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# Discharge Properties of MST Neurons That Project to the Frontal Pursuit Area in Macaque Monkeys

Anne K. Churchland and Stephen G. Lisberger

Howard Hughes Medical Institute, Department of Physiology, W. M. Keck Foundation Center for Integrative Neuroscience, Neuroscience Graduate Program, University of California, San Francisco, California

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**Churchland, Anne K. and Stephen G. Lisberger.** Discharge properties of MST neurons that project to the frontal pursuit area in macaque monkeys. *J Neurophysiol* 94: 1084–1090, 2005. First published May 4, 2005; doi:10.1152/jn.00196.2005. We have used antidromic activation to determine the functional discharge properties of neurons that project to the frontal pursuit area (FPA) from the medial-superior temporal visual area (MST). In awake rhesus monkeys, MST neurons were considered to be activated antidromically if they emitted action potentials at fixed, short latencies after stimulation in the FPA and if the activation passed the collision test. Antidromically activated neurons ( $n = 37$ ) and a sample of the overall population of MST neurons ( $n = 110$ ) then were studied during pursuit eye movements across a dark background and during laminar motion of a large random-dot texture and optic flow expansion and contraction during fixation. Antidromically activated neurons showed direction tuning during pursuit (25/37), during laminar image motion (21/37), or both (16/37). Of 27 neurons tested with optic flow stimuli, 14 showed tuning for optic flow expansion ( $n = 10$ ) or contraction ( $n = 4$ ). There were no statistically significant differences in the response properties of the antidromically activated and control samples. Preferred directions for pursuit and laminar image motion did not show any statistically significant biases, and the preferred directions for eye versus image motion in each sample tended to be equally divided between aligned and opposed. There were small differences between the control and antidromically activated populations in preferred speeds for laminar motion and optic flow; these might have reached statistical significance with larger samples of antidromically activated neurons. We conclude that the population of MST neurons projecting to the FPA is highly diverse and quite similar to the general population of neurons in MST.

## INTRODUCTION

Area MST has long been implicated in the generation of smooth pursuit eye movements. Lesions of MST impair the steady-state maintenance of pursuit (Dursteler et al. 1987). Microstimulation in MST during pursuit causes changes in smooth eye velocity (Komatsu and Wurtz 1989). Recordings in MST during pursuit suggest that single neurons are selective for the direction and the speed of pursuit (Erickson and Thier 1991; Newsome et al. 1988; Shenoy et al. 2002; Squatrito and Maioli 1997).

The role of MST in pursuit is not fully understood, however, partly because MST neurons show a wide variety of response patterns and partly because of the diversity of projection targets of MST neurons. MST neurons respond to simple visual stimuli such as laminar motion as well as more complex visual

stimuli such as combinations of spiral motion and expansion that might comprise major components of visual signals caused by self-motion (Duffy and Wurtz 1991; Graziano et al. 1994; Orban et al. 1995). Some neurons are also sensitive to eye position (Bremmer et al. 1997; Squatrito and Maioli 1996, 1997) and to vestibular stimulation (Bremmer et al. 1999; Duffy 1998). MST projects to areas believed to play a role in pursuit, such as the dorsal lateral pontine nucleus (Boussaoud et al. 1992; Glickstein et al. 1980) and the frontal pursuit area or FPA, (Schall et al. 1995; Tian and Lynch 1996a). However, MST neurons also project to areas that seem to be involved in other behaviors, including the dorsal terminal nucleus of the accessory optic system (NOT-DTN) (Hoffmann et al. 2002), the lateral intraparietal area (LIP) (Boussaoud et al. 1990), and the saccadic frontal eye fields or FEF (Schall et al. 1995; Tian and Lynch 1996a). To understand the role of MST in pursuit, it will be useful to know the physiological response properties of neurons that project to specific areas in the neural circuit for pursuit.

We used antidromic activation to identify neurons that were part of the projection from MST to the FPA (Schall et al. 1995; Tian and Lynch 1996a). We chose to identify MST neurons that project to FPA because a large body of evidence argues that the FPA is part of the circuit that drives pursuit eye movements. Microstimulation in the FPA with small currents causes changes in smooth eye velocity (Gottlieb et al. 1994; Tanaka and Lisberger 2002a; Tian and Lynch 1996b). Lesions of the FPA cause selective deficits in smooth pursuit in both Cebus and rhesus monkeys (Lynch 1987; MacAvoy et al. 1991; Shi et al. 1998). Our data reveal that there is impressive diversity in the response properties of MST neurons that project to the FPA.

