

26. Mouse Genome Sequencing Consortium. Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**, 520–562 (2002).
27. Anderson, J. P. *et al.* HRC is a direct transcriptional target of MEF2 during cardiac, skeletal, and arterial smooth muscle development *in vivo*. *Mol. Cell. Biol.* **24**, 3757–3768 (2004).
28. Dodou, E., Xu, S. M. & Black, B. L. mef2c is activated directly by myogenic basic helix–loop–helix proteins during skeletal muscle development *in vivo*. *Mech. Dev.* **120**, 1021–1032 (2003).

Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements We thank I. Ovcharenko, G. Loots and J. Schwartz for help with the identification and annotation of the gene deserts; D. Boffelli, L. Pennacchio, N. Ahituv, J. Bristow and other Rubin laboratory members for suggestions and criticisms on the manuscript; and H. Jacob for providing the clinical chemistry assays. Research was conducted at the E. O. Lawrence Berkeley National Laboratory and at the Joint Genome Institute, with support by grants from the Programs for Genomic Application, the NHLBI and the DOE.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to E.M.R. (emrubin@lbl.gov).

Neural correlates of mental rehearsal in dorsal premotor cortex

Paul Cisek & John F. Kalaska

Département de physiologie, Université de Montréal, C.P. 6128 Succursale Centreville, Montréal, Québec, H3C 3J7, Canada

Behavioural and imaging studies suggest that when humans mentally rehearse a familiar action they execute some of the same neural operations used during overt motor performance^{1–9}. Similarly, neural activation is present during action observation in many of the same brain regions normally used for performance, including premotor cortex^{6–9}. Here we present behavioural evidence that monkeys also engage in mental rehearsal during the observation of sensory events associated with a well-learned motor task. Furthermore, most task-related neurons in dorsal premotor cortex exhibit the same activity patterns

during observation as during performance, even during an instructed-delay period before any actual observed motion. This activity might be a single-neuron correlate of covert mental rehearsal.

The ‘direct matching hypothesis’^{10–13} suggests that understanding the observed actions of others not only involves a cognitive analysis of sensory inputs but also evokes a representation of the action in some of the same neural circuits that are engaged when the observer performs those actions, a form of covert simulation or mental rehearsal of the observed acts^{6,7,9,14}. At the single-neuron level, support for the hypothesis is provided by a select population of ‘mirror neurons’ in the ventral premotor cortex. These neurons discharge both while a monkey performs natural actions such as reaching, grasping or biting, and when the monkey observes someone else performing those same familiar actions directed at target objects^{10–13}. These findings indicate that premotor cortex (PM) might be involved not only in overt motor performance, but also in non-motor functions such as recognizing the nature and consequences of the actions of others, and attribution of agency^{1,12,14}.

However, activation of motor circuits during mental rehearsal occurs not only during the direct observation of an ongoing action but also whenever subjects are given information specifying a particular action^{9,14,15}. This predicts that the neural mechanisms involved in selecting and planning movements should also be activated, in advance of an observed action, as soon as the observer is given information that allows them to anticipate what will happen. Evidence that mental rehearsal can include anticipatory processes is indirectly supported by the finding that the oculomotor behaviour of humans is similar when they observe someone performing a block-stacking task and when they perform the task themselves¹⁶. In particular, the direction of gaze predicts what should happen next, indicating that the observers are mentally simulating the task to predict future events instead of just following the viewed events with their eyes as they occur. However, mirror neurons do not discharge in anticipation of a potential action when either the actor or target, or both, are viewed at rest, or during the observation of pantomimed actions¹². Furthermore, mirror neurons respond only to natural actions that are directly viewed, and not to their video images¹⁷. These findings indicate a specific role for mirror neurons in the interpretation of ongoing familiar actions of a

