hand. MEP activity was, indeed, activated to a lesser degree when the effector was less visible. This explanation would confirm previous TMS studies on action observation showing that an observer's M1 is activated on the basis of what is perceived [3–5,8,27]. Further studies in this direction are warranted to assess whether the differential levels of motor resonance reported here were because of worse lighting conditions or to the shadows cast on the moving effector. A study in which TMS over the superior temporal sulcus halt the processing of shadows might disambiguate this issue. It is important to remember that shadows seem to be processed by temporal areas of the human brain [24] and the superior temporal sulcus is a component of the mirror system [21].

To summarize, these findings indicate that the human motor system is not only involved in recognizing and mirroring actions performed by others but is also finely tuned to the presence of shadows. To what extent an observer's motor system might benefit from the additional information provided by shadows and how these can contribute to predicting others' actions are questions that future research will attempt to address and to answer.

Conclusion

Our data show that motor resonant activity is higher in the representation area of the abductor digiti minimi finger muscle when it is fully visible and lower when it is only partially visible. Three-dimensional motion stimuli such as shadowed biological motion yield different motor responses in an observer's mirror system.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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