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Neurons in primary motor cortex engaged during action observation

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Abstract

Neurons in higher cortical areas appear to become active during action observation, either by mirroring observed actions (termed mirror neurons) or by eliciting mental rehearsal of observed motor acts. We report the existence of neurons in the primary motor cortex (M1), an area that is generally considered to initiate and guide movement performance, responding to viewed actions. Multielectrode recordings in monkeys performing or observing a well-learned step-tracking task showed that approximately half of the M1 neurons that were active when monkeys performed the task were also active when they observed the action being performed by a human. These 'view' neurons were spatially intermingled with 'do' neurons, which are active only during movement performance. Simultaneously recorded 'view' neurons comprised two groups: approximately 38% retained the same preferred direction (PD) and timing during performance and viewing, and the remainder (62%) changed their PDs and time lag during viewing as compared with performance. Nevertheless, population activity during viewing was sufficient to predict the direction and trajectory of viewed movements as action unfolded, although less accurately than during performance. 'View' neurons became less active and contained poorer representations of action when only subcomponents of the task were being viewed. M1 'view' neurons thus appear to reflect aspects of a learned movement when observed in others, and form part of a broadly engaged set of cortical areas routinely responding to learned behaviors. These findings suggest that viewing a learned action elicits replay of aspects of M1 activity needed to perform the observed action, and could additionally reflect processing related to understanding, learning or mentally rehearsing action.

Introduction

During motor skill learning, observation and practice presumably engage neural mechanisms to create internal motor representations, which then provide the ability to accurately reproduce those voluntary actions. Mirror neurons, which are active both when an action is performed and when that same action performed by another is being viewed, have been proposed as one possible basis of action knowledge acquisition (Grafton *et al.*, 1997; Rizzolatti *et al.*, 2001; Rizzolatti, 2005; Umilta *et al.*, 2001; Buccino *et al.*, 2004; Rizzolatti & Craighero, 2004). Mirror neurons, according to the above definition, have been identified in the ventral premotor cortex (PMv) and inferior parietal cortex (Rizzolatti *et al.*, 1996a; Fogassi *et al.*, 2005) in monkeys. Indirect methods in humans suggest that mirror responses may occur in homologous areas in the human inferior frontal gyrus as well (Decety *et al.*, 1997; Buccino *et al.*, 2004; Iacoboni *et al.*, 2005; Aziz-Zadeh *et al.*, 2006; Molnar-Szakacs *et al.*, 2006). Because the mirror neuron system responds to the viewing of natural actions, and not abstractions such as static video images (Craighero *et al.*, 2007), it has been linked to action recognition and understanding (Rizzolatti *et al.*, 2001). Furthermore, a class of veridical mirror neurons predicts hidden goals to join nonidentical observed and executed actions that serve a common goal (Gallese *et al.*, 1996; Newman-Norlund *et al.*, 2007). Veridical neurons are also influenced by task complexity (Iacoboni *et al.*, 1999; Buccino *et al.*, 2004) and motivation or effector orientation (Maeda *et al.*, 2002; Cheng *et al.*, 2007). These findings demonstrate that motor learning through observation leads to widespread activation of parietofrontal circuits.

A large body of data has shown that the primary motor cortex (M1) is directly engaged in action generation. M1 neurons become active before a movement is performed, and this activity correlates with performed actions. M1 activity is necessary for the initiation and control of voluntary movement, and is presumably downstream of cortical areas containing mirror neurons that may eventually engage the M1 to enact movements. M1 has not been generally held to be part of the mirror neuron system (Gallese *et al.*, 1996; Iacoboni *et al.*, 1999; Fogassi *et al.*, 2001). However, a body of indirect and conflicting evidence from functional imaging, electroencephalography, magnetoencephalography, metabolic labeling and transcranial stimulation suggests that the M1 may be engaged during action observation (Fadiga *et al.*, 1995; Cochin *et al.*, 1998; Hari *et al.*, 1998; Nishitani & Hari, 2000; Baldissera *et al.*, 2001; Raos *et al.*, 2004, 2007; Montagna

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et al., 2005; Caetano *et al.*, 2007). Conversely, positron emission tomography scans have failed to find M1 labeling in mirror tasks (Rizzolatti *et al.*, 1996b; Decety *et al.*, 1997). The fact that some single-neuron studies had also failed to find M1 mirror neurons (Gallese *et al.*, 1996; Fogassi *et al.*, 2001) led to a conclusion that indirect methods may be detecting field potential activity related to mirror input to M1, and not spiking in response to action viewing (Hari *et al.*, 1998). However, transcranial magnetic stimulation above the precentral gyrus produces a larger response in muscles that are used in a task when the subject views another performing that task (Fadiga *et al.*, 1995), suggesting that M1 neurons are engaged during viewing.

Neurons engaged in the dorsal premotor cortex (PMd) (Cisek & Kalaska, 2004) and the M1 (Wahnoun *et al.*, 2006; Tkach *et al.*, 2007) associated with action viewing have been observed, but this activity has been interpreted as mental rehearsal of a learned motor action and not processes related to action recognition/understanding, which are the hallmark of mirror activity. Cisek & Kalaska (2004) carefully outlined the evidence for neurons in the PMd fitting best with mental rehearsal. In their study, neurons fired in anticipation of an abstraction of action instead of responding to it; both eye movements and licking suggested that the activity was elicited by an impending action that led to a reward. They concluded that the similar forms of single-neuron discharge in these two conditions reflected the rehearsal of the motor activity that would generate the reward if performed by the monkey. Wahnoun *et al.* (2006) and Tkach *et al.* (2007) found similar neurons in the M1, suggesting that neural activity observed in this area during viewing of only cursor motion is also mental rehearsal of action. More recently, it has been shown that M1 neurons are engaged in humans with paralysis who are explicitly rehearsing actions in the absence of purposeful movement (Hochberg *et al.*, 2006; Truccolo *et al.*, 2008). Whether these neurons would be engaged by viewing only was not tested, and testing their activity during movement performance in these people was not possible. In these studies, activity was not tested during viewing of a human producing the action, one feature of mirror neurons. Here, we demonstrate, in three monkeys, the existence of M1 'view' neurons that were engaged during the performance of a visuomotor task and also when viewing a human agent performing the same task. These 'view' neurons were spatially distributed among a set of simultaneously recorded neurons that were only active during movement. On the basis of differential timing, spatial distribution and directional tuning features of these neurons while movement was performed or viewed, we suggest that viewing an action elicits an internal or mental rehearsal in populations of M1 neurons, but that this population also reflects properties of mirror neurons at the same time.

Materials and methods

Recordings

Three female macaque monkeys weighing 4.5–6 kg (RN, CL, and LA) were studied in these experiments. Surgical multielectrode array implantation procedures and recording methods followed those previously reported (Paninski *et al.*, 2004; Suner *et al.*, 2005). Animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care, National Institutes of Health-approved facility, and the study was approved by the Animal Care and Use Committee of Brown University, and in accordance with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council). All operations were performed using standard sterile procedures in an approved animal surgical facility. Analgesics and antibiotics were administered postoperatively as needed, using established protocols and veterinary supervision, and have been reported elsewhere (Suner *et al.*, 2005). Briefly, the animal was sedated with ketamine hydrochloride (15 mg/kg) before surgery, and received antibiotic (Claforan 50 mg/kg), steroid (dexamethasone 0.5 mg/kg), and analgesic (buprenorphine 0.01 mg/kg). The animal's head was shaved and placed in a stereotaxic headholder. During the surgical procedure, deep and stable anesthesia was maintained with 1–2.5% isoflurane. Warm, lactated Ringer's solution was administered at a rate of 5–10 mL/kg/h. The surgery was performed under aseptic conditions, with continuous monitoring of heart rate, respiration rate, expired CO_2, arterial O_2 saturation and body temperature to maintain a stable and deep plane of anesthesia. After surgery, the animal was observed until it was spontaneously moving and holding its head upright. Buprenorphine (0.01 mg/kg) for analgesia was administered intramuscularly 8–12 h after the procedure, and was continued on subsequent days, together with antibiotic therapy (Claforan 50 mg/kg), under the direct supervision of a facility veterinarian who is highly experienced with nonhuman primate clinical care. After a suitable recovery period as specified by the veterinarian, and acclimatization to head restraint over a 2–4 week period, neurons were recorded simultaneously from a 96-microelectrode array chronically implanted in the M1 arm area. Electrodes were arranged in a 10×10 array (4×4 mm base), each spaced by 400 μm and 1 mm in length (Blackrock Microsystems, Salt Lake City, UT, USA). Electrode impedance ranged between 100 and 750 kΩ, at 1 kHz. Signals were recorded during sessions lasting for up to 3 h while the monkey either performed or viewed visually guided movement tasks. Waveforms were stored and spike sorted using OfflINE Sorter (Plexon, Dallas, TX, USA). Principal component clusters, autocorrelation functions, inter-spike interval distributions and signal-to-noise ratio (S/N) were used offline to classify each recorded waveform as a neuron, using the same criteria across all tasks. S/N was defined as the difference in mean peak-to-peak voltage divided by twice the mean standard deviation of waveforms at each of 50 sample time points over all acquired spikes and then averaged (Suner *et al.*, 2005). All cells with S/N = 3 were included for analysis. This comprised more than one cell from some electrodes.