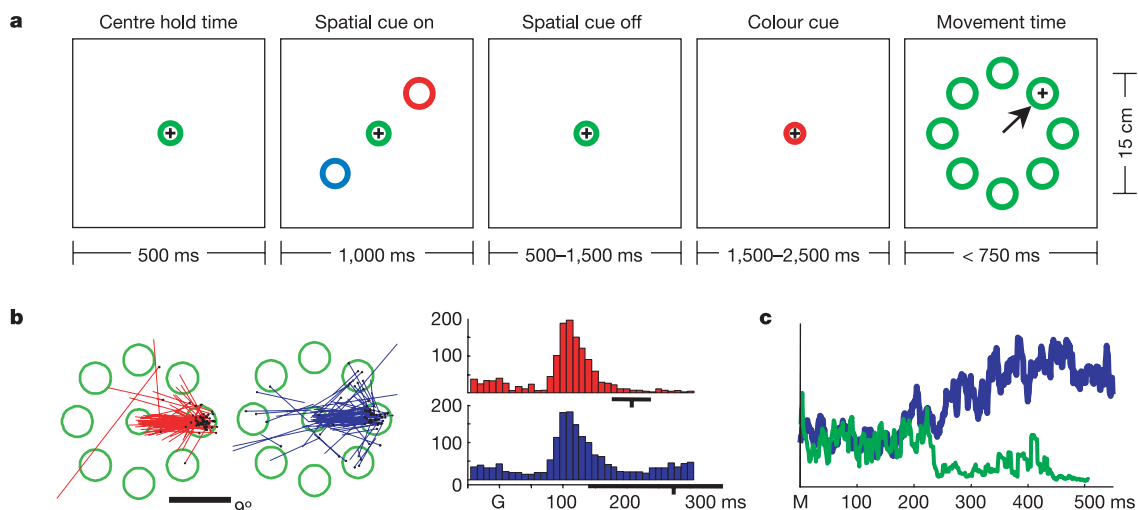


Figure 1 Behavioural data. **a**, Stimulus events in the two-target task. The cross indicates cursor location. **b**, Saccadic behaviour. Left: representative saccades 50–200 ms after GO when the right target was selected are shown as coloured lines (red, performance; blue, observation) terminating with black dots indicating fixation. Right: histograms of saccade onset relative to GO (G), during performance (red, upper) and observation (blue,

lower). Black bars indicate mean and s.d. of cursor motion onset. **c**, Electromyogram activity of mentalis (a lip muscle) during observation trials in which the experimenter moved to the correct target (blue) and to the opposite target (green). Activity is aligned on cursor motion onset (M) and averaged across trials.

directly observed actor, but they do not seem to be implicated in the prediction of impending actions or events based on arbitrary information.

In contrast, we report here that neurons in the dorsal premotor cortex (PMd) of monkeys might contribute to mental rehearsal of processes that normally occur before the onset of movement. Unlike mirror neurons, their activities predict the directionality of impending events before the onset of movement, they respond to abstract visual cues that have become associated with action only through training, and they do not require the actor to be in view. These PMd neurons emit very similar responses when the monkeys actively perform the motor task, indicating that passive observation of the artificial stimulus events might evoke a covert rehearsal of the same premotor neuronal operations that would normally lead to overt motor output.

Two monkeys learned a reaching task guided by visual stimuli presented on a computer monitor¹⁸ (Fig. 1a). During the first

instructed-delay period (spatial cue, SC), two colour-coded potential targets for movement were displayed briefly, and during the second delay period (colour cue, CC), one of them was designated as the correct target. The monkeys learned to make reaching movements in the appropriate direction in a horizontal plane, out of sight and out of the vertical plane of the screen, to move an on-screen cursor into the designated target (performance task). The monkeys usually made a saccade to the correct target just before reaching (Fig. 1b, left).