## METHODS

Many of our methods have been described in detail elsewhere (Tanaka and Lisberger 2002b). Briefly, data were collected from two adult rhesus monkeys (9–13 kg) using methods that had been approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco. All procedures were in compliance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. Surgical procedures were conducted using sterile procedure while the monkey was under general anesthesia with isoflurane. Analgesics were provided during postsurgical recovery under the supervision of veterinarians and veterinary nurses. A stainless steel or cilux head holder was installed over the skull and

Address for reprint requests and other correspondence: A. K. Churchland, Dept. of Physiology, Box 0444, 513 Parnassus Ave., Rm. S-762, San Francisco, CA 94143-0444 (E-mail: anne99@u.washington.edu).

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cemented to orthopedic titanium straps that had been secured to the bone with 6-mm-long titanium screws. At the same time, two cilix cylinders (Crist Instruments, Gaithersburg, MD) were positioned over the superior temporal sulcus and arcuate sulcus of the right hemisphere to allow access to MST and the FPA, respectively. During a separate procedure, a coil of wire was sutured to the sclera beneath Tenon's capsule (Ramachandran and Lisberger 2005) so that we could use the magnetic search coil method to record eye movements.

### Neural stimulating and recording

Sharp, 100–200 K $\Omega$  tungsten microelectrodes (Frederick Haer) were used for both stimulating and recording in the FPA. Electrodes were introduced through a guide tube inserted in a plastic grid (Crist Instruments) that fit snugly in the recording cylinder. We searched for pursuit-related regions by recording unit activity and looking for sites where eye movements were evoked by stimulation with 80-ms trains with intra-train frequencies of 300 or 500 Hz and intensities of 10–100  $\mu$ A. In electrode penetrations where superficial stimulation caused saccades while deeper stimulation caused pursuit, we were careful to position stimulating electrodes as far as possible from sites that elicited saccades. In other, generally more posterior locations, superficial stimulation caused pursuit and no saccades were observed, consistent with previous reports (Gottlieb et al. 1993, 1994; Tanaka and Lisberger 2002a).

At the sites we selected for antidromic stimulation, the properties of the eye movements evoked by stimulation conformed with previous papers (Tanaka and Lisberger 2002a). For the site illustrated in Fig. 1A, for example, stimulation delivered during sustained pursuit evoked an initial increase in eye speed followed by a decrease during all directions of pursuit.

In early experiments, three to five electrodes were implanted semi-chronically at appropriate stimulation sites by cementing them to the guide tube with epoxy and were left in place for  $\leq 4$  wk (Pare and Wurtz 2001). The integrity of the electrodes and the recording site were checked daily by examining the eye movements evoked by stimulation. Thresholds for eliciting eye movements generally increased over time, but the direction and nature of the eye movements rarely changed. In later experiments, three stimulating electrodes were introduced and removed daily. The latter method had the advantage of allowing the stimulating electrodes to be moved over the course of an experiment to optimize their location and produced a larger yield of antidromically activated cells.

Once the stimulating electrodes had been positioned, we searched for MST neurons that were antidromically activated using single biphasic pulses whose duration was kept short ( $\sim 0.15$  ms for each phase) to optimize axon activation and minimize the stimulus artifact. Activation thresholds ranged from 30 to 400  $\mu$ A; larger thresholds were more commonly observed when stimulating electrodes were glued in place because their position could not be changed to minimize the amount of current needed.

When stimulation repeatedly caused a spike in MST at a fixed delay, we attempted to isolate the neuron. Once the neuron appeared to be well isolated, as determined by the repeatability of waveforms and the presence of a refractory period, the collision test was performed to determine whether the activation was ortho- or antidromic. Stimulation in the FPA was triggered after variable delays following the occurrence of a spontaneous action potential (oblique arrows in Fig. 1, B and C). When the delay was long (Fig. 1B), the antidromic response persisted. If the collision test was passed, however, the antidromic response was abolished when the delay was short (Fig. 1C).