Electrode array location

Array insertion in the M1 arm representation was guided by surface landmarks identified intraoperatively. The array was placed as far posteriorly as possible, to be immediately anterior to the precentral gyrus and medial to a line reflected posteriorly from the genu of the arcuate sulcus at the level of the principal sulcus (Suner *et al.*, 2005). This location reliably provides recordings of neurons related to arm actions.

Tasks

We evaluated the same neuron population during the performance of a well-learned point-to-point arm-reaching ('do' condition) and then during observation of that same task as it was performed by a human ('view' condition). During sessions, monkeys were seated in a primate chair with the head fixed. Monkeys were trained to make step tracking arm movements that were instructed by visual targets displayed on a vertically oriented computer monitor placed approximately 57 cm in front of the monkey. The monkey held a low-friction/low-inertia two-link manipulandum that allowed horizontal two-dimensional arm motions across a planar surface (Fig. S1, A and B). Hand position was

determined from a sensor in the manipulandum handle that moved across a 30-cm digitizing tablet (Wacom Technology, Vancouver, WA, USA). The position was sampled at 167 Hz with an accuracy of 0.25 mm, and recorded to disk. The hand position on the tablet was represented by a cursor of 0.6° (1.5-cm tablet radius) displayed on a monitor. The task was based on a standard center out format that required movement from a center start position, after a fixed hold, to one of eight targets placed equidistantly in a circle (Fig. S1, D). On a particular trial, a randomly selected target was displayed, which also served as a go cue. The monkey was required to begin movement to the target within 200 ms, and after 300 ms (CL) or 500 ms (RN and LA) of target hold, the monkey returned to the start position (Fig. S1, A and B). The short-duration (< 500 ms) hold period was used to reduce movement preparation and to circumvent movement of the eyes to the goal before the hand. Following four to six movements (randomly determined) to a set of targets, a juice reward was delivered. If a target was not acquired, the trial aborted and a target reappeared at a new position to begin the next trial. For the 'do' condition, the monkey's hand motion controlled the cursor; in the 'view' condition, the experimenter stood alongside the monkey to the side of the tablet, ipsilateral to the monkey's 'moving' arm, and moved the manipulandum to perform the identical task. In one session for monkey CL, the experimenter's moving hand was contralateral to the monkey's 'moving' arm. All actions accomplished by the experimenter involved the same apparatus and recording room, immediately following or preceding the 'do' task. In the 'view' task, the monkeys received rewards (one per four to six trials) while watching a human perform the task. In one experiment, in addition to the standard 'view' condition, the monkey observed parts of the 'view' task either with the monitor occluded ('view' device task), or while the hand performing the task was hidden from view ('view' screen task).

Frame of reference for muscle activity

The monkey's arm was always positioned in abducted posture for the 'do' task. It was adducted during the 'view' task in two monkeys (CL and LA), whereas in one (RN) it remained in the abducted posture for both tasks as a control for postural effects between both conditions. In order to restrict arm motion during viewing, monkeys were required to hold one or two lever arm hand-switches in the closed position using a sustained finger flexion motion. During the 'view' task, CL held switches in both hands, LA closed a switch with the 'moving' hand, and RN simply rested the 'moving' hand. In the latter case, a barrier blocked access to the manipulandum.

Task learning

Training and practice on the 'do' condition task spanned more than 1 year (typically five sessions per week). During a 1–2-month period prior to data collection, each monkey was exposed to four view sessions and performed the task in the same session. The data reported here were obtained after approximately one additional month of task exposure and data collection, during a single session for each monkey in which 111 (RN), 120 (CL) and 72 (LA) neurons were recorded simultaneously. Each single recording session ('do' and 'view' tasks) contained 400 (CL and LA) and 900 (RN) trials.

Preferred direction (PD) analysis

To evaluate the similarity of neural activity in the 'do' and 'view' tasks, we compared features of neural activity on a single-cell and population basis. We analysed only those trials that fell within one standard deviation of mean movement time. We first identified all neurons that showed a significant change in firing rate associated with cued movement, and then identified all neurons of this set that were directionally tuned. We measured differences in firing rate during movement and similarity of PD on a cell-by-cell basis. We also compared the relative timing of firing with respect to movement onset in the two conditions. Movement onset and end were defined using a hand speed threshold-crossing criterion of 0.2 cm/s (200–360 ms). The modulation of movement-related activity in each condition was determined by comparison of the firing rates around the times of real and observed movement [Kruskal–Wallis (KW) test, $P < 0.05$, mean firing rate comparison for intervals before and after movement onset]. The firing rates between 'do' and 'view' conditions were compared using a nonparametric test (KW test, $\alpha < 0.05$).

We determined the PD of each neuron on the basis of a cosine fit to the peak firing rate during the movement or viewing period across the eight directions. We applied a nonparametric bootstrap test (1000 samples) to define the 95% confidence interval for the cosine fit model (Amirikian & Georgopoulos, 2000), and to assess the statistical significance of cosine tuning (Cisek *et al.*, 2003). To identify a significant change in PD across conditions, we used a nonparametric bootstrap statistic (KW test, $P < 0.001$) to compare the PD in the view condition against a distribution derived from multiple recalculations of PD from subsets of trials in the 'do' condition (Cisek & Kalaska, 2004). Shifts of more than ± 25° around the mean PD were usually significantly different (Amirikian & Georgopoulos, 2003). We defined cells tuned only in the 'do' task as 'do' or movement-related cells, and those directionally tuned in both the 'do' and 'view' tasks as 'view' neurons. The population of view cells with PDs that were not significantly different in the view and 'do' conditions (bootstrap procedure, $P > 0.001$) were called similar PD (sPD) neurons. View cells for which PDs changed between the 'do' and 'view' conditions ($P < 0.001$) were called different PD (dPD) neurons.

Analysis of firing rate timing changes

The use of simultaneous multielectrode recording allowed direct within-session comparison of each neuron's timing and activation with respect to others in the population during identical conditions of motivation, attention, intention, and other general factors. Cross-correlations between the 'do' and 'view' conditions were performed separately for the reaction time (RT) and movement time (MT) intervals, allowing both the strength of similarity (correlation coefficient) and timing relations (lag of the correlation coefficient peak) to be evaluated. The RT interval began at the go cue and ended when the center of the moving cursor left the go cue target border; the MT was defined as the interval from when the hand speed exceeded 0.2 cm/s to when it dropped below that speed at the goal location. These timing relationships were used to provide an index of task involvement, where selective lags suggest that a population is more dominated by sensory feedback, and leads suggest a stronger central or predictive drive. Differences in the peak activation time during RT and MT behavioral epochs were used to identify shifts between 'do' and 'view' conditions. With these criteria, the RT and MT behavioral intervals did not overlap in time. Changes in the timing of firing were determined by the peak of the firing rate cross-correlogram of each neuron between the 'do' and 'view' conditions, computed for each interval. Timing differences were represented as a lead or lag in the peak time, calculated in 2-ms bins of the instantaneous firing rate during the MT and RT intervals respectively. A lead (positive time values) indicated

that M1 neurons fired earlier in the 'do' condition than in the 'view' condition, whereas a lag (negative time values) indicated that the cells fired earlier in the 'view' condition. We evaluated timing separately for sPD and dPD neurons, to test whether these groups had systematic and different timing shifts during viewing. For dPD cells, we performed correlations using their PDs. We compared the firing latency during viewing of human movements to the target closest to the new PD (i.e. the direction of maximum firing during viewing) with the firing latency during the monkey's movement to the PD target during the 'do' condition.