After lengthy training and weeks of neuronal recording with the performance task, we presented the monkeys with a novel situation. In a separate block of trials (observation task), the monkeys sat quietly and watched the usual task-related events unfold on the monitor while the task was performed by an unseen party. To encourage the monkeys to remain attentive to the observed events, they received a juice reward whenever the unseen party performed a trial correctly. The monkeys spontaneously exhibited two predictive

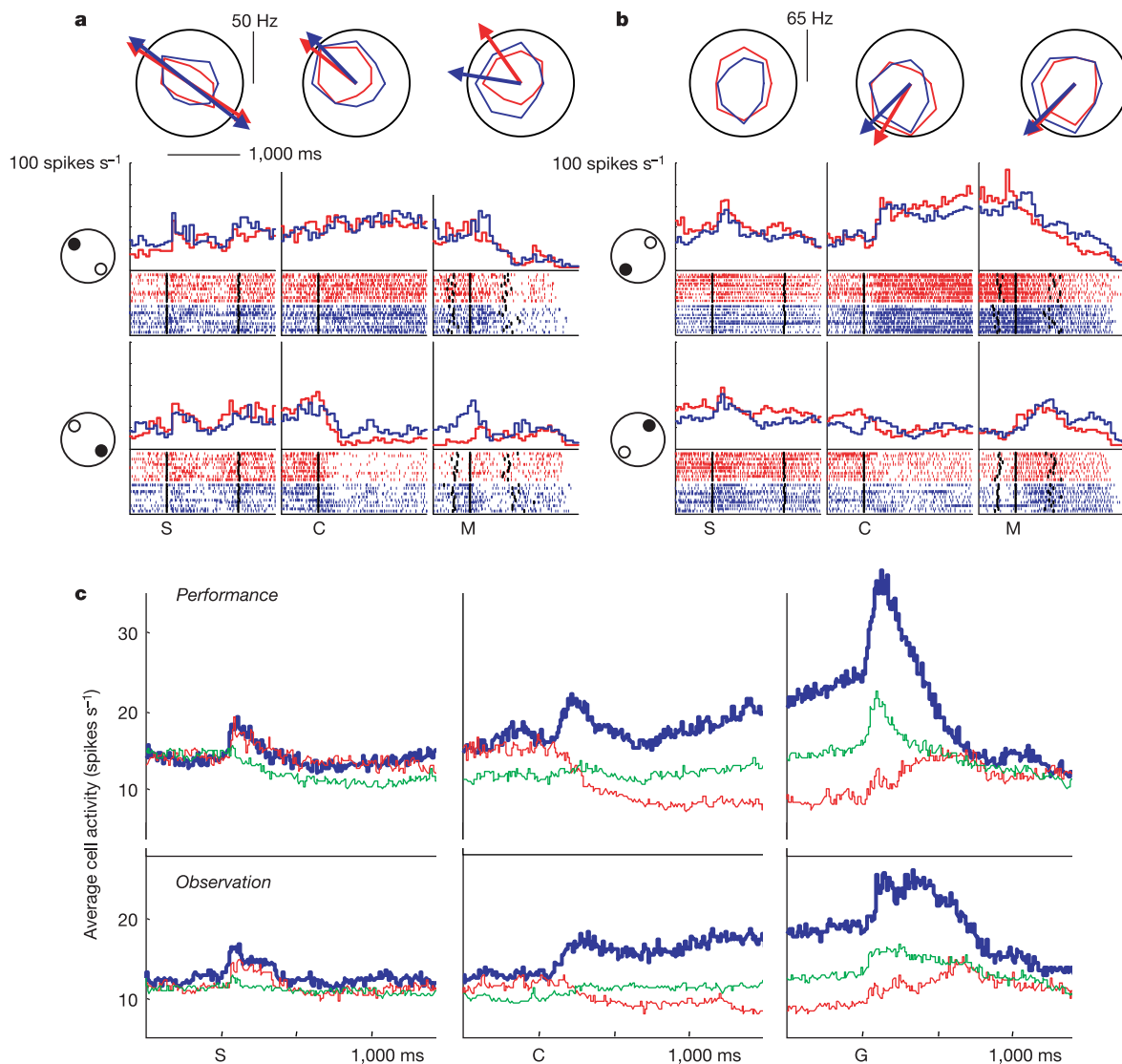


Figure 2 Neural data. **a**, Activity of a PMd cell during performance (red) and observation (blue). In the rasters, coloured marks indicate action potentials and black marks indicate behavioural events (from left to right: SC onset (S) and offset, CC onset (C), GO, movement onset (M) and offset). Data are shown for trials in which the cell's preferred target (upper) or the opposite target (lower) were specified by the CC, as indicated by the circular diagrams on the left. Polar plots show the cell's directional tuning function during SC, CC, and movement time (MT). Arrows indicate the preferred direction (or axis) of statistically

