

Asymmetric fMRI adaptation reveals no evidence for mirror neurons in humans

Angelika Lingnau^a, Benno Gesierich^a, and Alfonso Caramazza^{a,b,1}

^aCenter for Mind/Brain Sciences (CIMEC), University of Trento, 38100 Mattarello, Italy; and ^bDepartment of Psychology, Harvard University, Cambridge, MA 02138

Edited by Edward E. Smith, Columbia University, New York, NY, and approved April 24, 2009 (received for review February 28, 2009)

Neurons in macaque ventral premotor cortex and inferior parietal lobe discharge during both the observation and the execution of motor acts. It has been claimed that these so-called mirror neurons form the basis of action understanding by matching the visual input with the corresponding motor program (*direct matching*). Functional magnetic resonance imaging (fMRI) adaptation can be used to test the direct matching account of action recognition by determining whether putative mirror neurons show adaptation for repeated motor acts independently of whether they are observed or executed. An unambiguous test of the hypothesis requires that the motor acts be meaningless to ensure that any adaptation effect is directly because of movement recognition/motor execution and not contextually determined inferences. We found adaptation for motor acts that were repeatedly observed or repeatedly executed. We also found adaptation for motor acts that were first observed and then executed, as would be expected if a previously seen act primed the subsequent execution of that act. Crucially, we found no signs of adaptation for motor acts that were first executed and then observed. Failure to find cross-modal adaptation for executed and observed motor acts is not compatible with the core assumption of mirror neuron theory, which holds that action recognition and understanding are based on motor simulation.

embodied cognition | motor action recognition | motor theory of action recognition

A specific type of visuomotor neuron in macaque ventral premotor cortex and inferior parietal lobe discharges when the monkey both observes and executes a particular motor act (e.g., grasping a peanut) (see also refs. 1–3). These neurons fire even in the absence of visual stimulation, e.g., to the sound associated with a particular motor act (4) or if the motor act is partially hidden (5). It has been suggested that these so-called “mirror neurons” form the basis of action understanding. According to the “direct matching hypothesis” (6, 7), we understand actions directly through their motor simulation. Activation of our own motor programs in turn provides access to the meaning associated with the observed action. Mirror neurons are assumed to provide the key element in translating the sensory input into the associated motor program (6, 7), although the exact mechanisms involved in this translation are still unknown.

Neurophysiological (8–10) and brain imaging (6, 11–16) studies suggest that a similar action mirroring system may exist also in humans. Recent functional magnetic resonance imaging (fMRI) studies using action observation or imitation tasks demonstrated activation in areas in the human ventral premotor (vPM) and parietal cortices assumed to be homologous to the areas in the monkey cortex containing mirror neurons (6, 11, 14, 17); for a review see ref. 13.

However, direct evidence for the existence of mirror neurons in humans is still lacking (18, 19). There are 2 conditions that must be fulfilled by any study that aims to address the existence of mirror neurons in humans. First, it must be demonstrated that execution and recognition of a specific motor act activate a common set of neurons in so-called mirror neuron areas (con-

dition I). Importantly, this overlap must be act specific (18). Second, it must be demonstrated that activation of neurons within potential mirror neuron areas results from direct activation and not from a prior nonmotor categorization on the basis of inferences about potential motor acts from minimal visual cues, e.g., seeing a hand move toward a familiar graspable object, inviting the inference that the actor’s intention may be to grasp the object (condition II).

fMRI adaptation (20–22) provides a tool to test for the existence of movement-selective neurons in humans (condition I). The idea behind this approach is that cortical areas that contain mirror neurons should adapt if the same motor act is repeated, irrespective of whether this motor act is repeated within the same (“within-modality adaptation”) or across different modalities (“cross-modal adaptation”).

Shmuelof and Zohary (15) and Hamilton and Grafton (16) demonstrated within-modality adaptation for the repeated observation of the same grasping movement in the anterior intraparietal sulcus, but did not investigate cross-modal adaptation. Dinstein et al. (23) identified several cortical areas that adapt if the same motor act is repeatedly observed or executed, but found no sign of cross-modal adaptation (23). More recently, Chong et al. (24) reported adaptation in the right ventral inferior parietal lobe for actions that were executed and then observed, but failed to find adaptation for actions that were first observed and then executed. Thus, the only 2 studies that met condition I (23, 24) produced mixed results.

One way to meet condition II is to use arbitrary (nonmeaningful) motor acts that cannot be recognized early in the act itself, to prevent visual categorization from minimal initial cues. The only 2 studies that met condition I failed to meet the second condition because in both cases meaningful acts were used. Furthermore, the movements used in the 2 studies were associated with an object (e.g., “click pen”), such that any cross-modal adaptation could have been caused by adaptation of object-related properties associated with the movement instead of the movement itself.

Despite the lack of evidence for a mirror neuron system in humans, mirror neurons have been claimed to be involved in a variety of cognitive functions including empathy, mind reading, and the development of language (6, 13, 25–27). Such claims would be undermined if we failed to find evidence for the involvement of mirror neurons in action understanding.

The study presented here is a unique unambiguous test of the existence of mirror neurons in humans because it meets the 2 elementary conditions outlined above. We studied within- and cross-modal adaptation for simple intransitive motor acts that are not associated with a particular meaning, such that any

Author contributions: A.L., B.G., and A.C. designed research; A.L. and B.G. performed research and analyzed data; and A.L., B.G., and A.C. wrote the paper.