Waveforms from the collision test were always recorded so that off-line analysis could be used to confirm observations made during the experiment. We computed the probability that stimulation triggered on a spontaneous spike would cause the neuron to fire. For long delays, this probability was always close to 1. For shorter delays, we concluded that a neuron was antidromically activated as long as the probability was

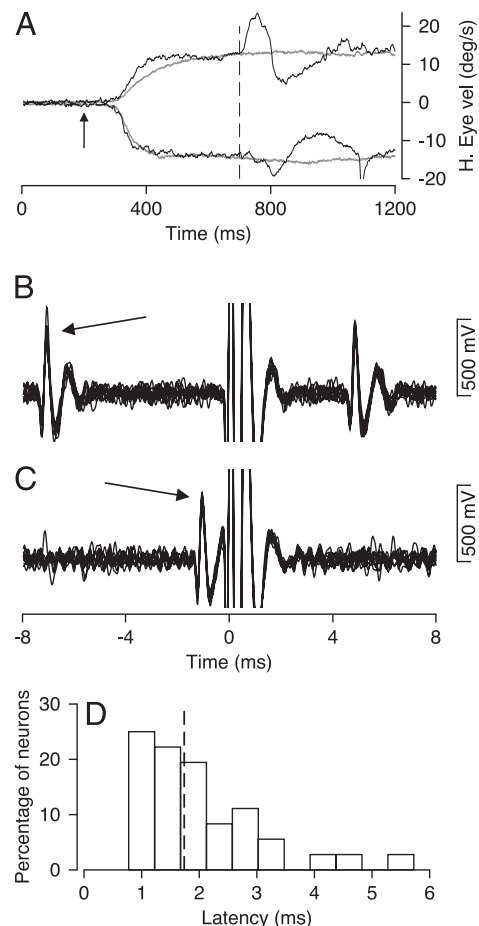


FIG. 1. Properties of stimulation sites in frontal pursuit area (FPA) and responses of medial-superior temporal (MST) neurons to antidromic stimulation in the FPA. *A*: horizontal eye movements evoked by stimulation at a representative site in the FPA during horizontal pursuit. The black and gray traces show eye velocities during pursuit with and without stimulation in the FPA. Vertical dashed line shows the time of onset of stimulation. Upward arrow shows the time of onset of target motion at 20°/s. *B*: 10 superimposed traces showing reliable activation of the neuron when stimulation in the FPA followed a spontaneous action potential by 7 ms. *C*: 10 superimposed traces showing the absence of antidromic activation due to collision when stimulation in the FPA followed a spontaneous action potential by only 1 ms. In *A* and *B*, the oblique arrows point to the action potentials that were used to trigger stimulation in the FPA. *D*: histogram showing the distribution of the latencies between stimulation in the FPA and the beginning of the action potentials for antidromically activated neurons. Vertical dashed line indicates the median latency of 1.74 ms.

$\leq 0.25$ . We also examined waveforms off-line to further confirm that our activated units were well isolated. We were conservative in only including for analysis those neurons that, in addition to the constraints imposed by the collision test (see preceding text), had repeatable waveforms and clear refractory periods. We identified 37 neurons that passed the collision test. In addition, we found, on an almost daily basis, neurons that were activated at repeatable latencies but failed the collision test. These were most frequently deemed to be recordings from multiple units and were not included in our sample. We found two units that were activated and clearly were well isolated, yet failed the collision test. These were probably orthodromically activated and were not included in our sample.

### Experimental design

After determining whether a given MST neuron was activated antidromically from the FPA, we ran a series of experiments to

determine the neuron's functional response properties. Visual stimuli were presented in blocks of trials that either required the monkey to track smoothly moving small targets or to fixate during presentation of moving stimuli.

First, a series of tests was conducted to determine the optimal size and receptive field placement for visual stimuli. Thereafter, visual stimuli consisted of a  $30 \times 30^\circ$  patch containing 500 dots that occupied most of the contralateral visual hemifield except in a few rare sites where smaller patches were able to drive neural responses more effectively. Patches were usually placed in the contralateral visual hemifield except for a few neurons the responses of which were larger for visual motion in the ipsilateral hemifield. Once optimized spatially, the patch was moved in each of eight directions to determine the preferred direction. Subsequent trials assessed the relationship between firing rate and image speed by presenting stimulus motion in the preferred direction at speeds that varied from either 2 or 8 up to  $128^\circ/\text{s}$ .