significant unimodal (or bimodal) tuning (bootstrap, $P < 0.01$). **b**, Activity of a second cell in the same format. **c**, Population histograms collected as the average activity of cells during trials to each cell's best tested direction (blue), opposite direction (red) or orthogonal directions (green). The best tested direction was defined using the preferred direction from the reaction time of performance, and only cells with significant tuning in that period were included ($N = 28$).

behaviours¹⁶, showing that they attended to the task events and knew when it was being performed correctly. First, after the GO signal but before the onset of observed cursor motion, the monkeys made a saccade to the correct target, demonstrating that their eye movements were guided by knowledge of what should happen next¹⁶. Saccade latencies were similar to those made when the monkeys performed the task themselves (Fig. 1b). Second, whenever the unseen party caused the cursor to move in the correct direction, the monkeys vigorously licked the reward tube long before the reward was delivered, but quickly stopped licking when it moved in the wrong direction (Fig. 1c), again long before the reward would have been delivered. This predictive behaviour by both monkeys demonstrated that they attended to task events, assessed their correctness¹⁵, judged the likelihood of impending reward, and acted on that judgment by licking the juice tube or not¹⁹.

The direct-matching hypothesis suggests that these behavioural phenomena are evidence of covert mental rehearsal of the learned motor task associated with the visual events, and predicts that individual neurons in arm-movement-related motor regions express qualitatively similar activity in both the observation and performance conditions. Furthermore, neural responses during observation should occur even before the onset of cursor motion and should signal the required direction of motion.

In accord with these predictions, the large majority of task-related PMd neurons recorded from both monkeys exhibited strikingly similar activity patterns during both performance and observation (Fig. 2), even before the onset of cursor motion. The responses were found in the first cell tested during observation in each animal. Of the 42 PMd cells eventually tested during observation, 38 were directionally tuned during one or more instructed-delay periods in the performance task, and 32 of 38 (84%) were also tuned during one or more instructed-delay periods in the observation task. For example, the cell in Fig. 2a discharged as soon as the target in the upper left was identified as a potential movement target during the SC period, regardless of whether the monkey intended to perform the associated actions (red rasters and histograms) or simply observed the instructional stimuli while the unseen party performed

the task (blue rasters and histograms). The cell in Fig. 2b discharged only after information was presented during the CC period that unambiguously selected the bottom left target, during both performance and observation conditions.

When the activity of PMd cells was pooled, the population signals during both performance and observation were strikingly similar (Fig. 2c). After the onset of the two SC stimuli, the activity of two populations of cells tuned to those directions (Fig. 2c, blue and red) increased relative to their activity for orthogonal directions (green). After the central colour change, activity tuned to the selected target (blue) increased while activity tuned to the unselected target (red) decreased. This showed that many PMd cells performed similar neuronal operations in response to the information provided by instructional cues during observation and performance.

Various control experiments and analyses were performed to determine whether these PMd responses simply reflected sensory driving, gaze-related or attention-related modulation, or overt motor outputs (see Supplementary Information). To summarize, it is unlikely that any of these alternative explanations could be the principal cause of the directionally tuned activity during observation.

After the GO signal, PMd cells often generated further task-related responses during observation (Fig. 2c). We investigated whether activity during the reaction time (RT), movement time (MT) and target hold time (THT) was related to the instructed cursor motion or to the motion that was actually observed. For a subset ($N = 20$) of the cells, the unseen party deliberately made errors after the GO signal, such as delaying movement or moving in the direction opposite to the correct target. Figure 3 illustrates the activity of one PMd cell, which became tuned just before and during movement. That cell showed remarkably similar responses whether the monkey (red) or the unseen party (blue) promptly performed correct responses. When the selected target was to the left, the cell became active shortly after the GO signal but before movement onset, even if the movement was delayed (Fig. 3, left, purple) and remained active until the correct leftward movement was completed. When the selected target was to the right, the cell was initially suppressed before movement onset, and then discharged only after

