

John A. Buford · Adam G. Davidson

Movement-related and preparatory activity in the reticulospinal system of the monkey

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Abstract Three monkeys (*M. fascicularis*) performed a center-out, two-dimensional reaching task that included an instructed delay interval based on a color-coded visuospatial cue. Neural activity in the medial pontomedullary reticular formation (mPMRF) was recorded along with hand movement. Of 176 neurons with movement-related activity, 109 (62%) had movement-related but not preparatory activity (M cells), and 67 (38%) had both movement-related and preparatory activity (MP cells). EOG analyses indicated that the preparatory activity was not consistent with control of eye movements. There were slight changes in electromyograms (EMG) late in the instructed delay period before the Go cue, but these were small compared with the movement-related EMG activity. Preparatory activity, like the EMG activity, was also confined to the end of the instructed delay period for 14 MP cells, but the remaining 53 MP cells (30%) had preparatory activity that was not reflected in the EMG. Peri-movement neural activity varied with movement direction for 70% of the cells, but this variation rarely fit circular statistics commonly used for studies of directional tuning; directional tuning was even less common in the preparatory activity. These data show that neurons in the mPMRF are strongly modulated during small reaching movements, but this modulation was rarely correlated with the trajectory of the hand. In accord with findings in the literature from other regions of the CNS, evidence of

activity related to motor preparation in these cells indicates that this function is distributed in the nervous system and is not a feature limited to the cerebral cortex.

Keywords Directional tuning · Motor planning · Reaching · Reticular formation

Introduction

In the last three decades, investigations of the motor functions of the reticulospinal system have focused on initiation and regulation of locomotion (Drew 1991; Drew and Rossignol 1984; Drew et al. 1986, 1996; Garcia-Rill and Skinner 1987; Gossard et al. 1996; Kably and Drew 1998a; Noga et al. 1988), postural control and participation in vestibular reactions (Bolton et al. 1992; Eccles et al. 1975; Matsuyama and Drew 2000a, b), and head movement for gaze control (Cowie and Robinson 1994; Cowie et al. 1994). Other data suggest a role in additional functions. For example, in non-human primates, lesion studies have demonstrated that posture and gross limb movements are severely impaired by lesions to the ventromedial descending systems, which include reticulospinal fibers (Lawrence and Kuypers 1968). Thus, the reticulospinal system may also contribute to voluntary reaching. However, to our knowledge, no one has employed the methods of behavioral neurophysiology to study this system during voluntary limb movements in the primate.

Anatomically, reticulospinal cells take origin from the medial pontomedullary reticular formation (mPMRF), including the nucleus reticularis gigantocellularis (NRGc), nucleus reticularis magnocellularis (NRMc), nucleus reticularis pontis caudalis, and nucleus reticularis pontis oralis (Kuypers 1981). Several anatomical and physiological studies have demonstrated that cortical motor and premotor areas contribute descending projections to the mPMRF (Canedo and Lamas 1993; He and Wu 1985; Jinnai 1984; Kably and Drew 1998a, b; Keizer and Kuypers 1984, 1989; Kuypers and Lawrence 1967;

J. A. Buford (✉)
Division of Physical Therapy, School of Allied Medical
Professions, The Ohio State University,
1583 Perry Street, Rm 516G,
Columbus, OH, 43210, USA
e-mail: Buford.5@osu.edu
Tel.: +1-614-2921520
Fax: +1-614-2920210

J. A. Buford · A. G. Davidson
Neuroscience Graduate Studies Program, The Ohio State
University,
Columbus, OH, 43210, USA

Peterson et al. 1974; Rho et al. 1997). A seminal paper by Keizer and Kuypers (1989) in *M. fascicularis* found that corticospinal cells with corticoreticular collaterals originated primarily from premotor areas rostral to the primary motor cortex (MI), probably including SMA proper (supplementary motor area) (Mitz and Wise 1987; Rizzolatti et al. 1990), the dorsal premotor cortex (PMd) (Tanji and Kurata 1985; Weinrich and Wise 1982; Weinrich et al. 1984), and the dorsal cingulate motor area (CMAd) (Backus et al. 2001; Picard and Strick 1996), but not pre-SMA (Matsuzaka et al. 1992; Rizzolatti et al. 1990). Since these cortical areas have been shown to display significant amounts of preparatory activity (Alexander and Crutcher 1990b; Crammond and Kalaska 2000; Godschalk et al. 1985; Kurata 1993; Weinrich et al. 1984; Wise et al. 1997), we suspected that neurons in the mPMRF would also display preparatory and movement-related activity in the context of a voluntary, skilled reaching movement.

The classic paradigm for eliciting and studying preparatory activity is the instructed delay task, in which an instruction is given indicating what movement should occur, but the subject must wait before moving (Weinrich et al. 1984). Neural activity during this waiting period, the instructed delay period, may be preparatory activity. Because neurons in this region may also be important for gaze control (Cowie and Robinson 1994), we kept the head restrained and employed an instructed delay task that was designed to dissociate hand and eye movements. The results show that many neurons in the mPMRF have

activity associated with reaching and that more than a third of these arm-movement-related neurons also displayed some level of preparatory activity. Preliminary results from these studies have been reported (Buford 1996, 2000; Buford and Anderson 1996; Ruffo and Buford 1997).

Materials and methods

Subjects and animal care

The subjects, three juvenile male *Macaca fascicularis* weighing 2.5–5 kg, were cared for in accordance with the *NIH Guide for the Care and Use of Laboratory Animals*. Procedures were approved by the ILACUCs of The Ohio State University and The University of Washington. Animals received water ad libitum in their cages and earned rewards (strained applesauce or flavored yogurt) for correct performance. Monkey chow and fruits and vegetables formed the balance of the diet. Surgery was performed under Isoflurane inhalation (1–2% endotracheal) anesthesia with ketamine hydrochloride (13 mg/kg i.m.) as a preanesthetic. Analgesics were given for 1 week after surgery, and antibiotics were given as a perioperative prophylactic.

Behavioral control

Apparatus

As illustrated in Fig. 1b, the subject sat in a primate chair at an apparatus that has been described previously (Inase et al. 1996). The visual display was provided by a 17" flat-screen computer monitor with the screen parallel to the work surface. A piece of mirrorized

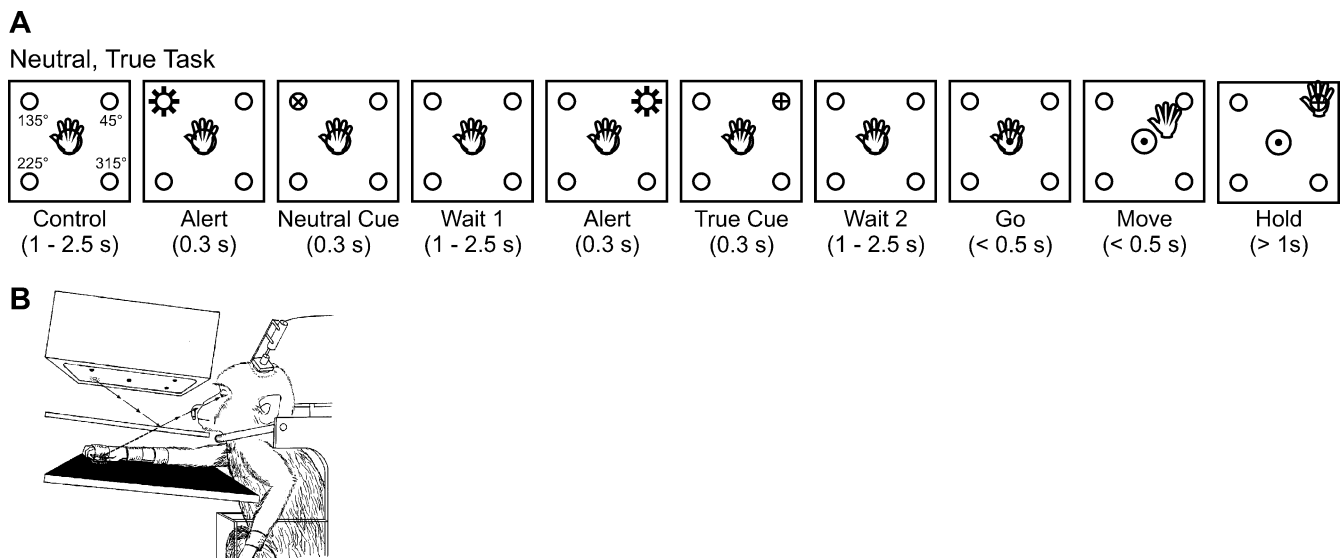


Fig. 1 a “Neutral-True” trial. Each *square* illustrates the display seen by the subject for an epoch in the trial, with the order proceeding from left to right. The silhouette of the *hand* indicates the proper position; times below each box indicate the duration (or range of durations) for each epoch. The default color for the four peripheral targets was blue-green. Home was red in between trials, when the hand was not on Home, and green during the trials, while the hand was waiting on Home. Prior to the Neutral Cue, a target (135° in this example) brightened (*Alert*) and then turned a shade of blue (⊗) for the Neutral Cue. After another waiting period, a second target (45°) brightened (*Alert*) and then turned a shade of green (⊕)

for the True Cue. After a third waiting period, the central “Home” target turned a shade of yellow-green (⊙) for a Go cue. After the Go cue, the subject was given time to react and reach to the target (*Move*), and then had to remain with his hand on the target (*Hold*) to receive his reward. The “True-Neutral” trials were similar, but the True Cue was first. Other types of trial are explained in methods. **b** Illustrates a profile of the subject in the apparatus, with the target presented on the computer monitor reflected by the mirrorized Plexiglas and appearing as a virtual image on the work surface; the *arrows* illustrate the light paths. The left hand was restrained at the subject’s side and the legs were contained in a box

Plexiglas placed halfway between the monitor and the work surface made images on the monitor appear as virtual images on the work surface. Illumination of the space below the mirror allowed the subject to see his arm along with the work surface and the virtual images. Rewards were pumped through a tube that allowed the monkey to lick a sip of applesauce for each trial without moving his head.