In pursuit trials, the target was a small dim spot presented in otherwise darkness. Trials began with the appearance of a fixation point that was  $7.5^\circ$  eccentric. After a fixation interval that varied randomly from 1,000 to 1,200 ms, the target stepped  $3^\circ$  further eccentric and immediately began moving toward the position of fixation at  $20^\circ/\text{s}$ ; other speeds were tried if necessary, but neurons responsive during pursuit almost always responded at  $20^\circ/\text{s}$ . When the monkey did not maintain an eye position that was within  $3^\circ$  of the target throughout the trial, the trial was terminated and the data were not included in analysis. In laminar flow trials, each trial began with 600 ms of fixation on a stationary spot, followed by the appearance of a stationary patch of dots. Two hundred milliseconds later, the dots in the patch began to move coherently at a single speed within a stationary virtual aperture and continued to move for 600 ms. The patch then was extinguished and the fixation light remained on for 200 ms. In optic flow trials, the moving stimuli consisted of large circular apertures with a radius of  $18^\circ$  containing 600 dots. Each trial began with 600 ms of fixation on a target that would be at the center of the patch. The patch then appeared and remained stationary for 200 ms before the dots in the patch moved for 1,000 ms either toward (contracting stimuli) or away from (expanding stimuli) the position of fixation. In different trials, the speed of the dots ranged from 10 to  $30^\circ/\text{s}$ ; all dots moved at the same speed for a given stimulus. In all trials, the monkey was rewarded for fixating or tracking within a  $4^\circ$  window for the duration of the trial and the trial was discarded if he failed the fixation requirements.

### Data analysis

Between 4 and 20 repetitions were recorded for each trial. The analysis interval began 100 ms after the onset of the neural response and ended at the end of the stimulus, thus capturing the entire sustained response but not the transient responses to the onset of stimulus motion. Responses to identical stimuli were averaged and the baseline firing was subtracted. To analyze the directional properties of the responses during pursuit or image motion, we treated each direction in the stimulus set as a two dimensional vector; the direction of the vector corresponded to the direction of the stimulus and the length of the vector corresponded to the size of the neural response. To determine the preferred direction, we summed the eight vectors for the eight directions of pursuit or image motion. Hotelling's  $T^2$  test was used to assess whether neural responses showed statistically significant modulation by stimulus direction (Batschelet 1981). We used this same approach to identify any skew in the distributions of preferred directions across our population: unit vectors corresponding to each neuron's preferred direction were summed together to generate a population vector (as in Fig. 4). The length of this vector is 0 when the preferred directions are uniformly distributed.

The relationships between firing rate and the image speed in responses to laminar motion and optic flow motion were analyzed

using the same basic approach. First, we performed an ANOVA using speed as the main factor and a criterion of  $P = 0.05$  to determine whether there was a statistically significant relationship between firing rate and speed. If there was, we quantified the relationship between firing rate and speed and assessed the best speed using an approach adopted by Liu and Newsome (2003) in MT: we fitted the responses with a smoothing cubic spline function (Shikin and Plis 1995). The knots of the spline were the speeds where we tested each neuron's response: from 2 or 8 to  $128^\circ/\text{s}$  for laminar motion and from  $-30^\circ/\text{s}$  (contraction) to  $+30^\circ/\text{s}$  (expansion) for optic flow. The smoothness of the spline was set so that the speed tuning properties from the fitted curves agreed well with visual inspection and the same smoothness was used for all cells.

### Comparison of units projecting to the FPA with overall population in MST

We combined two populations of MST neurons recorded from the same monkeys to obtain a sample of 110 recordings from the overall population of MST neurons for comparison with neurons that were antidromically activated from the FPA. Some neurons in the comparison population ( $n = 46$ ) were collected as we searched for antidromically activated neurons but were not activated by stimulation at the sites where we had positioned stimulating electrodes. The rest of the comparison population ( $n = 64$ ) was collected in the course of other experiments where we were not searching for antidromically activated neurons. Both subgroups used to assemble our sample of the general population in MST probably contained a few neurons that projected to the FPA. Nonetheless, we suspect that there were only a few projection neurons in the comparison sample because of the trying fact (see Movshon and Newsome 1996) that we were not able to demonstrate antidromic activation of the vast majority of neurons we encountered in MST, even when as many as five stimulating electrodes were positioned in the FPA.