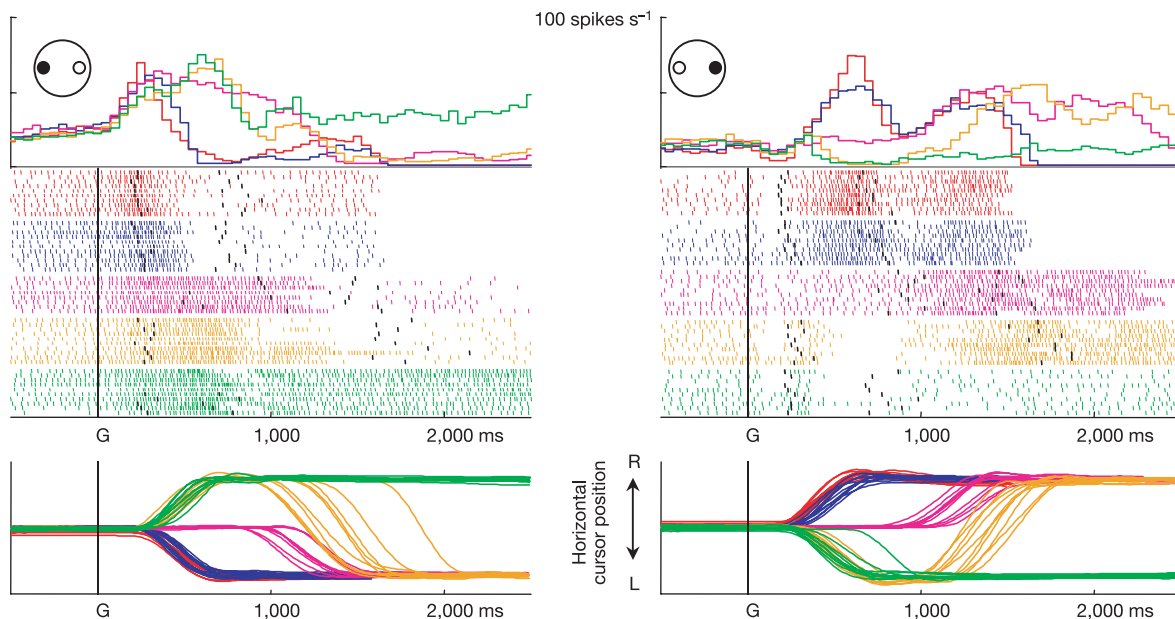


Figure 3 Activity of a PMd neuron during performance (red) and observation in trials in which the experimenter either moved promptly to the correct target (blue), delayed and then moved to the correct target (purple), moved to the opposite target and then reversed

(orange), or moved to the opposite target (green). Left: rasters, histograms and horizontal cursor position, aligned on the GO signal (G), during trials in which the correct target was on the left. Right: the same, for trials in which the correct target was on the right.

the correct rightward movement began (Fig. 3, right). In all cases, the activity of this cell during the unseen party's behavioural reaction time and for a brief time after cursor movement onset reflected the expected movement direction. Thus, even these early activity changes after the GO signal were centrally generated and were not a passive sensory response to cursor motion, which had not yet occurred. Later in the trial, cell activity often began to reflect the observed direction of cursor motion (see Supplementary Information for more quantitative analyses).

These PMd responses were very common. Our sole criterion for studying a cell during observation was that it was directionally tuned in the performance task. Nearly all (41 of 42, 98%) of the tested cells showed directional tuning during one or more of the movement periods in the observation task, and 32 of 42 (76%) showed directional tuning during one or more of the instructed-delay periods.

Although the phenomenon reported here in dorsal PM differs in several important ways from mirror neuron activity in ventral PM^{10–13,20}, it is also possible that they are functionally related. Both findings show that observation of external events can engage nominally 'motor' circuits, to generate motor representations of the actions associated with those events. This covert simulation or mental rehearsal of motor acts may contribute to nominally 'cognitive' functions underlying the assessment and understanding of observed events. For ventral premotor mirror neurons those events are natural and familiar, whereas for the PMd neurons they include sensory events whose relation to motor behaviour is acquired through learned stimulus–response associations.