To record hand position, the work surface was a Calcomp 1012 graphics tablet covered with a thin sheet of polycarbonate and black flat-finish vinyl to limit reflection and friction. A custom-made splint containing the electromagnetic coil for the graphics tablet was strapped to the ventral aspect of the forearm so that the position of the hand was recorded. The digital output of the graphics tablet was converted into X and Y analog voltage outputs at 12-bit resolution by a custom-built device. These analog signals of hand position were recorded by the Tempo for Windows data acquisition system (Reflective Computing, St. Louis, MO, USA) at 100 Hz, giving a final positional accuracy of about ± 0.25 mm. Although the subject was free to lift the arm from the work surface, the graphics tablet would not read hand position accurately unless the electromagnetic coil was parallel to and within about 1 cm of the work surface. In practice, subjects learned to slide their hand on the surface to succeed at the task. The hand position data were smoothed with a fourth order, zero-lag Butterworth digital filter with a high frequency cutoff set to 7 Hz. Tangential velocity, used to indicate hand speed, was calculated from the smoothed position data with a finite differences method. The alignment of the computer monitor with the graphics tablet was periodically checked and adjusted.

Instructed delay task

The motor task required a center-out planar reaching movement. The subject waited with his hand over a central, Home location, received an instruction cue for which of the four potential targets to go to, remembered the target during the delay period, and in response to a Go cue, slid his hand out to the proper target location as illustrated in Fig. 1a. The cues were presented as changes in color of the target or Home image. The potential targets remained visible in a background color during the waiting periods between cues.

There were four types of trial, but to explain the task, we will start with a Neutral-True trial. Each panel in Fig. 1a represents one stage of the trial; the temporal constraints for each stage are shown below each panel. During the control period (Fig. 1a, *leftmost panel*), the monkey waited with his hand over Home. Then, an alert cue was presented, with one of the four targets brightening to attract the monkey's attention. After the alert, the Neutral Cue was presented. For this, the target indicated by the alert became blue. The Neutral Cue served as a control to elicit eye movements, but did not tell the monkey which target he would be moving his hand to for the trial. After the Neutral Cue, the monkey kept his hand on Home (Fig. 1a, *Wait 1*) until another alert was followed by presentation of the True Cue, which was green. The True Cue indicated the proper target for the trial. After the True Cue, there was another waiting period, (Fig. 1a, *Wait 2*), which was the instructed delay period. Then, the monkey received the Go cue, which was a change in the color of Home from green to yellow/green. After the Go cue, the monkey moved his hand to the target that had been indicated by the True Cue, and then held his position over that target to be rewarded.

Neutral-True trials comprised 40% of successful trials. In another 40% of successes, the order of the cues was reversed so that the True Cue was first and the Neutral Cue was second; these were called True-Neutral trials. Preparatory activity was expected to begin after the True Cue, regardless of the order of the cues. With only these two trial types, however, it would be possible for a subject to succeed by noting only the location of targets that brightened in peripheral vision. With this information, a monkey could still make the correct choice by noting the color of only one of the cues. To provide an incentive for the monkey to look at both cues, Catch trials were used. In Catch trials, only one cue—Neutral or True—preceded the Go cue. If only the Neutral Cue was presented

(Neutral-Catch trial, 10% of successes), the hand had to remain on Home after the Go cue to elicit a reward. If only the True Cue was presented (True-Catch trial, 10% of successes), the hand had to move to the target after the Go cue. With this combination of True-Catch and Neutral-Catch trials, the monkey had to know whether the first cue was a True Cue or a Neutral Cue, and he had to be prepared to react after the first cue. The four types of trial were pseudo-randomly presented by the computer to achieve the desired ratios of trial types in the successful trials. With 100 successes per block, this produced about ten movements per target location for the Neutral-True and True-Neutral trials and 2–3 trials per target location for Neutral-Catch and True-Catch trials. The statistical analyses (described later) were conducted only on the True-Neutral and Neutral-True trials. The catch trials were used only to maintain behavioral control.

The task was computer controlled with the Tempo for Windows system, and visual displays for the subject were created with associated software (Videosynch, Reflective Computing). The centers of the targets were 5.08 cm away from the center of the Home image, and their locations are noted in standard Cartesian coordinates, as indicated in the *left panel* of Fig. 1a. With the hand over the target at 135° , the arm was almost fully extended. With the hand over the target at 315° , the angle of the elbow joint was about 90° .

Neural recording and analysis

Access to the reticular formation was through a stainless steel recording chamber (19-mm internal diameter) with its center targeted during surgery for Horsley-Clarke stereotaxic coordinates AP 0, ML 0, and DV -12 (Szabo and Cowan 1984). The electrode paths were in the frontal plane, but were tilted 10° left from the parasagittal plane, which helped avoid the major blood vessels of the midline. Recording electrodes were 0.2-mm tungsten wire that was insulated with a polyimide sleeve for most of the shaft and an epoxy-based electrical varnish for the last 10–15 mm between the end of this sleeve and the recording tip. Insulation was ground from the tips, which were electroplated with particulate iron (subject S) or with gold to produce recording impedances in the 100–200 K Ω range (measured with a 1-KHz 10-nA sine wave). Electrode positioning was controlled with an X-Y positioner and a manual hydraulic microdrive. The electrode was guided by a stainless steel 23 ga. cannula set to stop in the upper cerebellum. Extracellular somatodendritic action potentials were detected and converted to TTL pulses with an analog window discriminator (BAK DIS-2, Bak Electronics, Gathersburg, MD, USA). The firing rate was calculated as the inverse of the interspike interval.

During recording, cells with activity related to right (ipsilateral) arm movement were sought. These were recognized by a modulation in their firing rate around the time of movement. After recording from a cell was complete, the subject's arm, as well as other body parts, were gently manipulated to determine the cell's somatosensory receptive field. Visual, auditory, and gustatory stimuli were also provided at times in order to define the inputs of the cell. Cells with no definable somatosensory response were included if they did show task-related changes in firing. Cells with arm-related somatosensory responses were included, as were cells with responses to stimulation of other body parts as long as they also responded to manipulation of the right arm or shoulder girdle. Oculomotor and facial cells were excluded from analysis.

Custom-written software was used to locate task-related events (cue onset time, movement initiation and termination) within each trial. From these times, mean firing rates were calculated from the inverse of the interspike intervals for each fixed epoch in Table 1 (footnote a). Bursts and/or pauses in neural activity were also detected on a trial-by-trial basis with the Datapac software package (Run Technologies, Laguna Niguel, CA, USA). For this, a voltage threshold level was set just above (or below) the baseline firing rate for the cell and changes in firing (bursts or pauses) were detected as the rate crossed that threshold. Some manual adjustments were

required, as this process could not always succeed automatically. Onset and offset times were always placed at the time of an action potential. Examples of the detected onset and offset times are provided in Figs. 2 and 3. From these detected periods of activity, the mean firing rates as well as onset and offset times of neural activity were calculated for bursts or pauses detected after the True Cue and around the time of movement (Table 1, footnote b).

Data from the periods described in Table 1 were used in a repeated-measures ANOVA to determine if each cell had movement-related or preparatory activity. For all cells, the Control and Set periods were tested. In cases where there was a burst or pause after the True Cue that was detectable with the Datapac software, the Prep epoch was used to test for preparatory activity; otherwise, the True Cue epoch was used. Movement-related activity was defined by activity in Movement and Post epochs (biphasic activity) or in only the Movement epoch. A simple comparisons procedure was used to compare all epochs with Control. In addition to the repeated-measures ANOVA used to compare activity across epochs, the cue order (Neutral-True vs. True-Neutral) and target identity (1–4) were tested as main effects. These statistical analyses were conducted with SPSS with the significance level set to $P \leq 0.05$. The statistical reasoning for classifying a cell as having a significant preparatory or movement-related activity is explained in the results.

The directional tuning of each cell was also calculated from the average firing rates during the movement-related and preparatory periods using Eq. 6 from Amirikian et al. (Amirikian et al. 2000). This equation is based on a Von Mises function, which can assume the general case of a cosine function, but can also fit cells that are more broadly or more narrowly tuned, and cells with asymmetrical tuning about the preferred direction. The function was fit according to a least-squares criterion with SPSS. Goodness of fit was computed as a coefficient of determination, R^2 . To determine the overall relationship of cell discharge with the kinematics of hand movement, the firing rate predicted by directional tuning was entered into a multiple regression as an independent variable along with movement amplitude and peak movement velocity; the actual firing rate was the dependent variable. This analysis yielded an overall significance level for the regression, as well as individual significance levels for the components of the regression (Direction, Amplitude, and Velocity). Preparatory and movement-related activity were assessed for their relationship with hand kinematics through this analysis. The significance level of $P \leq 0.05$ was used for the overall regression and for each component.