Classifier procedure

Standard state classification methods were used to identify population direction information and to compare it during the 'do' and 'view' conditions. A probabilistic Bayesian classifier (Shenoy *et al.*, 2003) was applied to predict target location α ($\alpha = 1, 2 \ldots 8$), using the mean firing rate $f_i(\alpha)$ for each neuron with the number of spikes n_i ($i = 1 \ldots N$, for N neurons) during a time interval t in each movement direction. The conditional probability for the number of spikes n to reach direction α is $P(a|n) = C(t,n)P(a)[(\prod_{i=1}^{N} fi(a)^n)_t \exp(-t\Sigma_{i=1}^{N} fi(a))]$. Spike rate was assumed to have Poisson distribution. The spike distribution was normalized with factor $C(t, n)$ so that the sum of the probabilities was equal to one. $P(\alpha)$ is the prior probability for each direction. We used the highest probability to identify the direction α from the multidimensional distributions of probabilities. Randomly selected subsets of trials were used for the different test data, and each set was cross-validated for each target location. The chance level of the classifier performance is the minimal statistically significant movement prediction, defined from the eight possible movement directions (12.5%). Classification methods were also used to compare the similarity of models do(do) (i.e. how well does the 'do' condition model predict the do direction of movement) and view(view) of action present during the 'view' and 'do' tasks. Thus, a classifier created from data obtained when the task is performed should classify trials from the do or view periods equally well if the same population model operates in both conditions. We used a Bayesian classifier (BC) with cross-validation to compare direction information available in the population during the 'do' and 'view' tasks by means of the models do(view) and view(do). When the cells in the view period were used to classify the performed movement, this was a 'do prediction based on the view model', termed do(view). Similarly, a classifier created from 'do' activity predicting direction in the 'view' task was called view(do). Although the term 'direction' is used here, it is important to note for this analysis that goal and movement direction were indistinguishable. Prediction of the direction of the instructed movement goal was made using all cells, and two subsamples: (i) all sPD neurons; and (ii) all dPD neurons.

We also evaluated trajectory information available in individual cell firing using correlation methods. The ability to reconstruct movement trajectory from 'view'-related population activity was evaluated by calculating the cross-correlation between the firing rate of each neuron and the observed kinematic trajectory, as determined from cursor motion while a human performed the task in view of the monkey. This analysis was performed only for those cells that retained the same PD (sPD neurons) in the 'do' and 'view' tasks. We calculated the single-trial correlations as a Pearson correlation $c[t]$ between the viewed movement trajectory $X[i] = \{x[1], x[2], \ldots, x[n - t]\}$ and the rate histogram of each tuning cell $y[i] = \{y[t + 1], y[t + 2], \ldots, y[n]\}$ ($i = 1 \ldots n$, for n sampling points in the MT interval). Bins were set equal to the A/D sampling interval (6 ms) of the reference variable coordinates of the trajectory X_t, Y_t. Correlation values were calculated as the mean of all cross-correlation values for each direction–cell pairing.

Eye movements

We measured eye movements to determine their possible contribution to neural activity during the view task. Eye movements during task observation were recorded during one session in CL, using an infrared eye-tracking system (ISCAN model-200) with a spatial resolution of 0.06°, a temporal resolution of 240 Hz, and a sampling rate of 500 Hz (Cerebus; Cyberkinetics Neurotechnology Systems, Inc.). We evaluated 51 directionally tuned neurons to test the hypothesis that the directional tuning of the neurons was a result of directionally tuned eye movement during the view task.

First, we examined eye movements during an approximate 500-ms time interval from the go cue to the time when the next target was reached by the hand motion. We selected trials having a short fixation on the target during the reaction interval and where the instantaneous eye movement speed (differentiation of the oculomotor signals) did not exceed 10 times the maximum of its amplitude, to avoid artefacts (blinking or closed eyes). The first 100 ms after the go cue was excluded to avoid potential confounds of perisaccadic activity (Cisek & Kalaska, 2002). In all other ways, the oculomotor behavior of the monkey was unconstrained (Fig. S1, D). We calculated the correlation between the rate histogram of each cell $y[i]$ ($i = 1, \ldots, n$), and the reference variable $x[i]$ as eye movement parameters: x_t^e and y_t^e are coordinates of the trajectory; v_x^e and v_y^e are coordinates of the velocity; v_m^e is the magnitude of the speed; and v_τ^e is the tangential velocity of the eye movement.

Results

Behavioral performance

Task performance by the monkeys and the experimenter were similar. Monkeys completed ~88% of trials correctly [98% (RN), 90% (CL), and 88% (LA)] and showed <50° deviation from a straight trajectory at any time during the movement. Human-completed correct trials were also ~90%. Peak speed during the trials was not significantly different between the monkey (9.97 ± 4.65 cm/s) and the experimenter (11.15 ± 8.76 cm/s; Wilcoxon rank sum test, $P = 0.06$). Mean MT for the do task was 266 ± 62 ms (pooled for three monkeys), and that for humans in the view task was 285 ± 78 ms (not significantly different; Kolmogorov–Smirnov test, $P = 0.91$; Fig. S1, C).

Neural activity patterns

As typically encountered in the M1, many neurons recorded across the chronically implanted multielectrode array were active in association with limb movement during the center out task. More than one unit was identified per electrode for 33% of the electrodes in RN, 28% in CL and 17% in LA within a recording session (Fig. S2). On the basis of shape and waveform S/N, the same neurons appeared to be recorded within a session across all tasks (Suner *et al.*, 2005) (Fig. S3). A subpopulation of movement-related neurons remained active when the task performed by a human was being viewed (Figs 1, 3 and 5). Of 303 neurons recorded in the three monkeys, a total of 227 (mean, 75%; 80 of 111 in RN, 76 of 120 in CL, and 71 of 72 in LA) showed significant modulation around the time of movement (KW test, $H = 3.7$, $P = 0.03$; mean firing rate comparison for intervals before and after movement onset) and were directionally tuned when