Behavioural and functional imaging evidence suggests that mentally rehearsed motor actions obey the same constraints as overt actions^{3,5}, are similarly disrupted by lesions⁴, engage in a somatotopic manner⁶ many of the same brain areas (including PM) that are activated during overt motor behaviour^{2,7,9}, and facilitate the motor system in similar ways¹. The present findings indicate that mental rehearsal and overt motor planning involve very similar single-neuron operations. Mental rehearsal is still poorly understood, and may involve many of the processes associated with overt performance, ranging from representations of higher-level task goals and motor outputs to specific details of the limb movements. This study cannot distinguish the level of abstraction at which the PMd activity represented task-related events. However, when visual feedback is dissociated from the actual limb movements that produce it, PMd activity during the delay period covaries more closely with the directionality of visual feedback than with the direction of physical motor output^{21,22}. We propose that the activity reported here, in particular during the instructed-delay period, represents action in a coordinate frame related to desired cursor motions rather than specific limb movements^{21,22}. This is not a passive sensory representation. Through training, the cursor became a surrogate for the monkeys' arm, whose motion they control through arm movements. PMd generated a representation of intended motions of the controlled object (in this task, the cursor) in a coordinate framework related to the sensory feedback about the action^{21,22}, a variable of critical importance to the monkeys during both performance and observation. □

Methods

During the performance task, the monkeys controlled the motion of an on-screen cursor on a computer monitor positioned in front of them by moving the lower end of a two-degree-of-freedom pendulum in the horizontal plane with the arm contralateral to the cortical recording site. The monkeys could not see their arm, and task performance was guided entirely by the instructional cues and cursor motions on the monitor. During the observation task, the monkeys sat in the same position relative to the screen but both arms were lightly restrained, and the pendulum was moved by the experimenter who grasped it out of the monkey's sight. During the observation task, the monkeys received the usual juice reward each time the experimenter successfully completed a trial. The observation task did not form part of the training regimen before neural recordings. In both conditions, the monkey's gaze was unconstrained and eye movements were measured at 100 Hz with an infrared oculometer (Dr Bouis Devices, Karlsruhe).

During both tasks (Fig. 1a), each trial began when the on-screen cursor was moved into a central green circle (1.5 cm radius) for a 500-ms centre-hold time. Two coloured cue circles (radius 2 cm), one red and one blue, then appeared at two out of eight possible target locations on the circumference of a circle of radius 8 cm, for a spatial-cue-on period (1,000 ms) and then disappeared for a spatial-cue-off period (500–1,500 ms). Next, the central circle changed colour to either red or blue, for a colour-cue period (1,500–2,500 ms). This colour cue instructed which of the two memorized colour-coded spatial-cue locations was the selected target for the forthcoming movement. Finally, the central circle disappeared and green circles (radius 2 cm) appeared at all eight peripheral locations. This GO signal instructed the subject to move the cursor to the selected target.

Neurons were studied in both the performance and observation tasks in PMd in three hemispheres of the two monkeys. All recorded neurons were collected from the cortex along the rostral bank of the arcuate sulcus and rostral to the arcuate spur, spanning a region from 5 mm caudal to 3 mm rostral to the genu of the arcuate²³. All training, surgical and recording procedures were performed in accordance with institutional, Canadian and NIH guidelines. Further methodological details can be found in the Supplementary Information.

Received 2 August; accepted 13 September 2004; doi:10.1038/nature03005.