EMG recording and analysis

Selected electromyographic (EMG) recordings were made during task performance for all three subjects. In subjects C and D, initial recordings were made with surface and percutaneous fine-wire

electrodes; chronic intramuscular electrodes (multistranded stainless steel, Cooner AS-632) were implanted about half way through the data collection period according to published methods (Betts et al. 1976; Park et al. 2000). Muscles sampled on the right included biceps and triceps brachii, anterior and posterior deltoids, and upper and middle trapezius; the left upper and middle trapezius muscles were also implanted. The EMG data were sampled at 4.6 KHz by a Power1401 acquisition unit running Spike2 software (CED, Cambridge, UK). In subject S, occasional surface and percutaneous recordings were also obtained from the muscles described above, as well as from right vastus lateralis, lateral hamstrings and lumbar paraspinals. The sampling rate for subject S was 1,000 Hz. EMG averages were constructed with the Datapac software. Mean rectified EMG levels during the Control, Neutral Cue, True Cue, Set, and Movement epochs (Table 1) were compared with a mixed-model ANOVA like that used for the analysis of neural activity, with Epoch as a repeated factor and cue order and target direction as between subjects factors. The significance level was set to $P \leq 0.05$. A bonferroni correction was used for multiple comparisons among the means.

EOG recording and analysis

In the first subject (S), after criterion behavior had been attained, surface EOG recordings were used to characterize the pattern of eye movements for several days of behavior. The EOG electrodes were Ag-AgCl discs taped to the skin. Horizontal EOG's were positioned over the external canthus lateral to each eye at the level of the pupils, and vertical EOG's were positioned above and below the right eye on the brow ridge and just below the orbit. To account for drift in the DC EOG signals, the offset of the DC amplifiers was adjusted every few trials, so that the signal remained within the range of the data acquisition hardware. Custom software was developed to map the EOG into the workspace. The target zones were modeled as ellipses. A subject was considered to be looking at a target if the estimated direction of gaze was within 3° of the edge of that target's elliptical profile on the work surface.

The proportion of each epoch spent with the gaze directed at each location, as well as the number of times per epoch that the subject looked at each locus, were analyzed with a repeated-measures ANOVA like those above; epoch was the within-subjects factor and target and cue order were between-subjects factors. For these analyses, data for targets that were neither the True Cue nor the Neutral Cue for a given trial were pooled and called "other." The significance level for these analyses was set to $P \leq 0.05$.

Table 1 Epochs for analysis of neural firing rate

Epoch name	Reference times
Control ^a	First 500 ms after stable acquisition of Home
Neutral Cue ^a	300 ms starting with onset of Neutral Cue itself (not the alert)
True Cue ^a	300 ms starting with onset of True Cue itself (not the alert)
Set ^a	300 ms preceding the onset of the Go cue
Prep ^b	Onset to offset of preparatory response when present. If preparatory response is sustained and blends into movement-related change, then offset of preparatory response is onset of movement-related change.
Movement ^b	Onset to offset of initial (or sole) component of movement-related response. If preceding preparatory response continues and no additional modulation is noted with movement, then onset of hand movement itself is onset of movement-related firing epoch

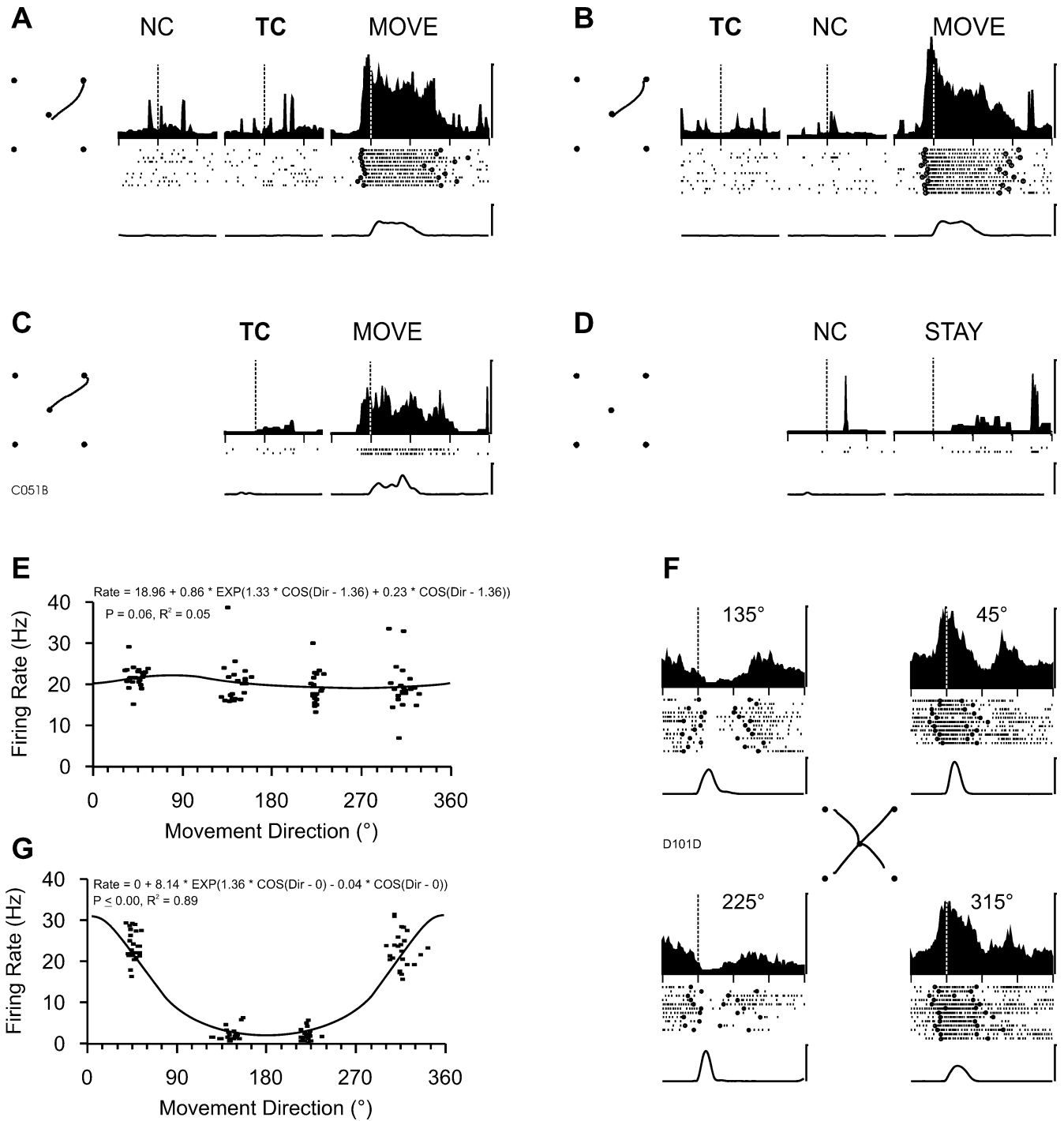


Fig. 2a–g Movement-related neural activity. **a–e** show data from a neuron (C051B) with movement-related activity that was not related to target direction. On the left in **a–d**, the average hand path is superimposed on the target locations. The three *time windows* in each panel show data around the Neutral Cue (NC), True Cue (TC), and movement (MOVE), with the cue and movement onsets shown by *dotted vertical lines*. The upper part of each time window illustrates the average firing rate for the cell, with tick marks at 500-ms intervals; the calibration bar for firing rate is 75 Hz. Below each time window, the rasters show action potentials for each of the 10–

12 trials per panel. *Circles* in the rasters show the onset and offset of the detected response for each trial. Below the rasters, mean hand speed is shown; the hand-speed calibration bar is 15 cm/s. For **a–d**, only data for movements to 45° are shown. The directional tuning of this cell is shown in **e**. In **f**, data from a cell (D101D) with significant directional tuning are shown. Only the movement-related period is illustrated, and the averaged hand paths for movements to each target are superimposed. The directional tuning curve for this cell is shown in **g**