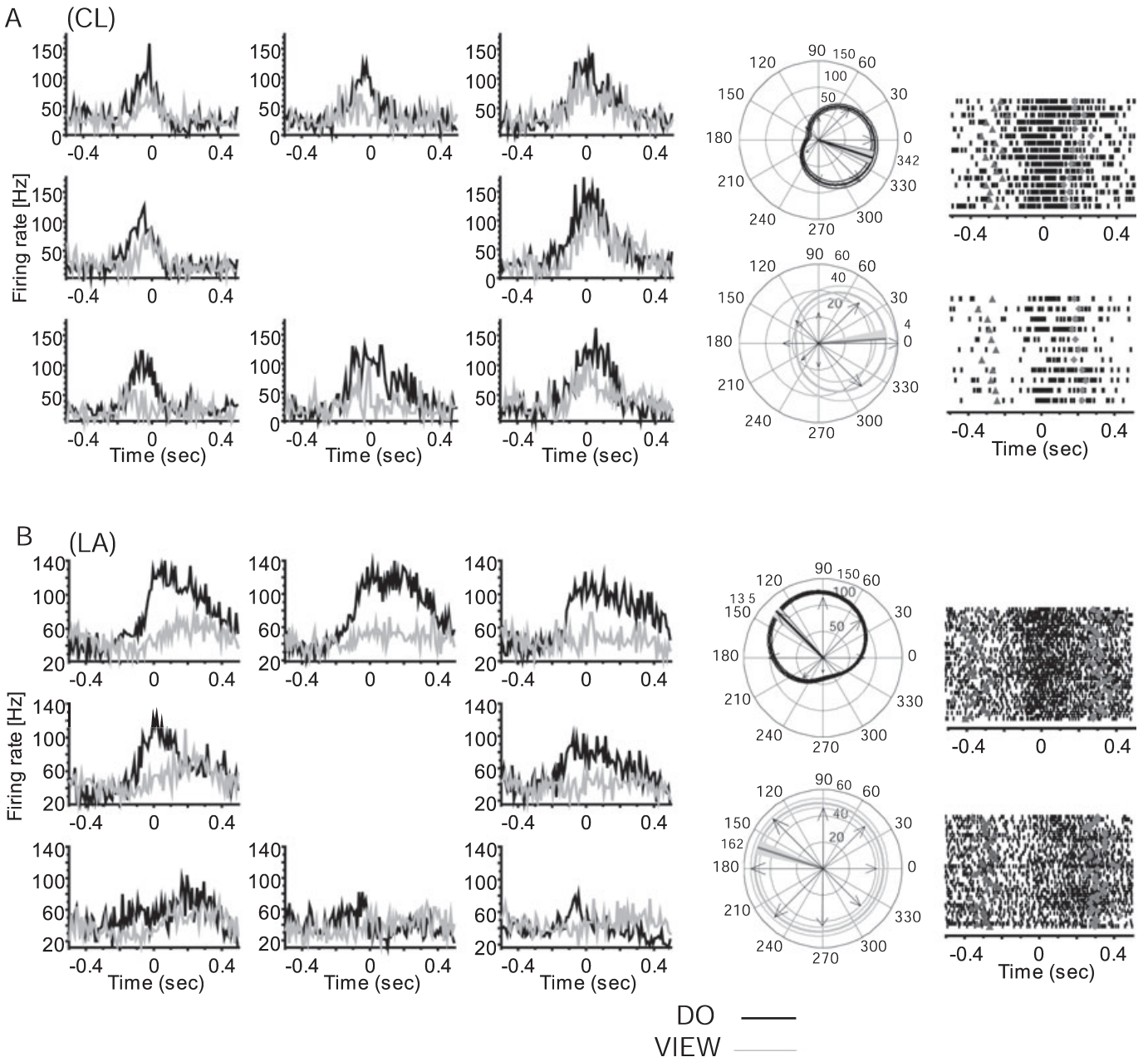

FIG. 1. Comparison of neural activity during performance and viewed action. Neurons in A and B show view task-related firing and directional tuning during action observation [preferred direction $(PD)_{(view)} = 4° \pm 8°$ (bootstrap, $P < 0.001$), $PD_{(view)} = 162° \pm 6°$ ($P < 0.001$)]. Left: perievent histograms of average firing across all trials aligned on the start of movement (time 0), showing 'do' (black) and 'view' (gray) task-related activity for sample primary motor cortex neurons in monkey CL (A) and monkey LA (B). Histograms are placed at the respective target locations. 'Do' and 'view' task-related firing for each of eight directions, where a rightward movement is towards 0° (middle–right histogram), and an upward movement is towards 90° (top–center histogram). Center: circular plots showing directional tuning in the 'do' condition (black, above) and the view condition (gray, below). The arrows indicate observed peak firing for each direction. The firing rate scale is reported near the 90° line; the circular plot shows the best fit cosine function with (95% confidence limit) for the best fit model; the thick straight line marks the PD and the gray shadow indicates the 95% confidence interval. (A) $PD_{(do)} = 342° \pm 7°$ (bootstrap, $P < 0.001$). (B) $PD_{(do)} = 135° \pm 5°$ ($P < 0.001$). Right: rasters showing firing rate for trials in each neuron's $PD_{(do)}$ (up) and $PD_{(view)}$ (down), aligned on the start of movement (0); the earlier triangles mark the go cue; the diamonds mark the end of movement. Note that firing in the view task is reduced and more variable than in the do task, but these neurons retain tuning and movement relationships across the two conditions.

the monkey performed the center out task. Of this task-engaged population, 54% (122/227; bootstrap, $H > 9.7$, $P < 0.001$) showed no significant modulation during the 'view' task (KW test, $H = 2.3$, $P = 0.06$; Fig. 2). However, the remaining 46% (105/227; bootstrap, $H > 11.5$, $P < 0.001$) of the 'do' task-engaged neurons also modulated and were directionally tuned during the 'view' task. Neurons directionally tuned in both 'do' and 'view' tasks were defined as 'view' cells (Figs 1 and 3; Fig. S4). All 'view' neurons changed firing rates and retained directional tuning in association with viewed action, although firing rates were significantly lower during viewing only, as can be appreciated both in trial rasters and in the histograms shown in Fig. 1. Mean firing rate during viewing across the population was significantly decreased to about half (46%; KW test, $H = 4.2$, $P = 0.02$) of that found in the do condition. PD changes and timing

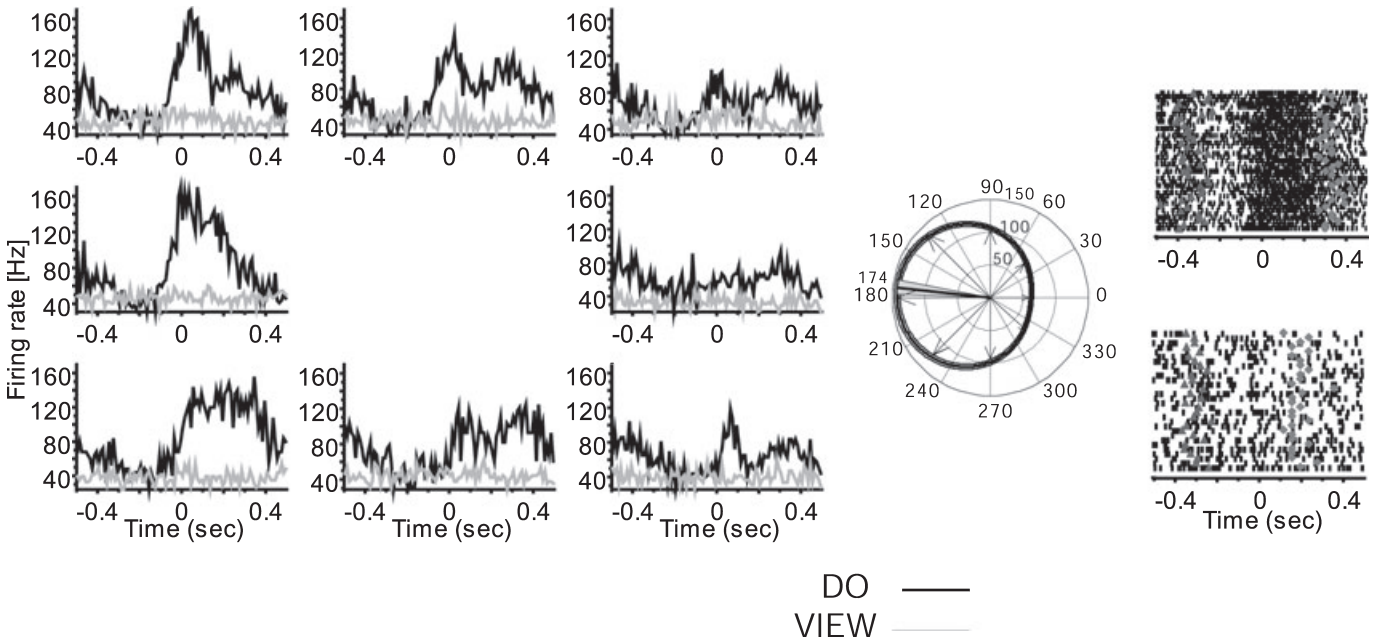

FIG. 2. Example of a primary motor cortex neuron that is only active during movement (same format as in Fig. 1). Note that this neuron is not modulated in the view condition (monkey LA).

TABLE 1. Comparison of 'view' neurons that retain and change their preferred direction (sPD and dPD, respectively)

	sPD neurons	dPD neurons
Percentage of view population	38% (40/105)	62% (65/105)
PD distribution (do, view)	Uniform	Not uniform
CC (RT)	0.77 ± 0.01	0.69 ± 0.02*
CC (MT)	0.72 ± 0.02	0.66 ± 0.01*
L (RT) (ms)	−16 ± 8.4	−10.3 ± 7.2
L (MT) (ms)	−4.8 ± 6.2	+2.5 ± 3.7**

Preferred direction (PD) distribution for each condition separately. CC, correlation of view firing rate with that in the do task; L (ms), leads/lags during the view task with respect to the do task; RT, reaction time; MT, movement time. *$P < 0.05$, dPD compared to sPD; **$P < 0.05$, lag in RT as compared with MT for dPD neurons. Different preferred direction; sPD, similar preferred direction.

differences between conditions separated two apparent subclasses of 'view' neurons, as summarized in Table 1. A minority of 'view' neurons maintained their PD (sPD cells) between the 'do' and 'view' conditions, but most shifted their PDs (dPD cells). Overall, 38% (40/105) of 'view' cells retained a similar PD (sPD; Fig. 1) in both conditions (bootstrap, $H < 1.6$, $P > 0.2$) and 62% of 'view' neurons (65/105; bootstrap, $H > 15.3$, $P < 0.001$) had different PDs (dPD cells) in the 'view' and 'do' conditions (Fig. 3; Fig. S4). The locations of these two populations were mostly nonoverlapping; the incidence of both types of cells being detected at one and the same location constituted only 7% (7/105) of 'view' cells. A previous study using the same 100-multielectrode array and a similar task showed that the somewhat randomly sampled population of MI arm area neurons had a roughly uniform distribution of PDs (Maynard *et al.*, 1999). In agreement with that study, we found that the sPD population had a uniform distribution of PDs in both 'do' and 'view' tasks (Table 1). However, the PDs were not uniformly distributed in either task for the dPD population (circular test, $P < 0.01$) (Fisher, 1993). In addition, dPD neurons showed a significant shift in their PD (Kuiper test, $P < 0.01$) (Fisher, 1993), in which PDs on average flipped approximately to the opposite direction (mean shift, 187° ± 84°) between the 'do' and 'view' conditions (Fig. S4, B). Because arm postural shifts from shoulder abduction to adduction made between the 'do' and 'view' tasks in two monkeys, rather than viewing alone, might have generated the PD rotations seen in dPD cells (Fig. 3), we compared PD shifts for one monkey in which the arm maintained the same posture in both the 'do' and 'view' conditions. The amount and direction of PD shift showed a similar distribution of direction change whether or not a postural shift was made (Fig. S5), suggesting that the selective rotation of PD of dPD cells during viewing was not the result of shoulder angle changes.

The regular 10 × 10 arrangement of electrodes in the recording array made it possible to evaluate whether there was an underlying spatial organization of 'view' neurons. The maps of the array location and distribution of cell features show that, in all three monkeys, there was no specific grouping of 'view' neurons across this 4 × 4 mm patch (Fig. S4, A). This demonstrates that neurons active during viewing and action were intermingled with action-selective neurons, at least within this part of the M1 arm area.