### Location of recordings

We were not able to localize the sites of recordings on the basis of histology because the two monkeys used in this study are still in use for other experiments. However, several observations suggest that our recordings were localized mainly in the dorsal subregion of MST known as MSTd. First, the mean depth of activated neurons was  $7,420 \mu\text{m}$ ; almost all activated neurons were between 5,000 and  $9,000 \mu\text{m}$  consistent with depths reported elsewhere for MSTd (Komatsu and Wurtz 1988a). Second, most of the neurons we recorded had large receptive fields. We quantified receptive field size by determining the spatial locations where responses were 3 SD above baseline response. Mean receptive field size was  $23.3 \times 16.6^\circ$  (over half the area of the screen). Several factors make it difficult to compare our receptive field sizes with those reported elsewhere. First, our screen measured only  $32 \times 40^\circ$  so the largest receptive fields contributing to our average were much smaller than those reported in other papers (Duffy and Wurtz 1991). Second, we counted locations in the visual field as part of the receptive field only when responses during the stimulus exceeded those during the baseline by 3 SD. Occasionally, neurons were described in on-line notes as having large receptive fields but turned out to have only a small receptive field by our strict criteria. Had we included all parts of the visual field where firing rate exceeded baseline by only 2 SD, our mean size would have been  $30 \times 22^\circ$  (almost the size of our screen) instead of  $23 \times 16^\circ$ . Finally, Komatsu and Wurtz (1988a) describe MSTd receptive fields as simply " $>14^\circ$  per side," in keeping with our observations. Thus even though we cannot rule out that part of our sample comes from the lateral part of MST (MSTl), our observations on the size of the receptive fields in our sample do not contradict the likelihood that we recorded primarily from MSTd.

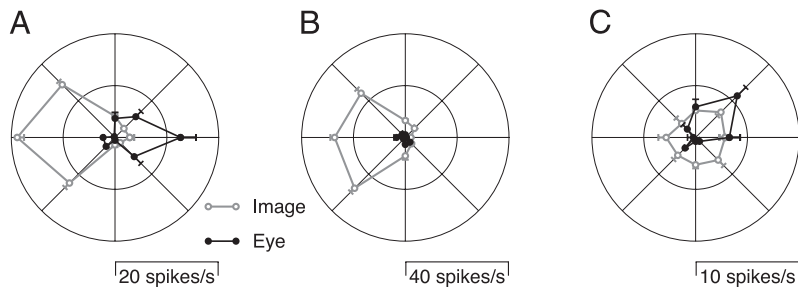


FIG. 2. Polar graphs showing tuning curves of 3 antidromically activated MST neurons. Black and gray curves and symbols show data for pursuit across a dark background and for image motion during fixation. Each symbol indicates the end of a vector, where the angle of the vector is the direction of eye or image motion and the length of the vector is proportional to response magnitude. Error bars indicate SE.

## RESULTS

We identified 37 MST neurons in two monkeys that were classified as antidromically activated because they were activated by stimulation in the FPA and passed the collision test (Fig. 1, *B* and *C*). As shown in the histogram of Fig. 1*D*, the latencies for antidromic activation ranged from 0.76 to 5.31 ms with a median of 1.74 ms (vertical dashed line in Fig. 1*D*). These values were similar to the latencies that have been reported for antidromic activation of MST neurons from the brain stem (median: 1.8 ms) (Hoffmann et al. 2002), for cortico-cortical connections between LIP and the frontal eye field (mean: 2.3 ms) (Ferraina et al. 2002), and for connections between LIP and the superior colliculus (mean: 2.3–3.2 ms) (Pare and Wurtz 1997). Somewhat shorter conduction times were found between V1 and MT (mean: 1.3 ms) (Movshon and Newsome 1996).

### Direction tuning for image motion and eye motion

Of 37 antidromically activated neurons, 21 showed significant direction tuning for the laminar motion of a random dot texture during fixation (Hotellings  $T^2$  test,  $P < 0.05$ ), 25 showed significant direction tuning during pursuit in the dark (Hotellings  $T^2$  test,  $P < 0.05$ ), and 6 were not tuned under either condition (or for optic flow stimuli). Of the antidromically activated neurons that showed significant direction tuning under one condition or the other, 16 showed significant direction tuning under both conditions. Example tuning curves for each type of response pattern are illustrated in Fig. 2: the neuron in Fig. 2*A* preferred leftward image motion (gray curve) and rightward eye motion (black curve), the neuron in Fig. 2*B* preferred leftward image motion and was unresponsive during eye motion, and the neuron in Fig. 2*C* preferred right and upward eye motion and was responsive but not tuned for the direction of image motion.