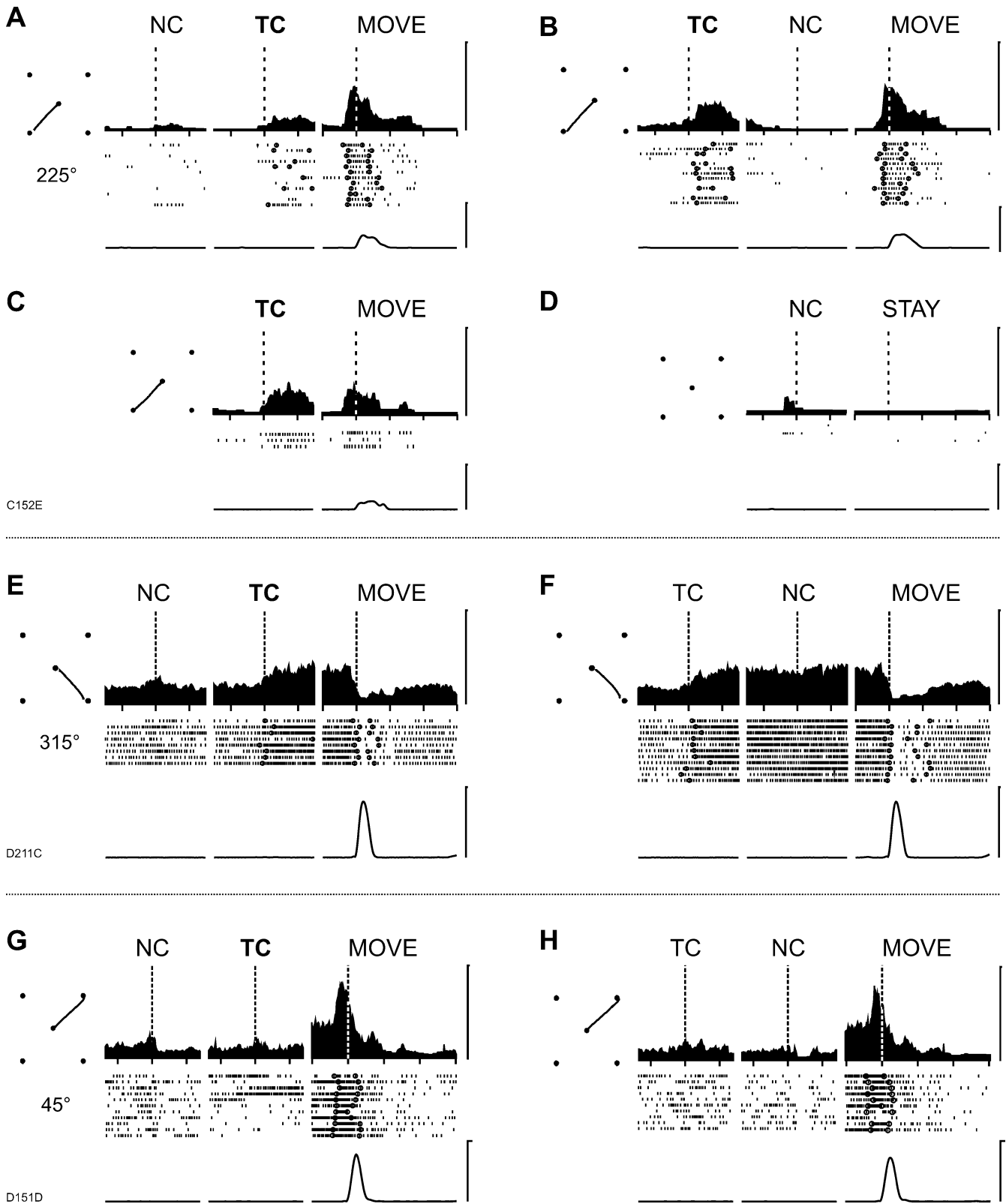


Fig. 3a–h Movement-related and preparatory activity in reticular formation neurons. In **a–d**, data are illustrated for a cell (C152E) with a transient preparatory response. Only movements to 225° are illustrated. The layout of panels **a–d** is that same as those in Fig. 2.

In **e** and **f**, data from a cell (D211C) with a sustained preparatory response are illustrated for movements to 315°. In **g** and **h**, a set-related response (D151D) is illustrated for movements to 45°. Rate calibrations are 100 Hz and hand-speed calibrations are 25 cm/s

Stimulation in the reticular formation

In order to locate arm-related regions, sites from which stimulation evoked right arm movement in addition to movements of other body parts were mapped in comparison with sites where cells with arm-related neural activity and somatosensory responses had been recorded. After the completion of a recording track, stimulation was performed at 0.5-mm increments as the electrode was retracted, and also at some additional depths where neurons had been recorded. The stimulus trains included 12 biphasic pulses (negative first, 0.2 ms per phase) applied at 333 Hz, with current ranging from 5–50 μ A, as controlled by a constant current stimulus isolator. Threshold was defined as the lowest current at which a minimally observable response was consistently produced.

Anatomical reconstructions

Subjects were euthanized with sodium pentobarbital (50 mg/kg i.v.) after preanesthetic with ketamine hydrochloride (13 mg/kg i.m.), followed by transcardial perfusion with phosphate-buffered saline followed by phosphate buffered 10% formalin. The brain was then removed and soaked in 10% formalin, followed by 10% formalin with 30% sucrose for cryoprotection. A block of tissue with the recording tracks was sectioned at 40 μ m and stained with cresyl violet. Recording positions were located based on electrolytic marking lesions (30 μ A, 30 s, DC negative current) that had been made at selected locations over the last 2 weeks of recording, along with physiological landmarks recognized during recording, especially the abducens nucleus. The nomenclature and locations of structures were based on the Template Atlas from the University of Washington, recently updated (Bowden et al. 2003), with locations also based on the atlas from Szabo and Cowan (1984).

Results

Movement-related activity

Patterns of activity

The most common pattern of movement-related activity, a monophasic movement-related increase, is illustrated in Fig. 2a–c. This cell had a low and somewhat variable baseline and consistent movement-related activity that led movement onset by an average of 103 ms. The movement-related burst had an early peak followed by slightly lower activity, and then a return towards baseline that outlasted the movement (Fig. 2b). There was little variation in the pattern with target direction, and directional tuning was not significant for this cell (Fig. 2e). This cell had no consistent activity after either of the cues. In the Catch trials, there was movement-related activity for the True-Catch trial (Fig. 2c) that was similar to those above. There was a slight increase late in the Neutral-Catch trial (Fig. 2d), which may have been associated with retrieval of the reward.

Four kinds of movement-related activity were observed. In 123 cells (70%), there was a monophasic increase in activity related to movement (e.g., Fig. 2a, b, *MOVE*). In 28 cells (16%), there was a monophasic decrease in activity related to movement (e.g., Fig. 3e, f, *MOVE*). In 17 cells (10%), there was biphasic modulation related to movement, either an increase followed by a decrease

($n=6$), a decrease followed by an increase ($n=7$), or a combination that varied with target direction ($n=4$). Finally, in eight cells (4%), there was a movement-related increase for some directions and a decrease for others (e.g., Fig. 2f). For 175 of the 176 cells studied, movement-related activity was significantly different from control across all four target directions. For the remaining cell, the neural activity increased during movement for some directions and decreased for others, canceling each other out in the average.

Timing of activity

On average, movement-related changes in neural activity preceded movement by 96 ± 138 ms. The onset and offset times of the movement-related activity were tested by linear regression for each cell to determine whether they were correlated with the onset of movement, the time of peak movement velocity, or the end of movement to the target. In 68% of the cells, at least one of these correlations was significant at the $P \leq 0.01$ level. Usually (58%), this was due to a correlation between movement onset time and activity onset time. The mean burst duration was 495 ± 311 ms, which was longer than the mean movement time (332 ms). In 68% of the trials, burst duration exceeded movement time.

Correlation with kinematics

To determine whether movement-related activity was related to the kinematics of hand movements, linear regressions were used to correlate the mean level of neural activity during movement with the firing rate predicted by directional tuning, the peak velocity of hand movement, and the amplitude of the movement. Overall, this regression was significant for 70% of the cells studied ($n=130$). In 80% of these ($n=108$), directional tuning was the only significant factor in the regression; amplitude and velocity made no difference. In some cells, movement amplitude ($n=16$) or peak movement velocity ($n=9$) were significant factors in the regression, but only two cells had significant regressions where directional tuning was not a factor. The goodness of fit, however, was rarely strong. Amirikian et al. (2000) defined an acceptable fit for directional tuning in cells from the primary motor cortex as an R^2 of 0.75 or better. That study included movements in 20 directions, offering a much better chance for a fit. However, even if a good fit for directional tuning in the present study was defined as an R^2 value ≥ 0.5 , only 29 cells had a good fit; only seven cells had an R^2 value ≥ 0.75 . For the full regression including directional tuning, velocity, and amplitude, there was little improvement: only 30 cells had an R^2 value ≥ 0.5 and only eight cells had an R^2 value ≥ 0.75 . For cells with a significant regression overall, the improvements in the R^2 values for the full regression averaged only 0.03 over that obtained from directional tuning alone.

Figure 2f illustrates movement-related activity for one of the cells with strong directional tuning. The directional tuning curve for this cell is illustrated in Fig. 2g. In this case, the R^2 for directional tuning was 0.89. Thus, although strong relationships between kinematics and firing rates were uncommon, some of the neurons in the mPMRF did have activity that correlated well with the kinematics of hand movement.

Preparatory activity

Patterns of activity

Sixty-seven cells (38%) also had preparatory activity, and these are referred to as MP cells (Movement and Preparatory); cells without preparatory activity are referred to as M cells (Movement only). Preparatory activity was defined by one of three mutually exclusive patterns: transient, sustained, or set-related. Cells with transient activity had a significant difference from Control only for the True Cue (or Prep) epoch, independent of cue-order (e.g., Fig. 3a vs. b). This was the most common kind of preparatory activity (Table 2). Cells with sustained activity had a significant difference only between Control and True-Cue (or Prep) when the True-Cue was second. But, with the True-Cue first, cells with sustained activity had a significant difference between Control and both the Neutral Cue and True Cue (or Prep) periods (e.g., Fig. 3e, f). Statistically, this produced an interaction between epoch and cue-order. This was about half as common as transient preparatory activity (Table 2). Finally, cells with set-related activity had a change in firing rate during the set period after the second cue but before the Go cue regardless of cue order (e.g., Fig. 3g, h). These were just as common as cells with sustained preparatory activity (Table 2).