Timing and firing pattern relationships

Firing rates of 'view' neurons in the 'do' task were significantly correlated with that present during 'view' trials, although these correlations were lower for those cells that changed their preferred direction (Table 1; Fig. S6, A). 'View' neurons also had similar firing patterns in the 'do' and 'view' tasks, although those neurons that retained their PD were significantly better correlated with direction than those that changed their PD, during both the movement period (KW test, $H = 6.2$, $P = 0.005$) and the go cue interval (KW test, $H = 3.7$, $P = 0.04$).

Both classes of 'view' neurons (sPD and dPD) showed a range of changes in peak firing time between conditions (Table 1; Fig. S6, B),

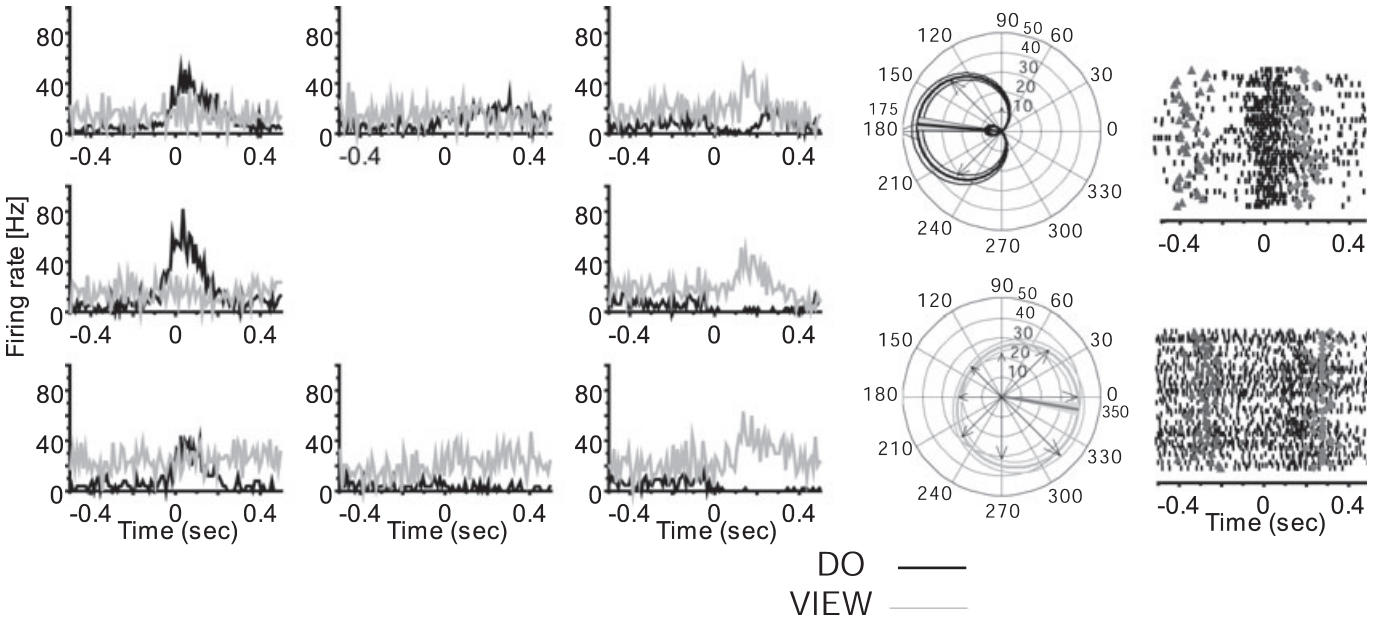

FIG. 3. Example of a 'view' neuron with changed preferred direction between the do and view conditions (same format as Fig. 1; monkey LA).

but as a population, they showed comparable times of peak discharge with respect to the go cue or to movement onset whether the monkey was performing or viewing the action, suggesting that view-related activity was predicting upcoming action. The onset of activity for the population of sPD neurons during viewed actions was not different from that during performed movement across the three monkeys. During the 'view' task, sPD neurons became active (16.3 ± 8.4 ms, mean ± standard error) earlier in the RT interval and the MT interval (4.8 ± 6.2 ms) than during the 'do' task, but neither shift was significant (KW test, $H = 1.3$, $P = 0.3$). Furthermore, dPD neurons reached their peak firing rate slightly, but not significantly, later (2.5 ± 3.7 ms) during the MT interval, and earlier (−10.3 ± 7.2 ms) during the RT interval. Although timing shifts for behavioral intervals RT and MT were not different across the 'do' and 'view' conditions within a class, sPD and dPD populations showed different timing shifts in the RT interval from those in the MT interval (Kolmogorov–Smirnov test, $P < 0.001$; Fig. S6, C). A test of the effect of trial interval on sPD and dPD cell activity indicated that there was a significant difference in timing shift pattern between RT and MT for dPD cells (KW test, $H = 4.8$, $P = 0.008$; Table 1; Fig. S6, B), but not for sPD cells ($H = 2.5$, $P = 0.06$). This greater spread in activation time only for dPD neurons suggests that those neurons that shifted their directional tuning also had changes in the firing pattern elicited by viewing, as compared with their pattern during movement. The temporal correlation of firing with viewed movement further supports a close relationship between movement and view activity.

Direction information in 'view'-active neurons

Classification methods were used here to test how well the performed or viewed movement direction could be predicted from the population activity on individual trials (see Materials and methods). Goal direction for movements was correctly predicted in 97, 98 and 85% of trials when movement was actually performed [RN, CL, and LA; Fig. 4; $BC_{(all)}$do(do) – 'do' model, tested on the 'do' condition], consistent with previous studies (e.g. Maynard *et al.*, 1999). Using a classifier model restricted only to sPD cell activity in the do condition, classifier success was moderately lower [$BC_{(sPD)}$do(do) = 88, 77, and

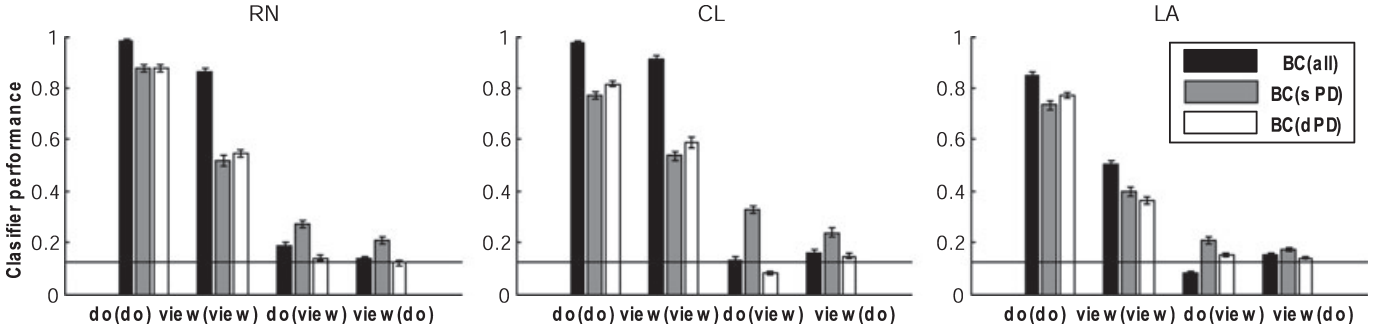

FIG. 4. Prediction of movement direction from primary motor cortex activity in the view and do conditions. Each bar shows the predicted direction of movement based on the entire population (all, black), those that retain the same preferred direction (PD) [similar PD (sPD); gray], and those that change their PD [different PD (dPD); white] in the 'view' and 'do' tasks for each of the three monkey (RN, CL, and LA). The Bayesian classifier (BC) has relative values in the range [0, 1], where 1 = perfect classification. Groupings show the results of different classifier models. The do(do) model predicts movement from a classifier built from data in the do task using a new do task trial; the view(view) model predicts viewed direction from 'view'-related activity; the do(view) model predicts movement in the 'do' task from 'view'-related activity; the view(do) model predicts viewed direction using a model created from the 'do' task activity. Horizontal line: chance level (12.5%).