Conflict of interest: The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹To whom correspondence should be addressed. E-mail: caram@wjh.harvard.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0902262106/DCSupplemental.

observed adaptation effect could not be attributed to adaptation of the same semantic representation or the same object. Furthermore, to ensure that participants would not be able to guess the target motor act from initial features of a movement, we used 8 different unpredictable movements that could be distinguished from each other only at a relatively late phase of the movement [see [supporting information \(SI\) Fig. S1A](#)] (28, 29).

We found adaptation for executed motor acts, when these were preceded by execution or observation of the same motor act, as would be expected if a previously executed or observed motor act were to prime the subsequent execution of that act (24). Importantly, we found no sign of adaptation when motor acts were first executed and then observed. These data are not compatible with the recent findings by Chong et al. (24). In line with the study by Dinstein et al. (23), our data do not support the direct matching account, according to which neurons exist that selectively respond to actions irrespective of whether these are observed or executed. Our data are compatible with the assumption that responses in mirror neuron areas reflect the facilitation of the motor system because of learned associations between semantic representation of actions and their generating motor programs (19).

Results

Visuomotor Regions of Interest. We identified seven *visuomotor* regions of interest (ROIs): left intraparietal sulcus, left and right superior parietal lobule, right ventral premotor cortex, left dorsal premotor cortex, and left and right lateral occipital cortex (Fig. 1, [Table S1](#)). Dorsal premotor cortex was defined as being located anterior to the precentral gyrus (30).

Within these ROIs, we examined the blood–oxygen level dependent (BOLD) response for “same” and “different” motor act trials. If the same motor act was repeatedly observed, the BOLD response adapted in the left lateral occipital cortex (Fig. 2A). If the same motor act was repeatedly executed, the BOLD response adapted in the left intraparietal sulcus, the left superior parietal lobule, the dorsal premotor cortex, and the lateral occipital cortex (Fig. 2D). If the same motor act was first observed and then executed, the BOLD response adapted in the left intraparietal sulcus, the left superior parietal lobe, and the left dorsal premotor cortex (Fig. 2C). No adaptation was found if the same motor act was first executed and then observed (Fig. 2B).

Next, we identified the following *motor* ROIs: left inferior parietal lobule, left intraparietal sulcus, left superior parietal lobule, left ventral premotor cortex, left dorsal premotor cortex/supplementary motor area (SMA), and the cerebellum (see [Table S1](#)). Within these ROIs, we examined the BOLD response. If the same motor act was repeatedly executed, the BOLD response adapted in the left intraparietal sulcus, the left superior parietal lobule, and the left dorsal premotor cortex/SMA (Fig. 3D). Furthermore, if the same motor act was first observed and then executed, we found adaptation in all examined motor ROIs, including the left thalamus and the cerebellum (Fig. 3C). No adaptation was found in motor ROIs if the same motor act was repeatedly observed (Fig. 3A) or if the same motor act was first executed and then observed (Fig. 3B).

Next, we identified 2 visual ROIs: left and right extrastriate body area (EBA) (see [Table S1](#)). We found adaptation for motor acts that were repeatedly observed in both the left and the right EBA (Fig. S2A). Furthermore, the BOLD response adapted in the left EBA if the same motor act was repeatedly executed (Fig. S2D).

To directly compare our results with the study by Chong et al. (24), we performed the same analysis within the left and the right inferior parietal lobe on the basis of average talairach coordinates reported in previous studies (see [Data Analysis](#) and [Table S2](#)). We found a similar pattern of results as described above: if

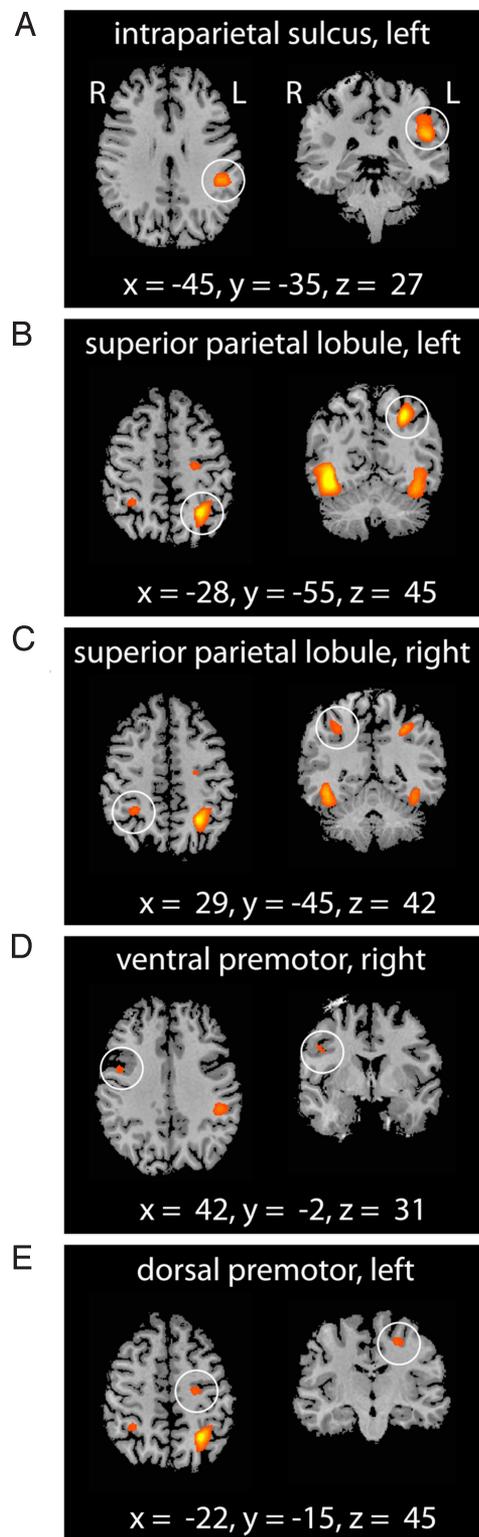


Fig. 1. Visuomotor ROIs (see text for details). The statistical map is superimposed on the anatomical image of one of the participants.