Figure 3a, b illustrates the activity of a cell with transient preparatory activity. Prior to the True Cue, this cell had a low and somewhat variable baseline. After the True Cue, there was transient activity regardless of whether the True Cue was the first (Fig. 3b) or second (Fig. 3a) cue in the trial. This cell responded in a similar manner during the True-Catch trials (Fig. 3c) as for the complete two-cue trials (Fig. 3a, b), but had no activity during the Neutral-Catch trials (Fig. 3d, *right*), where there

was neither a True Cue nor a movement. Cells with similar activity after the True Cue and Neutral Cue were considered eye-movement related and excluded from the sample. Examination of the rasters suggests that the movement-related activity was more consistent than the preparatory activity. This was evident for many of the MP cells.

Figure 3e, f illustrates sustained preparatory activity. This cell became more active once the True Cue was presented, and this increased activity was sustained until the time of movement, when the cell's activity decreased. The pattern was similar regardless of whether the True Cue was first (Fig. 3f) or second (Fig. 3e). The Neutral Cue had no effect on the activity. The reader may note that the cell illustrated with sustained activity had a relatively high background firing rate, but the cell illustrated with transient activity had a very low background rate; this is just a coincidence. There were no significant differences in control, preparatory, or movement-related firing rates for the three kinds of MP cells listed in Table 3.

Figure 3g, h illustrates set-related activity. This cell's activity increased well after both cues had been presented, regardless of their order. This was evident as a higher baseline activity during the period prior to movement than had been present after either of the cues. The rasters indicated that, like the transient preparatory activity in Fig. 3a–c, the set-related activity in Fig. 3g, h was not present in every trial. Around the time of movement, there was a further increase in the activity of this cell.

Across all of the arm-related M and MP cells studied, the mean firing rate during the Control period was 19.0 ± 16.5 Hz. During Movement, the mean firing rate was 34.2 ± 26.3 Hz. The mean firing rate of preparatory activity in MP cells was 26.8 ± 20.3 Hz, about half the mean increase in discharge seen during the Movement period. There was no difference between M and MP cells in either the Control or Movement-related firing rates, burst durations or in the prevalence of significant regressions between firing rate and the kinematics of hand movement. There was also no difference in the proportion of M vs. MP cells with movement-related bursts that were temporally correlated with the onset of movement.

For most MP cells (78%), the preparatory activity was an increase from baseline. Likewise, 82% of the movement-related activity was an increase from baseline. When the changes from baseline of the preparatory and movement-related activity were compared for each cell, most (72%) preparatory activity changed in the same manner as the movement-related activity, i.e., both were an increase

Table 2 Types of preparatory responses observed

Type of response	Change from control		Total
	Increase	Decrease	
Transient	28 (42)	10 (15)	38 (57)
Sustained	12 (18)	3 (4)	5 (22)
Set	13 (19)	1 (1)	14 (21)
Total	53 (79)	14 (21)	67 (100)

Values are numbers of cells, with percentages in parentheses. The types of preparatory responses are described in the text

Table 3 Responses to micro-stimulation

Location	N	Percent
Arm	17	14%
Shoulder	66	55%
Axial	15	13%
Other	3	3%
NO	18	15%
Total	119	100%

NO no response observed

or both were a decrease. This is illustrated in Fig. 4 as data in the upper right and lower left quadrants. Figures 3a, b and Fig. 3g, h show examples where both were an increase. In the remaining cases, the preparatory and movement-related changes were in the opposite direction. Figure 3e, f illustrates an example with a preparatory increase but a movement-related decrease. The *shaded area* in Fig. 4 encompasses the 28% of points for which the size of the preparatory activity exceeded the size of the movement-related activity. For most cases (72%), the symbols are in the unshaded areas, where the movement-related activity exceeded the preparatory activity.

Timing of activity

The timing of preparatory activity could only be determined on a trial-by-trial basis using the Datapac software in 42 (63%) of the MP cells. This included 77% of the cells with transient preparatory activity, but only 41% of the cells with sustained and set-related activity. In 41 of these 42 cells, the onset time of the preparatory activity was correlated with the onset time of the True Cue at the $P \leq 0.01$ level. The preparatory activity followed the True Cue at a mean onset latency of 398 ± 736 ms. The large standard deviation reflects the inclusion of a few cells with set-related activity that began very long after the

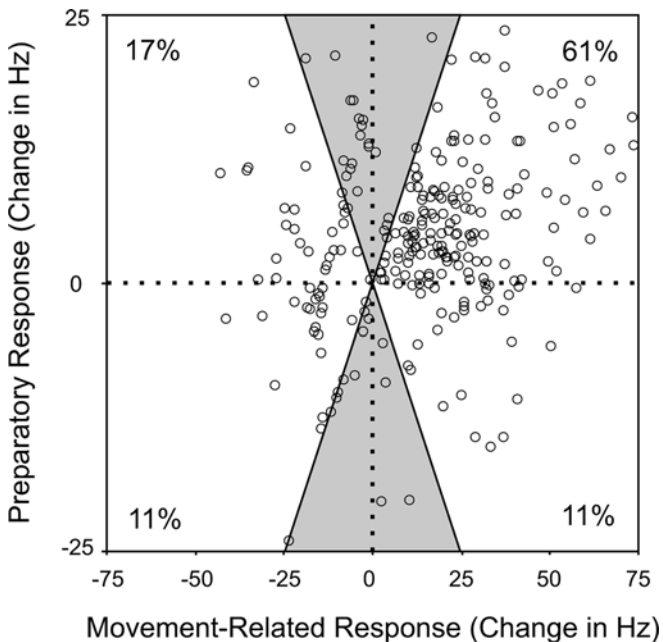


Fig. 4 Change from baseline for movement-related vs. preparatory activity. Values plotted are the difference between the preparatory response (Prep or True Cue) and the control on the vertical axis versus the difference between the movement-related response and control on the horizontal axis. One point is plotted for the overall mean difference for each MP cell for each direction. Percentages are shown for the proportion of cells in each quadrant. The *shaded area* shows where the preparatory response was larger than the movement-related response, and the *unshaded area* shows where the movement-related response was larger. Note difference in horizontal and vertical scales

True Cue. The median of the distribution was 187 ms, with most onsets between 0 and 300 ms. The presence of some very short onset latencies was probably due to the alert cue, which preceded the actual True Cue by a fixed-latency of 300-ms (Fig. 1), allowing the subject to anticipate within this short interval the onset of the True Cue. In less than 5% of the preparatory activity for individual trials, the cell had already become active before the True Cue. The shortest mean response latency to the True Cue for any MP cell was 90 ms.

Correlation of preparation with movement

Significant relationships between preparatory firing rates and movement kinematics were rare. Only five cells had a $R^2 \geq 0.5$ for directional tuning of the preparatory activity, and only one cell had an $R^2 \geq 0.75$.

Control studies

Eye movement

As a test to see whether the patterns of neural activity record could be explained by eye movement, a sample of EOG recordings from subject S was processed to map eye position onto the workspace as described in the methods. As illustrated in Fig. 5a, b, the subject typically scanned the targets and Home to gather information as needed throughout each trial. Eye movements were much more frequent than hand movements; this record included 23 saccades in association with three hand movements. In this trial, he scanned the targets early in the trial (1–2.5 s), looked at the Neutral Cue after it was presented (~2.5 s), and then began scanning again. He looked at the True Cue when it was presented (TC at ~4.2 s), and then began looking mostly at Home, presumably in anticipation of the Go cue. He was looking at Home when the Go cue was presented (~5.5 s), and then he looked at the target and moved his hand to the target. After completing the required hold on the target, he looked back at Home and put his hand there to begin the next trial.

Analysis of the EOG data for 7 days of behavior (more than 2,500 trials) from subject S showed a consistent pattern of eye movement. As shown in Fig. 5c, the subject spent the most time looking at Home when it was likely to change (Wait 1, Wait 2, Go & Hold). In the panel labeled “Home (Wait 1)” in Fig. 5c, there was no difference in the behavior toward Home by trial type. This suggests that as intended, the subject did not know if he was facing a catch trial; if he had, he should have paid more attention to Home during this period for the catch trials. Finally, the subject’s behavior towards the True Cue is presented in the panel labeled Target in Fig. 5c. The subject spent the most time looking at the True Cue when it was presented, and especially during the hand movement to the target. Although the proportion of the Neutral Cue epoch spent looking at the Neutral Cue was not different from Control,