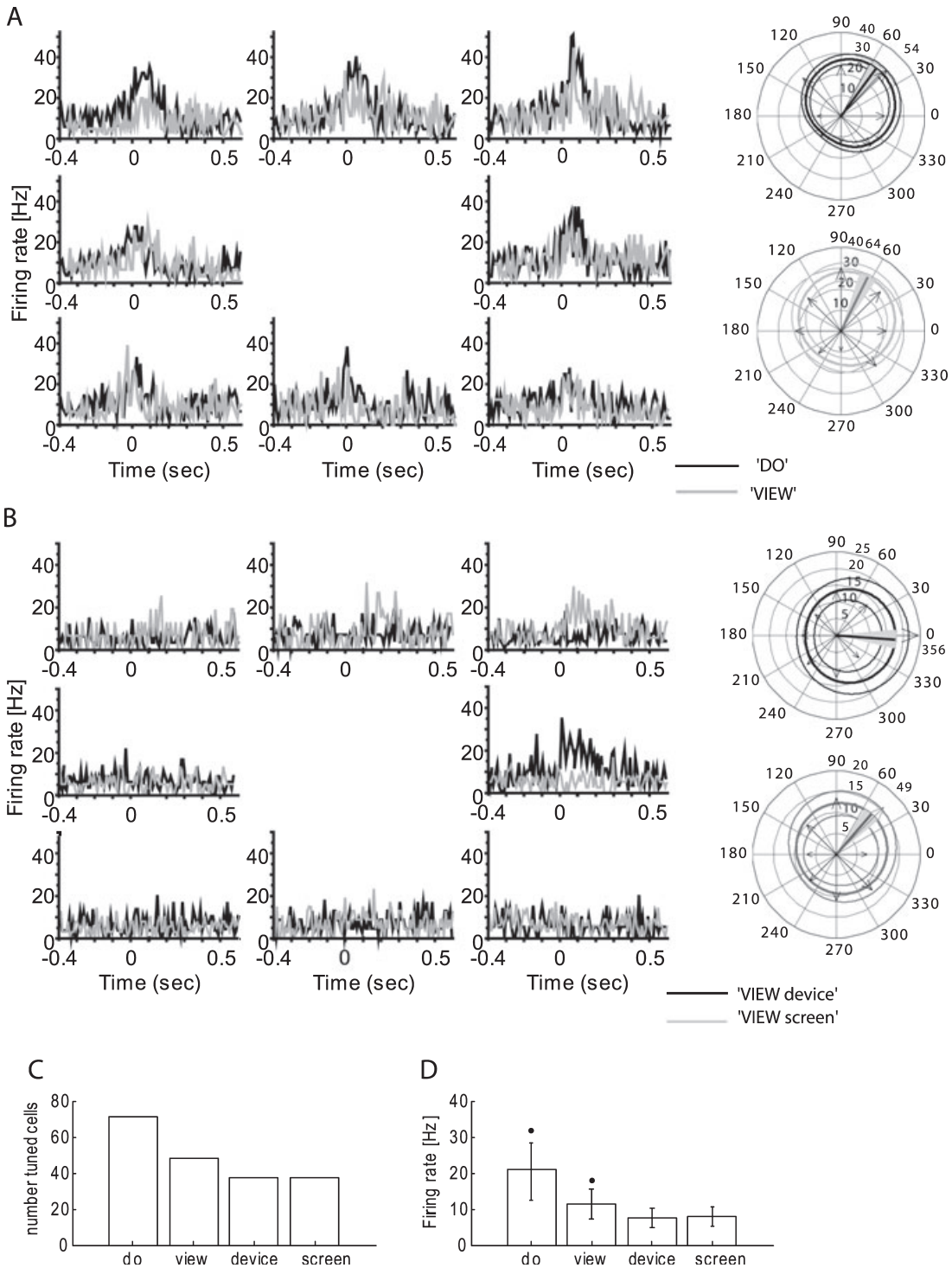

FIG. 5. Primary motor cortex activity for full and partial task conditions. (A and B) Histograms for a single neuron during (A) the 'do' task (black) as compared with the full 'view' task (gray), and (B) viewing only hand actions, termed 'view device' (black), or viewing only the screen, termed 'view screen' (gray). 'View' activity is markedly reduced and highly variable when only subcomponents of the task are present. The circular plots are in the same format as in Fig. 1. The preferred direction (PD) for each condition, marked with a thick line: (A) do, similar PD $(sPD)_{(do)} = 54° \pm 7°$; full view, $sPD_{(view)} = 64° \pm 8°$; (B) 'view device', $PD_{(view\ device)} = 356° \pm 9°$; 'view screen', $PD_{(view\ screen)} = 49° \pm 9°$; (C) number of tuned cells; (D) mean firing rate in full and partial task conditions. *Significant difference in the firing rate during the 'do' condition from that during the other three conditions, as well as during the 'view' condition from the 'do', 'view screen' and 'view device' conditions (Kruskal–Wallis test, $P < 0.05$).

74%; Fig. 5) than when all cells were used, perhaps because of the smaller dataset. However, the direction classification for this subpopulation $BC_{(sPD)}do(do)$ was significantly higher (KW test, $H = 15.8$, $P = 0.0001$) than for the dPD cells [$BC_{(dPD)}do(do)$], except for RN, where classification success was not different ($H = 0.84$, $P = 0.5$). These results demonstrate that the recorded population contained information about direction, or a correlate of direction, when the movement was performed.

The ability to predict observed direction was next evaluated using activity present during viewing [termed a view(view) comparison]. When the 'view' task model was applied to 'view' trials, classification success was reduced, but remained well above the 12.5% chance level in all three monkeys [$BC_{(all)}$view(view) = 85%, 91% and 50% for RN, CL and LA, respectively; Fig. 4]. Classification success using the entire population was significantly higher (KW test, $H = 10.3$, $P < 0.0005$) than achieved by either the sPD or dPD subpopulations in each monkey. When only those cells that retained the same PD across the 'view' conditions (sPD cells) were considered, classification success was also reduced, but remained above chance level [$BC_{(sPD)}$view(view) = 52, 54, and 40%). Direction classification success for dPD cells exceeded that for sPD cells in RN and CL, but not for LA [view(view); KW test, $H = 11.9$, $P < 0.0003$]. Thus, these results demonstrate that activity patterns during viewing contained substantial information about viewed actions, although less than predicted by the activity of neurons in the same region when the monkeys performed these actions.

Classification methods were next used to evaluate whether activity during viewing was the same as that during performance. If the same model operated in both conditions, a classifier created from data obtained when the task was performed should classify trials from do or 'view' periods equally well. This hypothesis was not supported. In general, classification success when predicting the direction of performed movement on the basis of activity during viewed actions, termed do(view), was at chance levels either when all cells in the view period were used to classify the performed movement or when only dPD cells were used in the classifier [Fig. 4; $BC_{(all)}$do(view), $BC_{(dPD)}$do(view)]. This suggests that the M1 was operating differently during viewing and performing the task. 'View' neurons preserved some features of movement during viewing but changed others, suggesting that these neurons were operating in a different modes in the two conditions. However, a classifier model from view cell activity incorporating only sPD cells was about twice the chance level in predicting direction [$BC_{(sPD)}$do(view) = 27, 33, and 21%], suggesting that this subpopulations of neurons retained a similar model during viewing and performing the task. The classifier $BC_{(sPD)}$, created from 'do' task activity, predicted direction in the 'view' task at about 1.7 times the chance level [$BC_{(sPD)}$view(do) = 21, 24, and 17%; KW test, $H = 9.2$, $P < 0.0009$]. Although the models built and tested on the same task were successful, these results indicated that M1 'view' sPD neurons transformed viewed action into a partial realization of the activity necessary to produce that movement.

In this task, activity related to goal or direction was not specifically separated. To attempt to disambiguate direction-related and goal-related activity, we computed the correlation of firing rate with trajectory for 15 sPD cells in CL. Firing rates of 'view' neurons were significantly correlated with observed arm and cursor trajectory during viewing, $c_m = 0.38 \pm 0.17$ (mean ± standard deviation), with single-cell correlations ranging from 0.8 to 0.03 across the cells. This suggests that 'view'-related activity carried information related to the details of the viewed movement trajectory and not just its goal.

Task contributions

Contribution of 'view' task components

During the 'view' condition, the monkey could observe at the same time the task evolving on the screen, which reveals a goal and an abstraction of the arm's action (i.e. cursor motion), its own nonmoving arm holding a stable position, and the experimenter's moving hand and manipulandum. In CL (Fig. 5), we evaluated how screen and hand components of the task separately influenced 'view' neuron activity. We compared the activity of cells: (i) during full view of 'do' or 'view' tasks; (ii) during separate observation of a human performing the task with the monitor occluded ('view' device); and (iii) with the hand and manipulandum hidden, and only the monitor visible ('view' screen). As compared with the full 'view' condition, M1 neurons showed significantly less firing, but retained weak directional tuning, when only task components were being viewed ('view device' and 'view screen'; Fig. 5). In this test, 40% of recorded neurons (48/120) were directionally tuned when the entire task was being observed (Fig. 5A and C). The number was reduced when only part of the task was evident [31% (37/120) for 'view device', and the same number for 'view screen'; Fig. 5C]. In addition, the overall mean population firing rate was about one-third lower when only components of the task were present (7.8 ± 5.2 Hz for view device, and 7.6 ± 5.2 Hz for view screen) as compared with the full view (11.4 ± 9.1 Hz; KW test, $H = 5.1$, $P = 0.006$; Fig. 5D). Firing rates in the 'view device' and 'view screen' conditions were not significantly different (KW test, $H = 2.4$, $P = 0.06$). These data indicate that viewing any component of the task continued to activate some M1 neurons weakly, but that viewing the entire task produced significantly greater activation in a larger population of cells.