the same motor act was repeatedly executed, the BOLD response adapted in the left inferior parietal lobule (Fig. S3D). Furthermore, if the same motor act was first observed and then executed, we found adaptation in the left and the right inferior parietal lobe (Fig. S3C). We found no sign of adaptation for observed motor acts that were preceded by observation (Fig. S3A) or execution (Fig. S3B) of the same motor act.

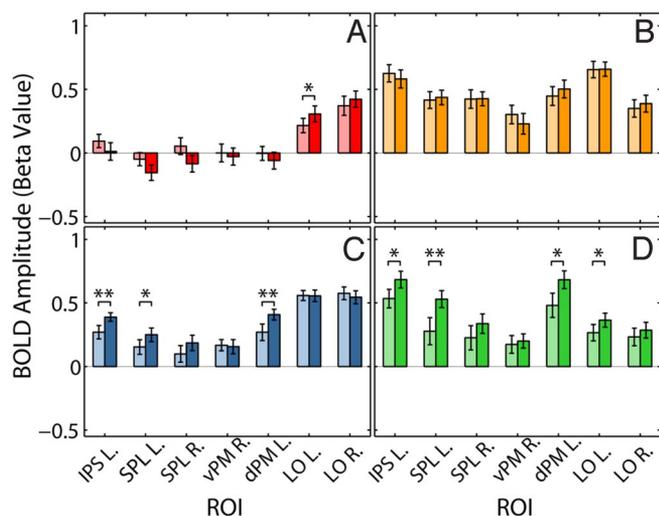


Fig. 2. Average BOLD response in visuomotor ROIs (see *Materials and Methods* and Fig. S5). (A) S1, observation; S2, observation. (B) S1, execution; S2, observation. (C) S1, observation; S2, execution. (D) S1, execution; S2, execution. Light colors, same motor act; dark colors, different motor act. IPS L., left intraparietal sulcus; SPL L., left superior parietal lobule; SPL R., right superior parietal lobule; vPM R., right ventral premotor cortex; dPM L., left dorsal premotor cortex; LO L., left lateral occipital cortex; LO R., right lateral occipital cortex. *, $P < 0.05$; **, $P < 0.01$. Error bars show the standard error of the mean (SEM).

Behavioral Data. The video recordings of the participants' hand motor acts revealed that across all conditions, participants made 2.3% errors on average (Table S3). In 1.9% of the trials, participants executed motor acts incorrectly or executed different motor acts instead. Participants executed motor acts when observation was requested in 0.4% of the trials. The number of error trials was generally larger when the motor acts in Video S1 and Video S2 were different.

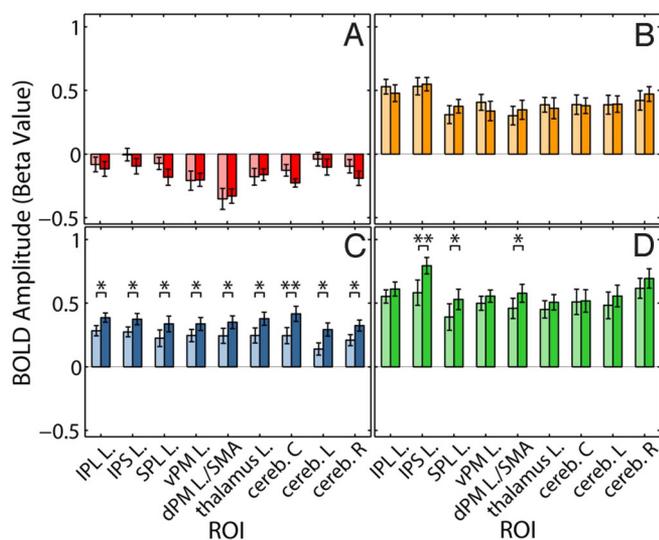


Fig. 3. Average BOLD response in motor ROIs (see also Fig. S6). (A) S1, observation; S2, observation. (B) S1, execution; S2, observation. (C) S1, observation; S2, execution. (D) S1, execution; S2, execution. IPL L., left inferior parietal lobule; IPS, left intraparietal sulcus; SPL, left superior parietal lobule; vPM, left ventral premotor cortex; dPM/SMA, left dorsal premotor cortex/supplementary motor cortex; cereb. C, central cerebellum; cereb. L, left cerebellum; cereb. R, right cerebellum. *, $P < 0.05$; **, $P < 0.01$.

Discussion

Asymmetric Adaptation: No Support for the Direct Matching Account.

We demonstrated that executed motor acts preceded by the observation of the same motor acts led to BOLD adaptation in intraparietal sulcus, superior parietal lobe, and dorsal premotor cortex. Crucially, the opposite pattern was not found: the observation of a motor act preceded by execution of the same motor act did not lead to BOLD adaptation.

Cross-modal adaptation for repeated movements that are first observed and then executed likely reflects a facilitation of the motor system (8, 31, 32). This facilitation is likely to be because of the covert generation of a motor command during action observation (see also refs. 19 and 33). If the same motor act is first observed and then executed, the same motor command that has been covertly generated during action observation can be used for overt motor execution. This might lead to benefits for the selection, preparation, and execution of the required motor command and thus to a reduced BOLD response in the corresponding cortical regions.