Figure 3 compares the distributions of direction preferences in the population of antidromically activated neurons with those of the comparison population of neurons. In these graphs, the direction preferences of the samples from the two monkeys are shown with different symbols but have been plotted together because they were not statistically different. Inspection of Fig. 3 illustrates that direction preference was similar for our activated and comparison populations. For image motion during fixation (Fig. 3, *A* and *B*), the distribution of preferred directions in the antidromically activated neurons (*A*) showed a weak bias for contraversive motion that did not reach significance (Rayleigh test:  $P = 0.244$ ) with the same situation ( $P = 0.377$ ) in the 70 of 110 comparison neurons that showed significant direction tuning (*B*). When we examined the direction tuning for image motion of only those comparison neurons

that we encountered while explicitly searching for activated neurons, we found a similar weak contraversive bias that was not significant ( $n = 20$ ,  $r = 0.25$ ,  $P = 0.32$ ). For pursuit across a dark background (Fig. 3, *C* and *D*), the distribution of preferred directions in the antidromically activated neurons (*C*) showed a weak downwards bias that did not reach significance (Rayleigh test:  $P = 0.301$ ), whereas there was a weak contraversive bias (Rayleigh test,  $P = 0.853$ ) among the 80 comparison neurons had significant direction tuning for pursuit (*D*). When we examined the direction tuning for pursuit of only those comparison neurons that we encountered while explicitly searching for activated neurons, we found a weak contraver-

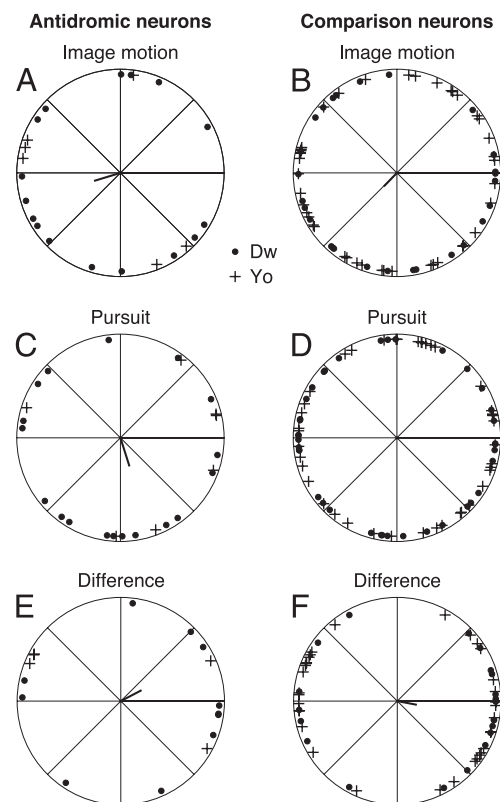


FIG. 3. Comparison of preferred directions for image motion and eye motion in MST neurons. *Left* and *right*: data from antidromically activated and control populations, respectively. *A* and *B*: preferred directions for image motion during fixation. *C* and *D*: preferred directions for pursuit across a dark background. *E* and *F*: difference in preferred directions for image vs. eye motion. In each polar plot, the + and • show neurons from monkeys Yo and Dw. Each symbol indicates the end point of a vector, where the angle of the vector indicates the preferred direction. All vectors had the same length, so that response magnitude is not represented. The short vectors in the middle of the graph show the average preferred direction and response magnitude across the population of neurons in the graph.

sive bias that was not significant ( $n = 31$ ,  $r = 0.21$ ,  $P = 0.236$ ).

Comparison of the preferred directions for image motion and eye motion in individual neurons also failed to reveal striking differences between antidromically activated and comparison neurons. In Fig. 3, *E* and *F*, each symbol shows data from one neuron and plots the difference in the preferred directions for image motion during fixation and pursuit across a dark background. A number of neurons were similar to the example in Fig. 2*A* in that their preferred directions differed by  $\sim 180^\circ$ , but other neurons had preferred directions that were quite similar and differed by closer to  $0^\circ$ . This pattern is clearest in the larger comparison sample of neurons, where the difference between preferred directions in the two conditions tended to cluster around  $0$  or  $180^\circ$  but were unlikely to be  $90$  or  $270^\circ$ . In other words, the preferred directions were usually either aligned or opposed. Rao's spacing test (Batschelet 1981) revealed that the bimodality was statistically significant in Fig. 3*F* ( $P < 0.01$ ), but not in Fig. 3*E*.