Contribution of other behavioral variables

The task used here involved other associated behaviors that might influence neuronal activity, including gaze shifts, reward contingencies, and limb actions. Although not described for the M1 arm region, the ability of the eyes to move freely would allow gaze shifts to contribute to 'view'-related activity (Cisek & Kalaska, 2002). To test this hypothesis, we compared eye position, recorded with an infrared eye tracker, and firing during the 'view' task in CL. While the monkey was viewing the task, its gaze was directed variously at the screen, the moving hand, or other locations (Fig. S1, D). The mean correlation, c_m, for all cells between the eye movement trajectory and rate histograms of the tuned cells was weak and not significant (mean ± standard deviation: $x_t^e = 0.015 \pm 0.0086$; $y_t^e = 0.0154 \pm 0.059$). Likewise, correlation with eye movement velocity (x and y), eye speed and tangential velocity was not significant. The highest c_m for any single cell did not exceed 0.018. From these data, we concluded that eye movements in this task were not directly correlated with neural activity (Fig. S1, D; see Materials and methods), and could not account for the 'view' task-related modulation in the M1 arm area.

We next addressed whether 'view' activation was sensitive to the laterality of the viewed arm. 'View' neurons were found in both hemispheres contralateral to the arm used in the 'do' task, which was the right hemisphere in one monkey (CL; 31% of all PD neurons were 'view' neurons; 37/120 on the basis of one recording session) and the left hemisphere in the other two (RN, 32%, 35/111; LA, 46%, 33/71), where the monkey viewed the experimenter's arm performing the task alongside the same arm as used by the monkey in the 'do' condition. In one monkey (CL), 'view' activity existed whether the experimenter's moving hand was located contralateral (40%, 48/120) or ipsilateral (31%, 37/120) to the arm used by the monkey to perform the task. Despite the limited sample here, this finding suggests that both hemispheres are engaged during action observation, and that the precise view of the agent may not be essential to evoke this activity.

'View'-related firing could also have been influenced by the expectation of rewards. Our task design made it possible to evaluate the influence of reward expectancy during 'view' trials, because reward was never delivered on the first three trials, and was randomly

delivered on the subsequent fourth to sixth trials. This design has increasing probability of reward across trials 4–6 (33, 66, and 100%). Firing rates during unrewarded trials (the first three trials) were significantly greater than during the rewarded trials (trials 4, 5, or 6) (KW test, $H = 4.9$, $P = 0.007$), indicating that the view influence on cell activity diminished as reward expectancy increased. Thus, reward was not a correlate of increased firing.

Finally, uncontrolled hand motions during task viewing could account for view-related activity. We controlled for covert hand movements by requiring that the monkey maintain a hand-switch in a closed position using finger flexion with one (LA) or both hands (CL) during viewing, thus preventing both overt mimicry of the viewed action and the active engagement of the same limb in another action during viewing. Review of the hand video for RN, in which there was no required hold, showed virtually no hand motion during task trials, and none of the rare movements was systematically related to viewed action. In this case, the monkey simply held a plastic plate that was part of the chair. Thus, it is unlikely that activity was the result of systematic hand motion during viewing.

Discussion

These experiments reveal that a substantial subpopulation of MI neurons is actively engaged both when a well-learned skilled action is being performed and when a human performing that same action is being viewed. Using simultaneous multielectrode recording methods, we have demonstrated that 'view' neurons are interspersed within a larger population of movement-related neurons that are only active when action is performed. Firing is more variable and of lower intensity during viewing, but is generally similar to the activity observed during performance, in that 'view' neurons modulate around the time of movement, retain directional tuning, and contain information about movement trajectory. Thus, activity during viewing resembles that generally observed during performed actions. However, differences among 'view' neurons suggest that there may be two subgroups, which we identified as those that retain (sPD) and those that change (dPD) their PDs across the two conditions. 'View'-related firing in the M1 for this well-learned task best represents action when the entire task, including the agent and the abstraction of the task, is viewed. These properties suggest that the M1, an area closely linked to movement production, is also involved in movement rehearsal or a related prospective activity when action performed by another agent is being observed.

We found that a substantial number, nearly half (46%), of all directionally tuned M1 neurons recorded were active during both viewing and movement. Previous studies found that approximately 70–90% of PMd neurons (Cisek & Kalaska, 2004) and 70% of M1 neurons (Wahnoun *et al.*, 2006; Tkach *et al.*, 2007) were engaged during viewing the motion of cursors that constituted an abstraction of a learned movement, without the monkey seeing either its own arm or the agent performing the viewed task. Thus, it appears that a smaller percentage of directionally tuned neurons may be engaged when an agent and an abstract representation of that task are being viewed than when the abstraction of a task alone is being viewed. The lower percentage of tuned, engaged neurons is not likely to be related to sampling biases, because similar nonselective array methods were used by Tkach *et al.* (2007) and Wahnoun *et al.* (2006). Task, training or reward contingencies might also contribute to variability in these responses. The body of studies so far shows that many M1 neurons are actively spiking when learned actions are being viewed, which helps to explain earlier stimulation and imaging studies that suggested M1 activation during various forms of observation using these indirect methods to measure cortical activity (Cochin *et al.*, 1998; Hari *et al.*, 1998; Nishitani & Hari, 2000; Baldissera *et al.*, 2001; Raos *et al.*, 2004, 2007; Montagna *et al.*, 2005; Caetano *et al.*, 2007).

Our results suggest that dPD and sPD groups may form two classes of 'view' neurons in M1 that have not been previously recognized. As compared with their activity during movement, dPD and sPD neurons differed in their tendency to shift PD and in their pattern of shift in peak firing time between the 'do' and 'view' conditions. We found that 38% of M1 'view' neurons retained the same directional tuning present during movement (sPD cells), but a majority (62%) showed marked tuning shifts (dPD). In our experiments, when monkeys viewed the task, dPD neurons as a population showed a significant change in their PD. These shifts occurred while other neurons in the same, simultaneously recorded population did not change. This selective effect on a subset of neurons rules out the possibility of a simple underlying global mechanism, and suggests one that is more selective with respect to these two conditions. The PD shift was not due to a postural change, because both types of neurons were also observed when the posture was held constant across the 'view' and 'do' conditions. Timing shifts also differentiated the dPD and sPD groups. Neurons that shifted their PD peaked later with respect to movement onset than neurons that retained the same PDs (Table 1), across the 'do' and 'view' conditions. Interestingly, dPD neurons did not have a uniform distribution of their PDs during either performance or viewing, whereas sPD neurons retained a regular distribution of PDs, even within our small sample of neurons. This collection of distinguishing features suggests that part of the view-selective population more closely mimics the learned action, because they retain the same properties across conditions, whereas another set is engaged in a different manner during viewing. However, the mechanism that leads to this segregation is not clear.

The sPD/dPD subpopulation hypothesis is further supported by the superior direction decoding of the monkey's performed movement using 'view' period activity of the sPD neurons as compared with the dPD cells. That is, sPD activity during viewing appeared to be a closer match to the actual activity produced when movement is performed. The dPD neurons in our data resemble the set of cells described by Wahnoun *et al.* (2006), which appeared to have different tuning for viewed and performed movements. By contrast, Tkach *et al.* (2007) found only a small subset of M1 neurons that shifted their PD between acting and viewing. These investigators encountered a greater percentage of 'view' neurons than in our study, and had a larger sample than Wahnoun *et al.* (2006), suggesting that they probably did not miss 'view' cells. We cannot readily attribute these differences to the task, as Wahnoun *et al.* (2006) and Tkach *et al.* (2007) both showed only an abstraction of action, whereas in our experiment monkeys viewed both the agent, a physical device that created abstract cursor motion, and a visual representation of that task on a monitor. Eye movements are not likely to have accounted for the differences between the 'do' and 'view' conditions because our monkeys looked freely at various aspects of the task. By contrast, in Cisek & Kalaska (2004) and Tkach *et al.* (2007), monkeys appeared to track the target cursor with eye movements, perhaps because other distractors, such as the experimenter, manipulandum, and screen, were not viewed in these other studies.

Finally, our monkeys were actively engaged in a secondary task, either voluntarily gripping of a plastic plate, or mandatory holding of a switch closed with the hand that would ordinarily have produced the observed movement. Monkeys were restrained in the other studies, a condition that may not preclude movements in the same way as in our task. In sum, these differences in attention, motivation, performance, visual tracking and complexity of scenes with regard to the agents and

abstractions of the task across studies suggest that a range of variables might influence the way in which the motor cortex is engaged by viewing. These factors may also account for the much more pronounced trial-to-trial firing variability evident during viewing, as can be seen by inspection of spiking rasters shown for this and other studies. None of the experiments to date can adequately rule out any of this assortment of features as potential sources of variance.