- Jeannerod, M. The 25th Bartlett Lecture. To act or not to act: perspectives on the representation of actions. *Q. J. Exp. Psychol. A* **52**, 1–29 (1999).
- Jeannerod, M. & Decety, J. Mental motor imagery: a window into the representational stages of action. *Curr. Opin. Neurobiol.* **5**, 727–732 (1995).
- Sirigu, A. et al. Congruent unilateral impairments for real and imagined hand movements. *Neuroreport* **6**, 997–1001 (1995).
- Sirigu, A. et al. The mental representation of hand movements after parietal cortex damage. *Science* **273**, 1564–1568 (1996).
- Papaxanthis, C., Schieppati, M., Gentili, R. & Pozzo, T. Imagined and actual arm movements have similar durations when performed under different conditions of direction and mass. *Exp. Brain Res.* **143**, 447–452 (2002).
- Buccino, G. et al. Action observation activates premotor and parietal areas in a somatotopic manner: an fMRI study. *Eur. J. Neurosci.* **13**, 400–404 (2001).
- Crammond, D. J. Motor imagery: never in your wildest dream. *Trends Neurosci.* **20**, 54–57 (1997).
- Jeannerod, M. Neural simulation of action: a unifying mechanism for motor cognition. *Neuroimage* **14**, S103–S109 (2001).
- Johnson, S. H. et al. Selective activation of a parietofrontal circuit during implicitly imagined prehension. *Neuroimage* **17**, 1693–1704 (2002).
- di Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V. & Rizzolatti, G. Understanding motor events: a neurophysiological study. *Exp. Brain Res.* **91**, 176–180 (1992).
- Fadiga, L., Fogassi, L., Gallese, V. & Rizzolatti, G. Visuomotor neurons: ambiguity of the discharge or 'motor' perception? *Int. J. Psychophysiol.* **35**, 165–177 (2000).
- Rizzolatti, G., Fogassi, L. & Gallese, V. Neurophysiological mechanisms underlying the understanding and imitation of action. *Nature Rev. Neurosci.* **2**, 661–670 (2001).
- Umiltà, M. A. et al. I know what you are doing. A neurophysiological study. *Neuron* **31**, 155–165 (2001).
- Jeannerod, M., Arbib, M. A., Rizzolatti, G. & Sakata, H. Grasping objects: the cortical mechanisms of visuomotor transformation. *Trends Neurosci.* **18**, 314–320 (1995).
- Ramnani, N. & Miall, R. C. A system in the human brain for predicting the actions of others. *Nature Neurosci.* **7**, 85–90 (2004).
- Flanagan, J. R. & Johansson, R. S. Action plans used in action observation. *Nature* **424**, 769–771 (2003).
- Ferrari, P. F., Gallese, V., Rizzolatti, G. & Fogassi, L. Mirror neurons responding to the observation of ingestive and communicative mouth actions in the monkey ventral premotor cortex. *Eur. J. Neurosci.* **17**, 1703–1714 (2003).
- Cisek, P. & Kalaska, J. F. Simultaneous encoding of multiple potential reach directions in dorsal premotor cortex. *J. Neurophysiol.* **87**, 1149–1154 (2002).
- Watanabe, M. et al. Behavioral reactions reflecting differential reward expectations in monkeys. *Exp. Brain Res.* **140**, 511–518 (2001).
- Kohler, E. et al. Hearing sounds, understanding actions: action representation in mirror neurons. *Science* **297**, 846–848 (2002).
- Shen, L. & Alexander, G. E. Preferential representation of instructed target location versus limb trajectory in dorsal premotor area. *J. Neurophysiol.* **77**, 1195–1212 (1997).
- Ochiai, T., Mushiaki, H. & Tanji, J. Effects of image motion in the dorsal premotor cortex during planning of an arm movement. *J. Neurophysiol.* **88**, 2167–2171 (2002).
- Cisek, P., Crammond, D. J. & Kalaska, J. F. Neural activity in primary motor and dorsal premotor cortex in reaching tasks with the contralateral versus ipsilateral arm. *J. Neurophysiol.* **89**, 922–942 (2003).

Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements We thank R. Ajemian, T. Drew and S. Wise for comments and suggestions on earlier versions of this manuscript. This study was supported by operating grants to J.F.K. from the Canadian Institutes of Health Research, the Human Frontier Science Program, and the New Emerging Team Grant in Computational Neuroscience (CIHR), and by NIH and FCAR postdoctoral fellowships to P.C.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to J.F.K. (kalaskaj@physio.umontreal.ca).