Direct evidence for the existence of mirror neurons would require finding adaptation in the condition in which execution is followed by observation (24). As in the recent experiment by Dinstein et al. (23), our study failed to reveal this kind of cross-modal adaptation. Our results, and those of Dinstein et al. (23), are not compatible with the study by Chong et al. (24), who reported adaptation for repeated motor acts that were first executed and then observed, but no adaptation for repeated motor acts that were first observed and then executed. As outlined above, the study by Chong et al. (24) cannot be taken as providing evidence for the existence of a direct matching mechanism because it used meaningful movements, most of which were associated with a particular object. Adaptation for motor acts that were first executed and then observed might have been caused by the repeated activation of the same semantic content associated with the cued actions or by the repeated activation of object-related representations associated with the movement, rather than by repetition of direct activation of mirror neurons. Furthermore, it is puzzling that Chong et al. (24) did not find adaptation in motor or premotor areas. The direct matching hypothesis depends crucially on the presence of mirror neurons in those areas, where motor programs are "stored." Only adaptation in those areas could justify the claim that actions are understood by simulation of the movement in the observer's own motor system.

Adaptation Within Modalities. We found that observation of the same motor act led to adaptation in the left lateral occipital lobe and the left and right extrastriate body area. The repeated execution of the same motor act led to adaptation in the left intraparietal sulcus, the left superior parietal lobe, the left dorsal premotor cortex, and the left lateral occipital lobe. These findings are in line with previous reports on adaptation for the repeated observation (15, 16, 23) and execution of the same motor act (23, 34). However, because mirror neurons should be sensitive to the repetition of the same motor act both within and across modalities, we were mainly interested in determining whether there are areas that show both within- and cross-modal adaptation. As pointed out above, we found no such areas.

Admittedly, using visual instructions in an execution task adds complexity to the task: participants have to process the instruction, translate it into the corresponding motor plan, and execute the motor act. We tried to limit task demands by training participants and by using visual instructions that are easy to understand. However, we cannot rule out that the activation we see during execution partially reveals the process of translating the visual instruction into the associated motor plan. Furthermore, it is possible that the adaptation for the repeated execution

of the same motor act we found in the left lateral occipital lobe might be caused by the repeated observation of the same visual instruction. Importantly, adaptation because of the visual instruction itself cannot cause cross-modal adaptation because the visual instruction is shown during execution, but not during observation.

Allowing participants to freely select movements without visual instruction poses a different order of interpretive difficulty: in addition to executing a movement, participants would need to decide which movement to perform, thereby involving additional processes like visual imagery and more complex decision making. Because the use of visual instructions allows precise control of the executed motor act and, thus, proper counterbalancing while keeping the need for additional high-level processes low, we chose to instruct participants on which movement to execute instead of leaving them to choose freely despite the disadvantages described above.

Neurophysiological Basis of fMRI Adaptation. The neurophysiological basis of fMRI adaptation is still controversial (21, 22, 35, 36). Therefore, the absence of fMRI adaptation for motor acts that are first executed and then observed must be interpreted with care. The use of fMRI adaptation to test for the existence of mirror neurons is based on the assumption that such neurons adapt. The absence of cross-modal adaptation might be because mirror neurons lack this property. This seems unlikely given that there are numerous papers showing adaptation for complex stimulus properties like objects (20), surface texture (37), and actions (16, 38), indicating that adaptation is not restricted to low-level stimulus properties.

Transitive Versus Intransitive Actions. It could be argued that intransitive movements are not suitable to activate the mirror neuron system, given that mirror neurons in monkeys have been reported to respond preferentially in the presence of a target object (2) or other predictive clues such as the sound of a nut cracking. Likewise, one might argue that only actions within the motor repertoire of the observer engage mirror neurons. However, if direct matching constituted a general principle for action understanding, it should also apply to intransitive actions and even to actions that the observer cannot perform. Furthermore, the “human mirror neuron system” is assumed to react to a wider range of actions, including intransitive and nonsymbolic actions (6, 8, 17, 39). Finally, the fact that the conjunction analysis for action observation and execution revealed most of the classical mirror neuron areas rules out that the actions used in the current study were unsuitable to engage those regions.

It is puzzling that mirror neurons in monkeys seem to be restricted to transitive actions. It could be argued that intransitive actions are meaningless movements to a monkey and, hence, do not activate its mirror neurons. However, such an explanation would have severe consequences for the interpretation of both the mechanism and the function of the mirror neuron response. The implication would be that mirror neurons are not activated by an unmediated direct matching mechanism. Rather, it would suggest that observed actions are “simulated” in the motor system only after their meaning has been inferred outside the motor system (28, 29).

In line with this view, the results reported by Chong et al. (24) showing adaptation only in the right inferior parietal lobe seem more compatible with an account of action understanding as being independent from the motor system and not requiring its simulation in this system (19). The inferior parietal lobe has demonstrated neither connections to the primary motor cortex nor direct connections to the spinal cord. Furthermore, data included in their SI Text revealed a tendency of fMRI enhancement in the typical mirror neuron areas, including the most critical area, the ventral premotor cortex. Taken together, the

claim that neurons in the right inferior parietal lobe hold the motor programs for the observed actions seems peculiar. A true simulation of an observed movement cannot be accomplished only with neurons outside the motor or the premotor cortex.