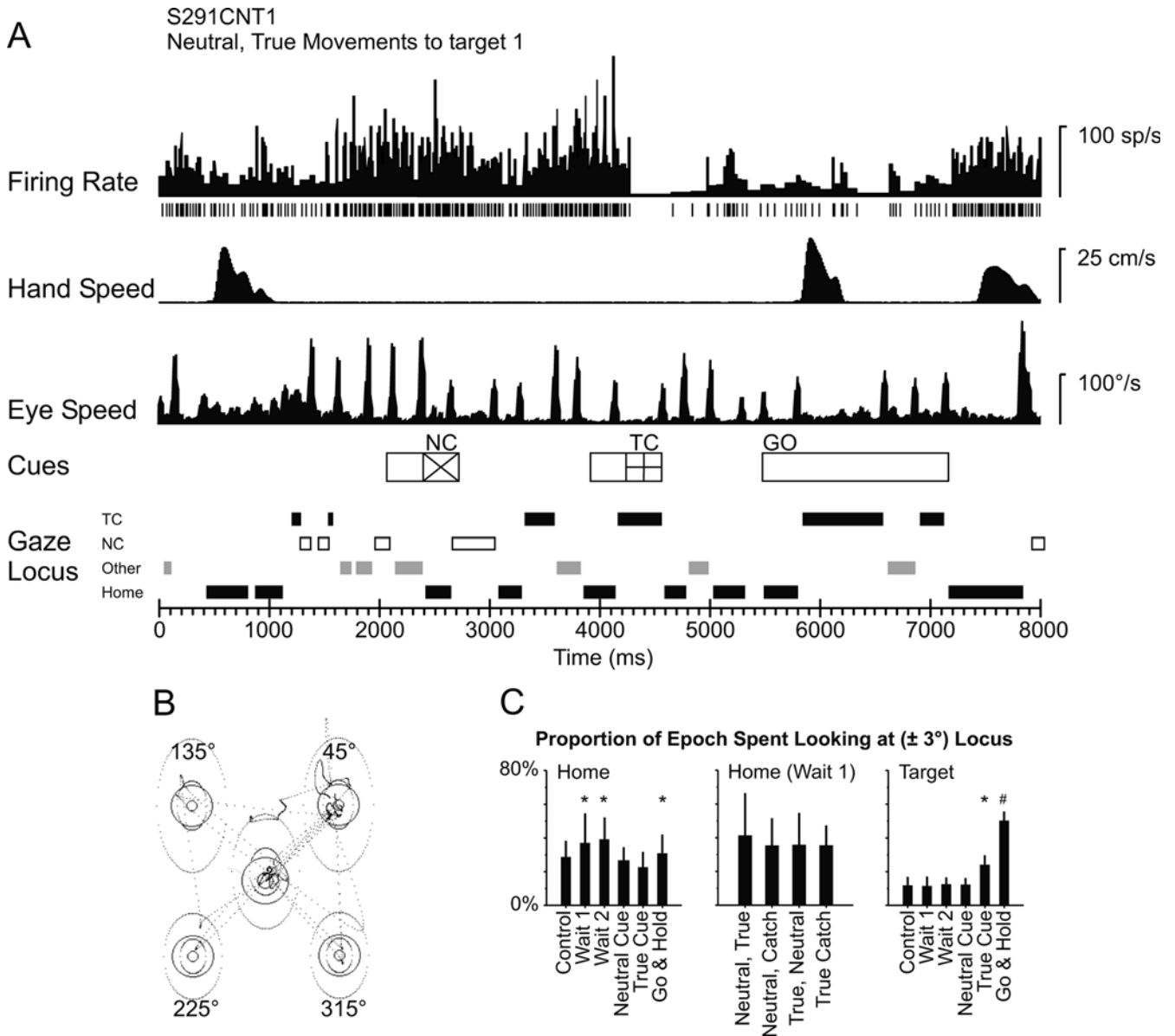


Fig. 5a–c Patterns of eye movements used by subject S during task performance. In **a**, a single trial is illustrated from start to finish, including the instantaneous firing rate for the cell (inverse of interspike interval), hand speed, eye speed, the presentation of the cues, and the location of the gaze. Details of EOG processing are provided in methods. For the record marked *Cues*, the times of the Neutral Cue (NC), True Cue (TC), and Go signals are indicated. For the Neutral Cue and the True Cue, the *open parts* of the boxes indicate the Alert signals, and the parts of the boxes marked by the x and + show when the cues were presented. This trial matches the example presented in Fig. 1, where target 2 was the Neutral Cue and target 1 was the True Cue. For “Gaze Locus,” the *small bars* on the line marked TC show when the subject was looking at target 1 (True Cue), NC shows when he was looking at the Neutral Cue (target 2),

Other shows when the subject was looking at either target 3 or 4, and *Home* indicates he was looking at Home. **b** Illustrates gaze location mapped onto the workspace for this trial. The *small circles* for target position in **b** show the actual targets. *Larger circles* around each target show a “halo” that was used to decrease the hand-position accuracy required while still providing a small visual target. *Ellipses* around each target show the estimate of ± 1 and $\pm 3^\circ$ of visual range as the subject looked down at these circles on the work surface. Targets are identified by their positions in standard polar coordinates, in degrees. **c** Shows the pattern of oculomotor behavior for this subject over 7 days of behavior, representing data from 2,500 trials. Bars are means and error bars are standard deviations; * and # indicate statistically significant differences among means

the mean number of times he looked at the Neutral Cue and True Cue when each was presented was not different. This suggests that the subject did look at the Neutral Cue when presented, but did not spend as much time looking at it as the True Cue.

EMG activity

EMG recordings were analyzed to determine whether the instruction cues elicited EMG activity in this task and whether there was a set-related increase in EMG activity in the instructed delay period. Data from surface, percutane-

ous, and chronic EMG recordings are shown in Fig. 6 for subjects C (Fig. 6a) and D (Fig. 6b, c). As illustrated, phasic muscle activity was confined to the movement-related period. For three blocks of trials for subject S, four blocks for subject C, and seven blocks for subject D, neither the Neutral Cue nor the True Cue elicited statistically significant EMG bursts from the muscles sampled. Although there was some EMG activity present around the times of the cues, there was no significant difference in the EMG levels after the cues compared with what was recorded during the Control period before either cue was presented. In the set period, however, there was often a slight difference in the EMG as compared with what was recorded in control intervals. In some cases, this difference was a slight elevation in the background level of EMG (e.g., Fig. 6a, AD). In other cases, the subjects seemed more careful to be still during the set period, whereas they may have made subtle adjustments at proximal joints during the preceding intervals, even though the hand remained in position over Home. This type of decrease is evident in Fig. 6b, where the EMG levels for BB, PD, and LUT were lower in the period just before the movement as compared with the levels earlier, during the times of the cues.

Thus, for the 14 cells with set-related preparatory activity (Table 2), the “preparatory activity” might well have reflected a change in EMG that was actually subtle motor activity. For the 53 cells with preparatory activity that was transient or sustained, however, the EMG patterns did not match the changes in neural activity during the instructed delay period, suggesting that these cells could truly be considered preparatory.

As illustrated in Fig. 6 and borne out by the statistical analysis, the most consistent and significant changes in EMG were associated with movement. As has been previously described for tasks of this sort (Moran and Schwartz 1999; Turner et al. 1995), movement-related EMG activity varied in amplitude and timing for movements to different targets (Fig. 6c). The proximal arm and shoulder muscles had long-lasting EMG activity that persisted during the hold period after the reach (e.g., ADIt, Fig. 6c). In the elbow muscles, biceps and triceps, there were shorter bursts that were mainly associated with the movement. For example, biceps was most active for movements to 135 and 225°, whereas triceps was most active for movements to 45 and 135° (Fig. 6c).

This variety corresponded to the variety of movement-related firing patterns observed in the neurons. For

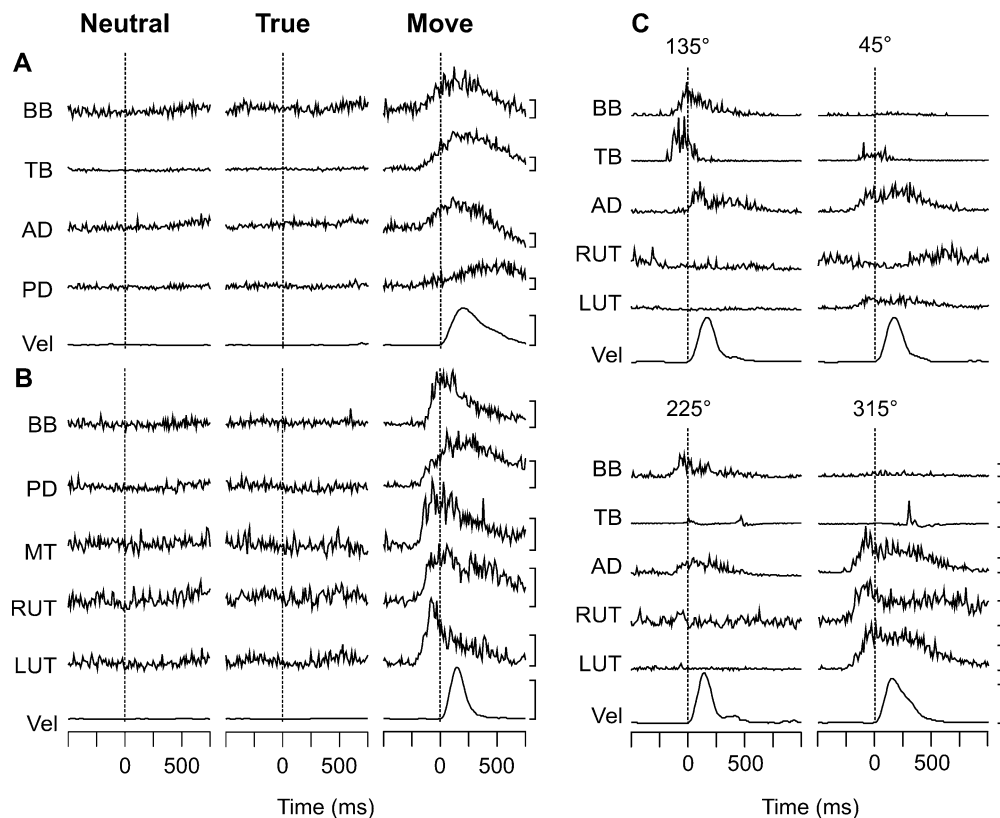


Fig. 6a–c Averaged EMG records for subjects C (**a**) and D (**b**, **c**). In **a** and **b**, EMGs were averaged for 100 contiguous successful trials from a single session. On the left, the column labeled *Neutral* illustrates EMG averaged around the time of the Neutral Cue, *True* illustrates EMG around the True Cue, and *Move* illustrates EMG during movement. In **c**, averages for movements to each target are illustrated. For each subject, a record of the hand speed (*Vel*) is shown along with the EMG's. All data were from the right arm