#### Relationship between firing rate and image speed

Of 31 antidromically activated neurons that were tested for laminar motion during fixation over a range of stimulus speeds, 19 had responses that were significantly related to the speed of image motion (ANOVA,  $P < 0.05$ ). The two example neurons used to obtain Fig. 4, *A* and *B*, had firing rates that depended strongly on stimulus speed, but the neuron in Fig. 4*A* had the largest response at the slowest speed we tested, whereas the neuron in Fig. 4*B* preferred faster speeds. For each neuron, the preferred speed was estimated as the value of speed at the peak of the cubic spline curve that provided the best fit to the data. Comparison of the preferred speeds among antidromically activated neurons with those of control neurons did not reveal large differences. For the antidromically activated neurons (Fig. 4*C*), there was a slight tendency for neurons to prefer slower speeds, but the population was sufficiently small that it is hard to tell conclusively. For the population of comparison

neurons (Fig. 4*D*), preferred speed was skewed toward faster speeds. The mean preferred speed of the comparison population was larger than that of the antidromically activated population ( $76.7$  vs.  $60.0^\circ/\text{s}$ ), but the difference did not reach statistical significance ( $P = 0.14$ , 2-tailed *t*-test).

#### Tuning for contracting and expanding optic flow

Of 27 antidromically activated neurons that were tested with optic flow stimuli, 14 had responses that were tuned significantly (ANOVA,  $P < 0.05$ ). For example, the neuron analyzed in Fig. 4*E* preferred stimuli that were expanding (positive values of speed) and gave the largest response at the fastest speed tested. The neuron in Fig. 4*F* preferred stimuli that were contracting (negative values of speed) and gave the largest response for contraction at  $10^\circ/\text{s}$ . For each neuron, the preferred speed was estimated as the value of speed at the peak of the cubic spline curve that provided the best fit to the data. In the population (Fig. 4*G*), 10 antidromically activated neurons preferred expansion while the remaining 4 preferred contraction. Three of the four neurons that were driven best by contraction preferred the slowest speed tested, whereas only one of those driven best by expansion preferred the slowest speed tested. Of 42 comparison neurons tested, 29 were tuned significantly (ANOVA,  $P < 0.05$ ). The comparison population (Fig. 4*H*) showed the same bias for expansion that we observed among the antidromically activated neurons, in agreement with the prior report of Graziano et al. (1994): 18 preferred expansion, whereas 11 preferred contraction. The distribution of preferred speeds among the comparison sample was skewed toward faster speeds regardless of whether flow field was expanding or contracting. Maintaining the signs for expanding and contracting optic flow, the mean preferred speed of the comparison population was larger than the mean preferred speed of the activated population ( $4.6$  vs.  $15.3^\circ/\text{s}$ ). However, the difference did not reach significance ( $P = 0.15$ , 2-tailed *t*-test).