It is worth noting, finally, that the inference of action goals from the context is indeed sufficient to activate mirror neurons in the monkey: if the crucial visual information about an ongoing action is hidden from the monkey behind a screen, the response of mirror neurons is modulated by the monkey’s prior knowledge about the presence or the absence of an object behind the screen, which allowed it to infer the goal of the hidden action (5).

Conclusion

A growing number of studies are challenging the assumption that action understanding requires the involvement of the corresponding motor circuits (19). Recently it has been demonstrated that action understanding of novel situations in humans does not rely on the mirror neuron system, but on brain areas involved in context-sensitive inferential processes (40, 41). Apraxic patients can be impaired in using particular objects despite being unimpaired in the recognition of visually presented actions (42). Developmental studies indicate that infants can distinguish between different types of actions (e.g., walking) that they are too young to perform themselves (43). Likewise, free-ranging rhesus monkeys recognize the functional properties of throwing despite not having the ability to throw themselves (44).

Our study provides an important extension of these reports by directly testing the predictions of the direct matching account in humans. Using motor acts that are not associated with a specific meaning or object, we demonstrated that the BOLD response adapts in several cortical areas, including intraparietal sulcus, superior parietal lobe, and dorsal premotor cortex, if a motor act is first observed and then executed. In contrast to the recent study by Chong et al. (24), but in line with Dinstein et al. (23), we found that simple motor acts that are first executed and then observed do not lead to adaptation. These findings challenge the direct matching account of action understanding, according to which neurons should exist that adapt if the same motor act is repeated, both when the motor act is observed and when it is executed. Our findings are compatible with the view that activation in mirror neuron areas reflects the facilitation of motor programs as a consequence of action understanding.

Materials and Methods

Participants. Twelve right-handed healthy volunteers (8 males) took part in the study (mean age, 27.4 years; range, 22–37 years). All participants gave written informed consent for their participation in the study. The experimental procedures were approved by the ethical committee for experiments involving humans at the University of Trento.

Visual Stimulation. Video clips were taken using a digital video camera (HDR-UX1E, Sony) and were processed by video editing software (Avid Liquid 7, Pinnacle Systems) and MATLAB (The MathWorks). Stimuli were back-projected onto a screen by a liquid-crystal projector at a frame rate of 60 Hz and a screen resolution of $1,280 \times 1,024$ pixels (mean luminance: 109 cd/m^2). Participants viewed the stimuli binocularly through a mirror above the head coil.

Stimulation was programmed with the in-house software “ASF” (available from jens.schwarzbach@unitn.it), based on the MATLAB Psychtoolbox-3 (45) for Windows.

Materials. For observation trials, video clips of 8 different simple right-hand motor acts were taken. Two versions were created for each motor act, showing either a male or a female hand. All video clips were cut to 2-s duration, color information was removed, and resolution was reduced to 432×346 pixels (visual angle: $6.05^\circ \times 4.85^\circ$). In each of the 8 videos the hand performed a different, but in all cases meaningless gesture (Fig. S1A). The hand always started from the same initial position and returned to the initial position. In the initial position the hand was located in the center of the video frame, entering from below and with the palm placed on a gray and featureless surface.

For execution trials, the hand motor act videos were modified using a

decomposition technique based on Fourier analysis to obtain full control over noise and contrast of pictures. Each image was decomposed by means of Fast Fourier Transformation (FFT) into a frequency and a phase image. Noise was then introduced by permuting the phase information of all pixels in the image. Finally, the grayscale image was reconstructed by means of inverse FFT (iFFT). Note that compared to other strategies for adding noise to an image (e.g., scrambling or replacing pixels with random intensity values), the adopted method leaves the spatial frequency content intact. It is assumed that thereby the images produce the same neuronal signal in low-level visual areas.

Superimposed in the center of each scrambled video clip, a gray rectangle ($4.04^\circ \times 3.03^\circ$) containing 1 of 8 different pictograms was shown (Fig. S1B). The pictogram indicated which of the 8 hand motor acts the participant had to execute. For each pictogram, 2 scrambled video clips were prepared, with the color of the pictogram being either black or white.

fMRI Adaptation Paradigm. The main experiment consisted of 4 event-related fMRI adaptation scans. Each trial lasted 5 s and consisted of 2 consecutively presented video clips (Video S1, adaptation stimulus; Video S2, test stimulus) separated by an interstimulus interval (ISI) of 1-s duration (Fig. S4). Both Video S1 and Video S2 could be either a hand motor act video ("observation", "O") or a scrambled hand motor act clip with a superimposed pictogram ("execution", "E") for 1 of 8 different hand motor acts.

The motor act in Video S1 and Video S2 could be either the same or different, resulting in a total of 8 different conditions (i.e., "OO-same", "OO-diff", "OE-same", "OE-diff", "EO-same", "EO-diff", "EE-same", "EE-diff"). In OO-same and OO-diff trials, the acting hand in Video S1 and Video S2 was identical in half of the trials and different in the other half of the trials. In EE-same and EE-diff trials, the pictogram was identical (e.g., black on white for both Video S1 and Video S2) in half of the trials and different (e.g., black on white for Video S1 and white on black for Video S2) in the other half of the trials.