unless otherwise noted. Records in **a** are from surface electrodes, **b** from chronic fine-wire, and **c** from percutaneous. *BB* biceps brachii, *TB* triceps brachii, *AD* anterior deltoid, *PD* posterior deltoid, *MT* middle trapezius, *LT* lower trapezius, *RUT* right upper trapezius, *LUT* left upper trapezius. Vertical EMG calibration bars: 10 μ V in **a** and **b**; 20 μ V in **c** for BB, TB, AD, and LUT, and 10 μ V for RUT. Vertical velocity calibration: 10 cm/s in **a** and 20 cm/s in **b** and **c**

example, the relatively long and omni-directional activity pattern of the cell illustrated in Fig. 2a–c bore some resemblance to the EMG patterns recorded from proximal muscles like posterior deltoids (Fig. 6). The brief and directionally specific pattern of the cell illustrated in Fig. 2f seemed to be the opposite of the EMG pattern typical for biceps. Biceps was active for reaches to 135 and 225°, which is when the cell illustrated in Fig. 2f had a decrease in activity. This is not meant to suggest that these specific cells controlled these specific muscles or their antagonists. Rather, the observation is that neural activity patterns in the mPMRF included a variety reflective of the muscle activity patterns for reaching.

Responses to somatosensory manipulation

Neural responses to somatosensory examinations were documented for 126 cells. Effective somatosensory stimulation usually required either passive movement of a body part or deep/firm palpation of body surfaces. Sixty-one cells (48%) responded preferentially to manipulation of the arm including the shoulder and/or elbow joints, 40 (32%) responded preferentially to the manipulation of the shoulder girdle (i.e., scapulothoracic joint), 21 (17%) responded preferentially to manipulation of the axial skeleton (trunk, tail, neck), and only four (3%) had no apparent response to somatosensory examination. Distal sites (forearm, hand) were ineffective. A Chi-squared analysis indicated no difference in the locations (ARM,

SHOULDER, AXIAL, or NO RESPONSE) of the most effective area for somatosensory stimulation for M vs. MP cells.

Responses to electrical stimulation

Electrical stimulation was applied at or near 119 of the 176 recording sites as another means to ensure that recording sites were indeed in arm-related regions of the mPMRF. The responses to electrical stimulation are presented in Table 3. As shown in the table, the predominant response to stimulation was contraction around the shoulder. This was a site of contraction for 66 of the 101 responses (65%) and from 55% of all of the sites tested. In many cases, movements evoked by stimulation were strong at the shoulder but also included weak tail or trunk movements; these sites were included in SHOULDER in Table 3. Only sites where the axial responses were alone or dominated over very weak shoulder responses were called AXIAL. Muscle twitches were not observed in the forearm or hand.

The mean threshold for a contractile response was 30 μ A. In rare cases, movements were evoked with currents as low as 5 μ A, but in practice, a “low” threshold was typically 10–15 μ A. Currents over 35 μ A were rarely required. Because the purpose of this investigation was to study areas related to reaching, the prevalence of arm- and shoulder-related areas reflects sampling bias, not the full capacity of the mPMRF. A Chi-squared analysis indicated

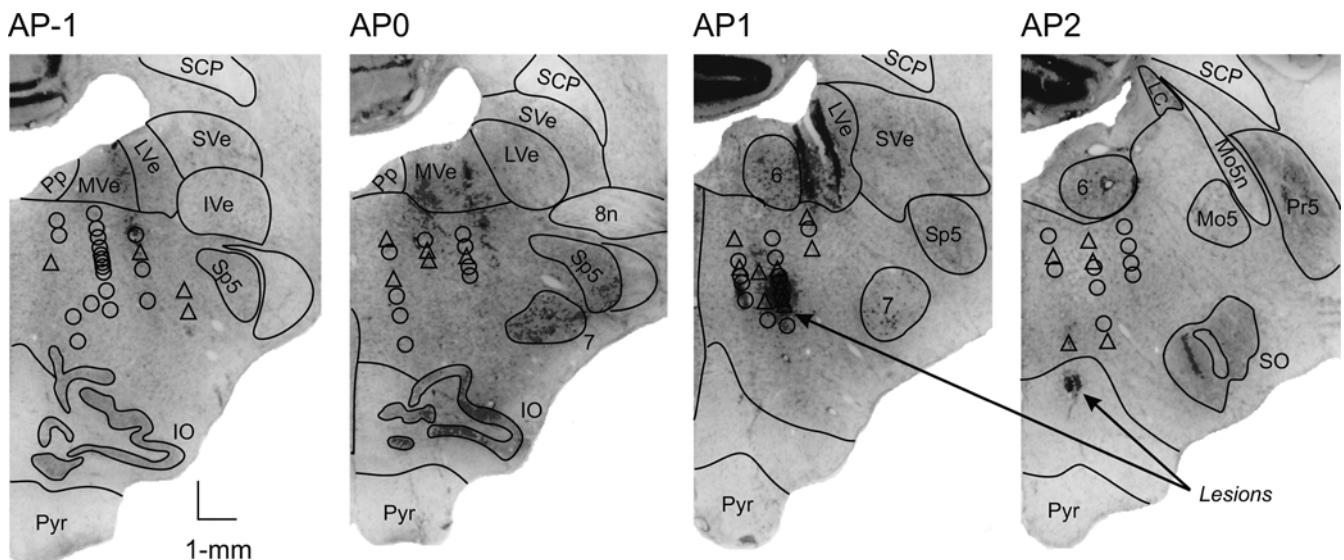


Fig. 7 Histological reconstruction of recording sites for subject D. Circles represent M cells, and triangles represent MP cells. AP planes are Horsley-Clarke stereotaxic coordinates based on the targeted location of the recording chamber. Sections were cut at 40 μ m and stained with Cresyl Violet and scanned at 1,200 dpi. Sections are illustrated at 5 \times actual size; the horizontal distance between tracks illustrated at AP0 was 1.0 mm. Electrolytic marking lesions are visible at AP1 and AP2, as indicated by the arrows. The lesion at AP2 was made where the electrophysiological monitoring during recording indicated that cellular potentials ended and fiber-like background activity began. The lesion at AP1 was made at a

location that was judged to be in the middle of a recording area that had yielded an abundance of data. *Pp* pedunculo-pontine nucleus, *MVe* medial vestibular nucleus, *LVe* lateral vestibular nucleus, *SVe* superior vestibular nucleus, *IVe* inferior vestibular nucleus, *SCP* superior cerebellar peduncle, *Sp5* spinal trigeminal nucleus, *IO* inferior olivary complex, *Pyr* Pyramid, *8n* vestibulocochlear nerve, *7* facial nucleus, *6* abducens nucleus, *LC* locus ceruleus, *Mo5n* motor component of trigeminal nerve, *Mo5* motor component of trigeminal nucleus, *Pr5* principal trigeminal nucleus, *SO* superior olivary complex

no difference in the region responding to electrical stimulation applied at the sites of M vs. MP cells.

Anatomical reconstructions

Anatomical reconstructions showed that the cells sampled were between 1 and 4 mm lateral to the midline, between 1 and 4 mm ventral to abducens, and between 2 mm anterior and 3 mm posterior to abducens, which was typically centered at around AP +0.5 in the stereotaxic coordinates. The areas where cells were recorded included NRGc, NRMc, and NRPC. No tracks were rostral enough to sample cells from NRPO. For the most part, cells were located in regions that would be expected to contain reticulospinal cells. Over the course of the studies, some activity was recorded dorsally in structures such as the pedunculopontine nucleus, and the vestibular nuclei, but these data were excluded. Some cells studied were as much as 4 mm lateral to the midline, such as the two cells at AP-1. Cells such as these were retained as long as they met the other criteria, since part of the NRPC, which extends this far laterally, may be present at this plane (Bowden et al. 2003). The main objective of the histological reconstructions was to determine whether there were separate locations for M vs. MP cells. Figure 7 illustrates the locations of M (*circles*) and MP (*triangles*) cells recorded in subject D. As illustrated, M and MP cells overlapped; there was no distinct zone where MP cells were concentrated. Similar reconstructions in subjects S and C showed the same relative locations of recording sites, and also showed no distinct locations for MP vs. M cells.

Discussion

Movement-related and preparatory activity

Several studies have demonstrated that the mPMRF is critical for postural support and other automatic movements such as locomotion (Drew et al. 1986; Kuypers 1981; Matsuyama and Drew 2000a, b; Matsuyama et al. 2004; Wilson and Peterson 1981). The present data suggest that the mPMRF system is also engaged in the preparation and performance of voluntary reaching. The arm movements were constrained to a relatively small workspace, with center-out movements like those used for the study of directional tuning in the primary motor cortex (Georgopoulos et al. 1986, 1988). The subjects sat in a primate chair with the left arm restrained at the side, the legs contained in a box, and the head also restrained. Even the right arm was restricted to reaching in front of the animal at shoulder level, with the hand and wrist affixed to a splint. Despite these constraints, it was not difficult to locate cells with activity that was clearly modulated during reaching. Our findings under these conditions suggest that even for reaching movements that do not include overt postural adjustments, descending commands from the

reticulospinal system are an important part of motor control.