'View' neurons are active both when a movement is performed and when that same action is observed, one hallmark of mirror neurons that have been identified in other cortical areas (Rizzolatti & Craighero, 2004). Neurons engaged in the premotor cortex when action is being viewed appear to form subcategories of neurons: those in the PMv responding to viewing actions, labeled mirror neurons (Rizzolatti & Craighero, 2004), and others found in the PMd that are related to the rehearsal of motor actions (Cisek & Kalaska, 2004). M1 'view' neurons appear to have features resembling both mental rehearsal and mirror neurons, and therefore cannot be easily categorized as either type of neuron.

Mirror and rehearsal 'classes' have been distinguished by their timing with respect to viewed action, how they respond to natural actions or abstractions, and contextual and reward sensitivity (Cisek & Kalaska, 2004; Rizzolatti & Craighero, 2004). Classically defined mirror neurons have features suggesting that they link cognitive aspects of transforming sensation to action (di Pellegrino *et al.*, 1992; Gallese *et al.*, 1996; Fadiga *et al.*, 2000; Rizzolatti *et al.*, 2001; Craighero *et al.*, 2007). Mirror neurons labeled 'congruent' respond in the same way to action observation and execution when the movement and the observed action coincide in terms of the goal and how the goal is achieved. They reflect cognitive aspects (e.g. goal and strategy) of the observed action. The subset of 'broadly congruent' mirror neurons appears to further generalize the goal of the observed action across many instances of the goal (Rizzolatti & Craighero, 2004). Similarly to mirror neurons, mental rehearsal neurons exhibit activity during action performance and observation, but they become active earlier, appearing to reflect a prospective mental rehearsal of an upcoming learned action (Cisek & Kalaska, 2004). Mental rehearsal is considered to be a replay of the internal movement plan, in which neurons re-enact their movement activity as if the learned action itself were being performed, but in a weaker way (Cisek & Kalaska, 2004). Consistent with the rehearsal hypothesis, Tkach *et al.* (2007) showed that M1 neurons fire during an exact replay of a learned cursor-tracking motion that the monkey had made earlier, much like our sPD neurons. The early activity of sPD 'view' neurons with respect to movement in our task is consistent with a role in mental rehearsal. However, dPD cells appear to have a new PD during viewing, a trait not consistent with a simple prospective function in which motor action is replayed. The slight, but not significant, tendency for dPD cells to shift to later times during the movement interval, and their timing differences as compared with sPD neurons, could suggest that the dPD subset has been modified by different processing systems, resembling the cognitive role attributed to mirror neurons. The evidence suggesting that these broadly tuned neurons both generalize the goal and show delayed activation (as compared with simultaneously recorded sPD neurons) could link this subset of M1 'view' neurons to action comprehension. Such response patterns can be explained by reference to the monkey's learning history. Overtraining made it possible for the monkeys to be able to reliably predict the movement's trajectory and goal on the basis of the ongoing motion of the cursor and the experimenter's arm (Catmur *et al.*, 2008).

We found that 'view' neurons became less active, became more variable and contained poorer representations of action when viewing reduced versions of the task (e.g. viewing only the agent performing the task or an abstraction of the task). Tkach *et al.* (2007) also noted that neurons were engaged in the M1 when only an abstraction of action, for example the motion of a cursor alone, was viewed. They also found that the activity diminished when pieces of the task were removed. These results suggest that 'view' activity could be evoking a rehearsal of the action that is dependent on viewing the entire scene of the task as learned in its original context, and that degradation of the task introduces greater uncertainty about the consequences of the viewed action, as reflected in reduced and more variable activity. This property is unlike the greater reliability of responses that appears to be evident for broadly congruent mirror neurons (Umilta *et al.*, 2001), and thus makes M1 'view' neurons less like mirror neurons.

Mental rehearsal neurons are sensitive to whether a trial is rewarded, which is presumably a driving force for rehearsal of the action. By contrast, mirror neurons respond to natural actions and their associated cues (Kohler *et al.*, 2002), during an interaction between a biological agent and some object, without the same reward sensitivity. In our experiments, M1 'view' neurons were influenced by reward expectancy, a property that makes them unlike mirror neurons but more like neurons engaged in mental rehearsal (Cisek & Kalaska, 2004). These investigators found that PMd neurons that were active during task viewing ceased firing as motivation and attention diminished, which was also noted in M1 'view' neurons by Tkach *et al.* (2007). We identified a change in 'view' activity related to reward expectancy, in that cells fired less as reward became more likely. This is the opposite of what might be expected; these cells more closely follow their movement activity for actions that are not rewarded. However, in our task, the monkey could be certain that the first three center out actions in a block of six would not be rewarded (by task design), but they were part of an attention-capturing sequence that would lead to an eventual reward. Alternatively, firing may have been related to the certainty of outcomes, which was high at the beginning of a block (no probability of reward) and was then variable on the last few trials. Thus, although the apparent sign of change varied, the coupling of firing rate to reward expectancy further suggests that these neurons are more like rehearsal neurons than classically defined mirror neurons. In summary, neurons engaged by action observation share features of mirror and mental rehearsal neurons that are consistent with the fact that the M1 is a target of dorsal and ventral premotor areas, as well as the parietal cortex, and therefore may reflect properties of each of these inputs.

The concept that M1 'view' activity only reflects mental rehearsal is challenged by our observations obtained using decoding methods. The decoding classifier created from the monkey's movement activity accurately predicted direction on single trials when the monkey performed the task. Similarly, 'view' activity was also reasonably good at predicting the direction of viewed action. Using the term 'representation' operationally, we can say that the activities during movement and viewing both contain a directional 'representation'. However, when these classifiers were tested on their respective conditions (i.e. view model on do trials or the converse) they performed poorly, indicating that the patterns of activity in the two conditions, although somewhat predictive, are not the same. A similar result was reported for the M1 by Wahnoun *et al.* (2006). Thus, this analysis directly tests the hypothesis that the M1 is simply unfolding the same learned motor pattern for mental rehearsal. The differences in these two classifier models suggest that dynamic changes occur in the functional organization of the M1 between performing and viewing, and that neurons during viewing are influenced in ways beyond the effects on those that are active during self-performance. Although only a speculation, this could be one way to attribute agency (i.e. who is the actor?) for viewed and self-performed movements.

Recently, it has been possible to examine activity in the M1 in humans with tetraplegia during viewing and attempted performance of

cursor motion (Hochberg *et al.*, 2006). Here, humans were explicitly asked to mentally rehearse movement while watching the motion of a cursor produced covertly by a human or by a computer, and this activity was then used to control a cursor in behavioral tasks. Neurons during this rehearsal of an abstract action were directionally tuned and led the cursor motion, demonstrating that rehearsal engages M1 neurons (Truccolo *et al.*, 2008), although the effect of viewing alone was not examined. Information in this model was directly demonstrated by showing that the direction of a target cursor could be predicted offline from this activity (Truccolo *et al.*, 2008), and that the human could use this model to control a cursor in a center out task (Hochberg *et al.*, 2006). These results further support the conclusion that M1 activity while viewing abstract action reflects mental rehearsal as well as knowledge of an abstract task.

In conclusion, 'view' activity in the M1 during arm movement observation has many features indicative of mental rehearsal of known actions. However, this explanation does not fully explain timing, PD shifts, sensitivity to task changes, or ensemble tuning properties. It is possible that these features reflect influences from more classic mirror neurons in the PMv and from other parts of the mirror system that project to the M1 (Matelli *et al.*, 1986), as well as rehearsal of the viewed action (Cisek & Kalaska, 2004; Hochberg *et al.*, 2006). Our results further confirm that the M1 is part of a large network of areas engaged in action processing, whether or not movement is produced. These observations have practical value for human neural prosthesis applications, because they point to differences in the nature of actual and viewed motor tasks and suggest that subgroups of M1 neurons may respond to viewing in different ways.

Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Task design and performance features.

Fig. S2. Example of spike waveforms of the units in the corresponding microelectrodes.

Fig. S3. Examples of recorded spike waveforms with signal-to-noise ratio (S/N) and resulting inter-spike interval (ISI) distributions.

Fig. S4. Spatial distribution and preferred directions (PDs) for primary motor cortex (M1) 'view' neurons.

Fig. S5. Preferred direction (PD) rotations between the 'do' and 'view' conditions projected into reach workspace.

Fig. S6. Comparison of correlation coefficients (CCs) and standard errors (±SEs) of neural firing between the 'do' and 'view' conditions.

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Abbreviations

BC, Bayesian classifier; dPD, different preferred direction; KW, Kruskal–Wallis; M1, primary motor cortex; MT, movement time; PD, preferred direction; PMd, dorsal premotor cortex; PMv, ventral premotor cortex; RT, reaction time; S/N, signal-to-noise ratio; sPD, similar preferred direction.

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