The intertrial interval (ITI) was jittered between 3 and 4.5 s (step size: 0.5 s). For the duration of ISIs and ITIs a blank gray screen was shown. Each scan consisted of 65 trials. Within each scan, each of the 8 conditions was repeated 8 times, with the exception of 1 randomly selected condition repeated 9 times. This ninth repetition was shown as the first trial of the scan and served for counterbalancing only. Hence, it was excluded from analysis. Counterbalancing was used to ensure that each condition was preceded by each other condition and itself exactly 1 time during a scan. Scans lasted 580 s, including a 15-s blank screen at the start and the end. Each participant completed $(65 - 1) \times 4 = 256$ trials, leading to $256/8 = 32$ repetitions per condition.

Functional Localizer. To identify cortical regions that are sensitive both to observation and to execution of motor acts, we used 2 separate functional localizer runs. Only those hand motor act clips showing the female hand were used. An additional set of 8 different foot motor act clips was prepared in an analogous manner to the hand motor act clips.

Both localizer runs were performed using a block design. Each block lasted 20 s and started with a written instruction ("hand," "foot," or "scrambled") displayed for 0.5 s. Subsequently, 8 video clips, each lasting 2 s and being separated by blank intervals of 0.5 s, were shown. Experimental blocks were separated by blank periods, each of them lasting 20 s. There were 4 blocks per condition, and the order of video clips within each block was randomized. Additional blank periods (20 s) were presented at the beginning and the end of the localizer scans.

The observation localizer involved observation of (A) right hand motor acts, (B) right foot motor acts, and (C) scrambled hand motor acts. Hand motor acts were identical to those used in the main experiment. Within each block, each motor act (or its scrambled version) was shown once per block in a randomized order. The order of blocks followed a fixed sequence (ABCABC).

The execution localizer involved execution of (A) right hand motor acts and (B) right foot motor acts. The corresponding blocks were constructed of scrambled hand motor act clips, with the superimposed pictograms corresponding either to the 8 possible hand motor acts or to the 8 possible foot motor acts. Each pictogram was shown once per block in a randomized order. The order of blocks followed a fixed sequence (ABAB).

Instruction and Training. *Main experiment.* Participants were instructed to execute the motor act corresponding to the pictogram whenever they saw a scrambled video with a pictogram. When they saw a video of a hand motor act, they were instructed to observe that motor act. Between motor act executions participants were told to keep the right hand in a relaxed position. The open palm of the right hand had to be placed on the thigh. Hence, during motor act execution participants always started from and returned to this position.

Either the day before the fMRI experiment or on the same day, participants

were trained to execute all hand motor acts associated with the pictograms before the experiment within the required time window of 2 s. Participants were also familiarized with the videos for motor act observation.

Localizer experiment. For the motor act observation localizer, participants received the instruction to observe the hand, foot, and scrambled motor acts while keeping their eyes at fixation. For the motor act execution localizer, participants were instructed to execute the hand or foot motor acts associated with the pictograms shown on the scrambled videos while keeping their eyes at fixation. Participants were familiarized with the foot videos and the associated pictograms before the experiment and received enough training to be able to reliably produce the required motor acts.

Movement Recording. To control participants' hand motor acts, a digital video camera (VP-D15i; Samsung Electronics) was placed outside the 0.5-mT line. Videos were analyzed offline (see *Data Analysis*).

Data Acquisition. We acquired data using a 4T Bruker MedSpec Biospin MR scanner and an 8-channel birdcage head coil. Functional images were acquired with a T2*-weighted gradient-recalled echo-planar imaging (EPI) sequence. We performed an additional scan to measure the point-spread function (PSF) of the acquired sequence, which served for distortion correction that is expected with high-field imaging (see *Data Analysis*). We used 34 slices, acquired in ascending interleaved order, slightly tilted to run parallel to the calcarine sulcus, with a TR (time to repeat) of 2000 ms (voxel resolution, $3 \times 3 \times 3$ mm; TE (time to echo), 33 ms; flip angle (FA), 73° ; field of view (FOV), 192×192 mm; gap size, 0.45 mm). For the main experiment, each participant completed 4 scans of 298 volumes. At the end of the experiment, we collected 2 additional functional localizer runs (execution localizer, 170 volumes; observation localizer, 250 volumes; all other scanning parameters were the same as in the main experiment).

To be able to coregister the low-resolution functional images to a high-resolution anatomical scan, we acquired a T1 weighted anatomical scan (MP-RAGE; $1 \times 1 \times 1$ mm; FOV, 256×224 ; 176 slices; GRAPPA acquisition with an acceleration factor of 2; TR, 2700 ms; TE, 4.18 ms; inversion time (TI), 1020 ms; 7° flip angle).

Data Analysis. Data analysis was performed with BrainVoyager QX 1.9.9 (BrainInnovation). The second run of participant 11 was excluded from data analysis because of several rapid head movements. In all remaining runs of all participants, 3D motion correction parameters revealed x, y, and z translation of <1.5 mm and x, y, and z rotation of <1 mm.

Video recordings. Videos were analyzed offline to verify that participants performed the correct motor acts. Trials were excluded from analysis when a requested motor act was executed incorrectly or not at all and when motor acts were executed without being requested (see Table S3).

Preprocessing. To correct for distortions in geometry and intensity in the EPI images, we applied distortion correction on the basis of the PSF data acquired before the EPI scans (46). Before further analysis, we discarded the first 4 volumes. Next, we performed 3D motion correction with trilinear interpolation and slice timing correction with ascending interleaved order, using the first slice as reference. Functional data were temporally high-pass filtered at 3 cycles/run length. A Gaussian kernel of 8 mm was applied to spatially smooth the images.

Next, we aligned the first volume of each functional run to the high-resolution anatomy. Both functional and anatomical data were transformed into Talairach space (47), using trilinear interpolation.