Perhaps the most interesting finding was the demonstration of preparatory activity in the mPMRF. We expected this because anatomical studies indicate that cortical motor areas just rostral to the primary motor cortex, which are known to display preparatory activity (Ashe et al. 1993; Crammond and Kalaska 2000; Godschalk et al. 1985; Tanji and Kurata 1985; Weinrich and Wise 1982; Wise et al. 1986), are a key source of input to the reticulospinal system (Keizer and Kuypers 1989; Kuypers and Lawrence 1967). Our physiological data supported this anatomical prediction.

Preparatory activity has been demonstrated at several other sites outside the cerebral hemispheres. These include the dentate nucleus of the cerebellum (Strick 1983), spinal interneurons during a wrist flexion-extension task (Prut and Fetz 1999), the globus pallidus (Turner and Anderson 1997), the striatum (Alexander and Crutcher 1990a, b), and pallidal-receiving areas of the thalamus (Anderson et al. 1993). Together, these results suggest that motor preparation is a distributed phenomenon, not a feature limited to the cerebral cortex (Prut et al. 2001).

EMG analyses indicated that the cells with set related activity had firing patterns that could have matched changes in muscle activation. As such, these cells cannot be considered purely preparatory within this dataset. Some might also question whether the cells with transient activity should be counted as preparatory, as these cells may have theoretically been responding to sensory aspects of the instruction stimulus. We have called these preparatory cells because they responded to the True Cue, not the Neutral Cue. Thus, they responded to the instruction stimulus only when it was relevant to the upcoming movement. The EOG analyses indicated that the patterns of preparatory neural activity observed were not consistent with the patterns of eye movements used to perform the task.

The proportion of reticular formation cells with preparatory activity is comparable to, though somewhat lower than reported in SMA, PMd, and CMAc (Alexander and Crutcher 1990b; Backus et al. 2001; Chen et al. 1991; Kurata and Wise 1988; Requin et al. 1990). Some cells with preparatory activity can also be found in M1 (Crammond and Kalaska 2000; Mushiake et al. 1991), though they are common only rostrally, close to PMd (Crammond and Kalaska 2000). Even in centers of motor preparation such as SMA and PMd, many cells show only motor activity and do not display preparatory activity (Chen et al. 1991; Kurata and Wise 1988). Our sample did not include any cells with preparatory activity that did not also have movement-related activity, but that should not be used to infer their absence from this part of the brain because we did not record from every cell we encountered: we focused on cells with clear movement-related activity. Cells showing only preparatory activity have constituted about 15–30% of the sample in studies designed to identify them in cortical motor areas (Alexander and Crutcher 1990b; Chen et al. 1991; Wise et al. 1986).

Few studies have identified subcortical output destinations of cortical cells with preparatory activity through methods like antidromic activation (Helminski and Segraves 2003; Sommer and Wurtz 2001; Turner and DeLong 2000). Except for Turner and DeLong (2000), who studied preparatory activity in corticostriatal cells from M1, these studies have been for control of eye movement. Thus, for most studies on the control of reaching, it is not known whether cortical cells with preparatory activity project to other cortical areas, to the basal ganglia, to neurons that project to the cerebellum, to the reticular formation, to the red nucleus, or to the spinal cord. The apparent prevalence of collaterals to the reticular formation from corticospinal cells in motor and especially premotor cortical areas (Kably and Drew 1998a; Keizer and Kuypers 1984, 1989; Rho et al. 1997) makes these cortical areas a likely source of motor and preparatory activity in the reticulospinal system. We hypothesize that MP cells receive input from cortical cells with preparatory activity, predominantly from premotor cortical areas, while M cells receive input from cortical cells without preparatory activity, from the premotor areas and the primary motor cortex. Arm-related activity has also been demonstrated in subcortical areas that project to the mPMRF, the mesencephalic reticular formation below the superior colliculus (Lunenburger et al. 2001; Stuphorn et al. 2000) and near the red nucleus (Gibson et al. 1998). These areas could also be sources of the activity studied here, along with cerebellar (interposed) and vestibular inputs to the mPMRF (Iwamoto 1990; Iwamoto et al. 1990; Keizer and Kuypers 1989; Kuypers 1981; Peterson et al. 1974).

As for the function of preparatory activity in the mPMRF, it seems likely that some of this activity is conveyed out of the mPMRF to the spinal cord, and this could be one of the sources of preparatory activity that has been demonstrated there (Prut and Fetz 1999), along with outputs from the cerebral cortex directly to the spinal cord. Although we did not antidromically identify the cells in this study as reticulospinal neurons, the well-known sampling bias towards those largest cells (Lemon 1984) makes it likely that they make up a substantial portion of our sample (Drew et al. 1986). As Prut and Fetz (1999) suggested, by having certain spinal interneurons activated in advance of descending commands, preparatory activity could serve to gate the motor commands for execution towards the specific pathways that would be used for control during that movement. Likewise, the reflex circuits expected to convey the afferent consequences of the movement could be primed so that the desired responses would be ready. Preparatory activity could also depolarize (to a subthreshold level) motoneurons that would need to be recruited during movement, and through interneurons, inhibit the antagonists. In addition to preparing the spinal cord, the preparatory activity may have reflected a preparation of the mPMRF itself, so that when movement execution began, the mPMRF was already primed to contribute. Some of this preparatory activity may also

have produced subtle postural adjustments that were undetected in this study.

The only other study showing something like preparatory activity in the mPMRF is recent work in the cat (Schepens and Drew 2003a, b). This study reported the activity of neurons in the mPMRF during the anticipatory postural adjustments (Horak et al. 1984, 1989; Massion 1992) preceding a reach and during the reach itself. As cats stood on four independent force platforms in a setup like that developed by Macpherson et al. (1987) and reached for food in a tube at shoulder level, Schepens and Drew identified two subsets of muscles, one with EMG onset latencies temporally correlated with the onset of the anticipatory postural adjustment, and the other with onset latencies correlated with the onset of the reaching movement; some muscles were associated with both. Neurons studied during the same task could largely be divided into the same categories, those with onsets correlated with movement onset, those with onsets correlated with the onset of the anticipatory postural adjustment, and those associated with both (Schepens and Drew 2003a).

Based on the present results, one could speculate that the MP cells might represent a population similar to those associated with anticipatory postural adjustments in the cat, whereas the M cells might be similar to those specifically associated with the reach in the cat. However, in the present task, there was a variable delay imposed between the instruction stimulus and the Go cue. In the task used by Schepens and Drew, the cats paced themselves after the Go cue, and the anticipatory postural adjustment and subsequent reach both followed this stimulus. Thus, the cells associated with the anticipatory postural adjustments in the cat might best be represented by the MP cells with set-related activity in the present study, because both were associated with task-related changes in EMG just before the reach.

Relationship with kinematics

Some directional tuning of the movement-related activity in the mPMRF was often present, but the goodness of fit was rarely strong. This lack of directional tuning is in contrast to strong directional tuning found in primary motor cortex (Evarts 1972; Georgopoulos et al. 1986, 1988; Moran and Schwartz 1999). Directional tuning of preparatory activity in the present study was less common and even weaker than directional tuning of the movement-related activity. In the dorsal premotor cortex, which projects to the mPMRF, preparatory activity commonly shows strong directional tuning (Alexander and Crutcher 1990b; Caminiti et al. 1991; Crammond and Kalaska 2000; Wise et al. 1997). Directional tuning has also been demonstrated in many other motor and somatosensory areas, including posterior parietal cortex (Kalaska et al. 1983), cerebellum (Fortier et al. 1993), thalamus (Anderson et al. 1993), striatum (Alexander and Crutcher 1990a),

globus pallidus (Turner and Anderson 1997), and corticothalamic cells in M1 (Ruffo and Anderson 2003).

Our main objective was to determine whether or not preparatory activity was present in these cells. This focus led us to limit the number of movement directions and other variations in kinematics. Thus, our experiment did not provide a thorough test for kinematic coding in this part of the brain. Although directional tuning might have been easier to reveal if we had used more directions of reaching, it seems doubtful that this alone would have made much difference. The mPMRF seems mainly dedicated to control of proximal muscles (Davidson and Buford 2004; Drew and Rossignol 1990; Lawrence and Kuypers 1968). Reaching like that employed in the present study may not have required much contrast in the neural activity of the mPMRF. To fairly test whether kinematic parameters such as direction or force (Fu et al. 1995) are encoded in this part of the nervous system, it would be better to study animals performing more extensive reaches with more explicit control of movement kinematics and kinetics.

Conclusions

Evidence in the literature shows that control of posture and whole body movement patterns is an important role for the reticulospinal system. The present results show that even for discrete, voluntary reaching, neurons in the mPMRF are strongly modulated. The new finding that preparatory activity exists in the mPMRF indicates that neural activity in this part of the brain is concerned with the early stages of motor control. These findings could support the hypothesis that the reticulospinal system is an alternative pathway for the voluntary control of reaching, which may be especially important for recovery of function after stroke and other injuries to the cerebral cortex (Freund and Hummelsheim 1985). The fact that there was little relationship between the activity of cells in the mPMRF in this study and the kinematics of hand movement is consistent with the fact that, after stroke, patients have great difficulty in controlling their distal segments. Thus, while reticulospinal outputs might serve to move the arm in gross patterns, activity in the mPMRF probably does not normally contribute to the details of accurate hand placement. Nonetheless, the presence of some cells with activity that was well correlated with the kinematics of the hand suggests that there is a latent potential for this function in the mPMRF, and expanding this potential could be one basis for recovery from stroke.

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