Statistical analysis. We identified ROIs that responded during both observed and executed motor acts, using a random effects (RFX) general linear model (GLM). Each predictor time course was convolved with a canonical hemodynamic impulse response function, where $\sigma = 2.5$ and $t = 1.25$ (22). The resulting reference time courses were used to fit the signal time course of each voxel. Parameters from 3D motion correction were included in the model. Finally, to identify voxels that are activated during both observation and execution (visuomotor ROIs), we carried out a conjunction analysis for observed and executed motor acts. To identify motor ROIs, we used a conjunction analysis for hand and foot motor acts exceeding the BOLD response of observation of scrambled movements. Visual ROIs were defined by the contrast of hand and foot observation versus observation of scrambled movements.

Multisubject analysis ($n = 12$) was performed using a random-effects analysis. Statistical maps were thresholded using a false discovery rate (FDR) (48) of 0.05 and a cluster threshold of 4 voxels.

Because fast event-related fMRI designs result in overlapping hemodynamic responses, we applied a deconvolution analysis (49) to our data. In brief, deconvolution analysis estimates the hemodynamic response function (HRF) on the basis of separate predictors for each condition and time point (TR). We

used 10 time points per condition, i.e., 20 s time locked to the onset of any given Video S1, with stimulus onset being $t = 0$ [TR]. Because peak latencies differed between the 4 main conditions (see Figs. S5–S7), we determined the latency of the absolute maximum separately for OO-diff, OE-diff, EO-diff, and EE-diff trials, using the following procedure. The peak latency for the same trials was chosen in each area as the absolute peak latency determined in the different trials of the corresponding condition (time windows used for averaging are marked in gray in Figs. S5–S7). The search for the peak was restricted to $t > 2$ to prevent detecting peaks within the initial falling part of the curve, especially in the conditions OO-same and OO-diff. Next, we computed mean percentage of signal change in a time window from $t_0 = \text{peak latency}$ to $t_1 = t_0 + 2 \text{ TR}$, separately for each condition. We compared the mean BOLD response for repeated and nonrepeated motor acts, using pairwise comparisons within ROIs (see Table S1).

- di Pellegrino G, Fadiga L, Fogassi L, Gallese V, Rizzolatti G (1992) Understanding motor events: a neurophysiological study. *Exp Brain Res* 91(1):176–180.
- Gallese V, Fadiga L, Fogassi L, Rizzolatti G (1996) Action recognition in the premotor cortex. *Brain* 119 (Pt 2):593–609.
- Rizzolatti G, Fadiga L, Gallese V, Fogassi L (1996) Premotor cortex and the recognition of motor actions. *Brain Res Cogn Brain Res* 3(2):131–141.
- Kohler E, et al. (2002) Hearing sounds, understanding actions: action representation in mirror neurons. *Science* 297(5582):846–848.
- Umiltà MA, et al. (2001) I know what you are doing. A neurophysiological study. *Neuron* 31(1):155–165.
- Iacoboni M, et al. (1999) Cortical mechanisms of human imitation. *Science* 286(5449):2526–2528.
- Rizzolatti G, Fogassi L, Gallese V (2001) Neurophysiological mechanisms underlying the understanding and imitation of action. *Nat Rev Neurosci* 2(9):661–670.
- Fadiga L, Fogassi L, Pavesi G, Rizzolatti G (1995) Motor facilitation during action observation: a magnetic stimulation study. *J Neurophysiol* 73(6):2608–2611.
- Gangitano M, Mottaghy FM, Pascual-Leone A (2004) Modulation of premotor mirror neuron activity during observation of unpredictable grasping movements. *Eur J Neurosci* 20(8):2193–2202.
- Nishitani N, Hari R (2000) Temporal dynamics of cortical representation for action. *Proc Natl Acad Sci USA* 97(2):913–918.
- Carr L, Iacoboni M, Dubeau MC, Mazziotta JC, Lenzi GL (2003) Neural mechanisms of empathy in humans: a relay from neural systems for imitation to limbic areas. *Proc Natl Acad Sci USA* 100(9):5497–5502.
- Fogassi L, et al. (2001) Cortical mechanism for the visual guidance of hand grasping movements in the monkey: A reversible inactivation study. *Brain* 124(Pt 3):571–586.
- Rizzolatti G, Craighero L (2004) The mirror-neuron system. *Annu Rev Neurosci* 27:169–192.
- Rizzolatti G, et al. (1996) Localization of grasp representations in humans by PET: 1. Observation versus execution. *Exp Brain Res* 111(2):246–252.
- Shmuelof L, Zohary E (2005) Dissociation between ventral and dorsal fMRI activation during object and action recognition. *Neuron* 47(3):457–470.
- Hamilton AF, Grafton ST (2006) Goal representation in human anterior intraparietal sulcus. *J Neurosci* 26(4):1133–1137.
- Buccino G, et al. (2001) Action observation activates premotor and parietal areas in a somatotopic manner: An fMRI study. *Eur J Neurosci* 13(2):400–404.
- Dinstein I, Thomas C, Behrmann M, Heeger DJ (2008) A mirror up to nature. *Curr Biol* 18(1):R13–R18.
- Hickok G (2009) Eight problems for the mirror neuron theory of action understanding in monkeys and humans. *J Cogn Neurosci* 21(7):1229–1243.
- Grill-Spector K, Malach R (2001) fMRI-adaptation: A tool for studying the functional properties of human cortical neurons. *Acta Psychol (Amst)* 107(1–3):293–321.
- Grill-Spector K, Henson R, Martin A (2006) Repetition and the brain: Neural models of stimulus-specific effects. *Trends Cogn Sci* 10(1):14–23.
- Krekelberg B, Boynton GM, van Wezel RJ (2006) Adaptation: From single cells to BOLD signals. *Trends Neurosci* 29(5):250–256.
- Dinstein I, Hasson U, Rubin N, Heeger DJ (2007) Brain areas selective for both observed and executed movements. *J Neurophysiol* 98(3):1415–1427.
- Chong TT, Cunnington R, Williams MA, Kanwisher N, Mattingley JB (2008) fMRI adaptation reveals mirror neurons in human inferior parietal cortex. *Curr Biol* 18(20):1576–1580.
- Gallese V, Eagle MN, Migone P (2007) Intentional attunement: Mirror neurons and the neural underpinnings of interpersonal relations. *J Am Psychoanal Assoc* 55(1):131–176.
- Rizzolatti G, Arbib MA (1998) Language within our grasp. *Trends Neurosci* 21(5):188–194.
- Gallese V (2003) The roots of empathy: The shared manifold hypothesis and the neural basis of intersubjectivity. *Psychopathology* 36(4):171–180.
- Csibra G (2008) Action mirroring and action understanding: An alternative account. *Sensorymotor Foundations of Higher Cognition. Attention and Performance XXII*, eds Haggard P, Rosetti Y, Kawato M (Oxford Univ Press, Oxford), pp 435–459.
- Kilner JM, Friston KJ, Frith CD (2007) Predictive coding: An account of the mirror neuron system. *Cogn Process* 8(3):159–166.
- Duvernoy HM (1999) *The Human Brain: Surface, Three-Dimensional Sectional Anatomy and MRI* (Springer, New York).
- Brass M, Bekkering H, Prinz W (2001) Movement observation affects movement execution in a simple response task. *Acta Psychol (Amst)* 106(1–2):3–22.
- Vogt S, Taylor P, Hopkins B (2003) Visuomotor priming by pictures of hand postures: Perspective matters. *Neuropsychologia* 41(8):941–951.
- Tkach D, Reimer J, Hatsopoulos NG (2007) Congruent activity during action and action observation in motor cortex. *J Neurosci* 27(48):13241–13250.
- Karni A, et al. (1995) Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature* 377(6545):155–158.
- Bartels A, Logothetis NK, Moutoussis K (2008) fMRI and its interpretations: An illustration on directional selectivity in area V5/MT. *Trends Neurosci* 31(9):444–453.
- Sawamura H, Orban GA, Vogels R (2006) Selectivity of neuronal adaptation does not match response selectivity: A single-cell study of the fMRI adaptation paradigm. *Neuron* 49(2):307–318.
- Cant JS, Arnott SR, Goodale MA (2009) fMRI-adaptation reveals separate processing regions for the perception of form and texture in the human ventral stream. *Exp Brain Res* 192(3):391–405.
- Shmuelof L, Zohary E (2006) A mirror representation of others' actions in the human anterior parietal cortex. *J Neurosci* 26(38):9736–9742.
- Gallese V, Keysers C, Rizzolatti G (2004) A unifying view of the basis of social cognition. *Trends Cogn Sci* 8(9):396–403.
- Brass M, Schmitt RM, Spengler S, Gergely G (2007) Investigating action understanding: Inferential processes versus action simulation. *Curr Biol* 17(24):2117–2121.
- Kilner JM, Frith CD (2008) Action observation: Inferring intentions without mirror neurons. *Curr Biol* 18(1):R32–R33.
- Mahon BZ, Caramazza A (2005) The orchestration of the sensory-motor systems: Clues from neuropsychology. *Cogn Neuropsychol* 22(3/4):480–494.
- Kamewari K, Kato M, Kanda T, Ishiguro H, Hiraki K (2005) Six-and-a-half-month-old children positively attribute goals to human action and to humanoid-robot motion. *Cogn Dev* 20:303–320.
- Wood JN, Glynn DD, Hauser MD (2007) The uniquely human capacity to throw evolved from a non-throwing primate: An evolutionary dissociation between action and perception. *Biol Lett* 3(4):360–364.
- Brainard DH (1997) The psychophysics toolbox. *Spat Vis* 10(4):433–436.
- Zeng H, Constable RT (2002) Image distortion correction in EPI: Comparison of field mapping with point spread function mapping. *Magn Reson Med* 48(1):137–146.
- Talairach J, Tournoux P (1988) *Co-Planar Stereotaxic Atlas of the Human Brain* (Thieme, New York).
- Genovese CR, Lazar NA, Nichols T (2002) Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage* 15(4):870–878.
- Dale AM, Buckner RL (1997) Selective averaging of rapidly presented individual trials using fMRI. *Hum Brain Mapp* 5:329–340.

We performed an additional ROI analysis in the left and the right inferior parietal lobule, using average Talairach coordinates reported in previous studies on motor act observation and execution (see Table S2). Event-related deconvolution analysis and averaged BOLD signals were computed within these 2 areas as stated above.

ACKNOWLEDGMENTS. We are grateful to Jens Schwarzbach for providing software for visual stimulation and Fourier scrambling and for helpful discussions. We also thank David Melcher and Marc Hauser for comments on the manuscript, Sara Fabbri for training participants, and Maxim Zaitsev for providing the point-spread function and modified EPI sequences. This research was supported by the Provincia Autonoma di Trento and the Fondazione Cassa di Risparmio di Trento e Rovereto.