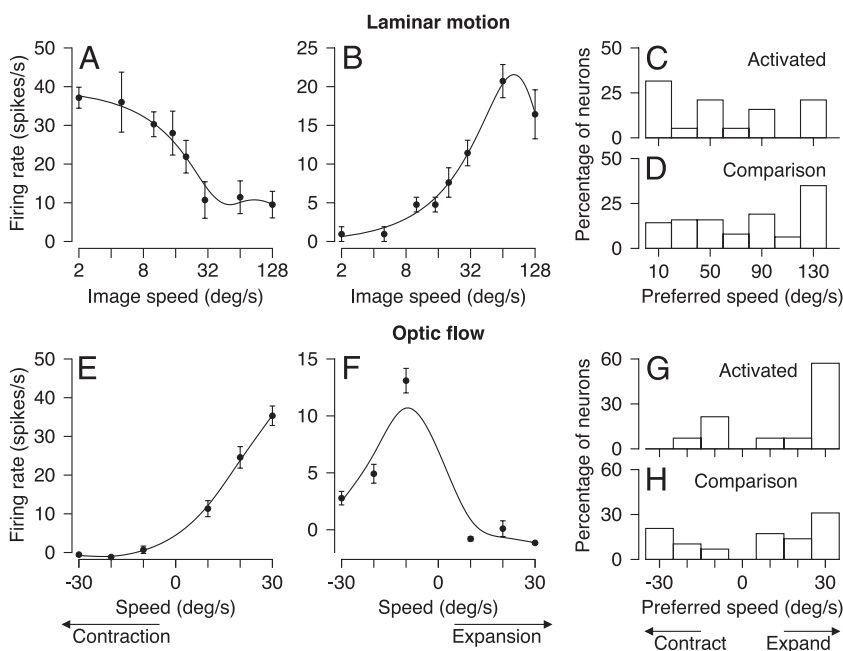


FIG. 4. Comparison of speed tuning (*A–D*) and responses to optic flow (*E–H*) of antidromically activated neurons and the general MST population. *A* and *B*: example speed tuning curves for image motion during fixation from two antidromically activated neurons. Symbols show the mean firing rates for each stimulus speed, after subtraction of the baseline firing rate. Error bars indicate SE. Solid curves resulted from fitting a cubic smooth spline to the data. *C* and *D*: distributions of preferred speeds for 19 antidromically activated neurons and 72 other MST neurons with significant speed tuning. Histogram bins are  $20^\circ/\text{s}$  wide; the first bin is centered at  $10^\circ/\text{s}$ . *E* and *F*: example optic flow tuning curves for 2 antidromically activated neurons. Symbols show the mean firing rates for each stimulus speed, after subtraction of the baseline firing rate. Error bars indicate SE. Solid line indicates cubic smooth spline fit to the data. *G* and *H*: histograms of preferred speeds of 14 antidromically activated neurons and 29 other MST neurons with significant tuning for optic flow stimuli. In all 4 panels, negative and positive values of speed indicate contraction and expansion of the optic flow stimulus, respectively.

## DISCUSSION

We set out to explore responses of MST neurons projecting to the FPA because the FPA clearly plays an important role in pursuit (Gottlieb et al. 1994; Lynch 1987; MacAvoy et al. 1991; Shi et al. 1998; Tanaka and Lisberger 2002b), and we hoped that identifying neurons according to their projections might highlight a functionally distinct subset of MST neurons. Comparison of the functional response properties of antidromically activated neurons with a sample of comparison neurons in MST failed to reveal response properties that distinguished the neurons projecting to the FPA. The distributions of preferred directions for eye motion across a dark background and for laminar visual motion during fixation were indistinguishable in the two groups of neurons, as was the difference in preferred direction for image and eye motion. There were differences between the control and antidromically activated populations in preferred speeds for laminar motion and optic flow, but these did not reach statistical significance. We conclude that the population of MST neurons projecting to the FPA is more similar to the general population of neurons in MST than it is different. Our data do not reject the possibility that the projection neurons are drawn uniformly from the general population, but it seems plausible that some of the small differences we saw could have reached statistical significance with a larger sample of antidromically activated neurons. Still there is good agreement between our finding that neurons sharing a projection target can exhibit diverse response properties and the results of parallel experiments done on the saccadic regions of the cortex (Ferraina et al. 2002; Sommer and Wurtz 2000, 2001; Wurtz et al. 2001).

The response properties of our comparison sample of neurons was in good agreement with prior reports, implying that our conclusions are based on an adequate sample of the overall population of MST neurons. Our observation that the population of MST neurons may have a weak bias for contraversive visual motion agrees with other studies that have examined the directionality of visual motion in MST (Komatsu and Wurtz 1988a; Shenoy et al. 2002). Our observation that the population of MST neurons does not exhibit a strong directional bias for pursuit agrees with other studies (Komatsu and Wurtz 1988a; Shenoy et al. 2002; Squatrito and Maioli 1997). Our finding that the preferred directions for image and eye motion were either aligned or opposed is consistent with prior studies: some reported that pursuit and visual responses tend to be opposed (Komatsu and Wurtz 1988b; Shenoy et al. 2002), whereas others suggest that they tend to be aligned (Chukoskie and Movshon 2002; Erickson and Thier 1992; Squatrito and Maioli 1997). However, all reports found both aligned and anti-aligned preferred directions and only the fraction of each varied across studies. We may have found a more even split because our comparison population was quite large ( $n = 110$ ) with 56 neurons having significant responses during both conditions or because we sampled across a wider area of MST. Finally, our control sample agrees with prior findings that the population of MST neurons is skewed toward faster visual speeds of laminar image motion (Kawano et al. 1994) and optic flow (Orban et al. 1995; Tanaka and Saito 1989).

Our observation, that neurons projecting to a pursuit area are responsive not only during pursuit, is perhaps surprising. Why might the FPA, an area that drives pursuit, receive signals from

MST about not only pursuit but also laminar visual motion and optic flow? One possibility is that the FPA combines the inputs from MST in a way that averages out or minimizes signals related to visual stimuli and allows the MST outputs related to pursuit have a disproportionately large effect on firing in the FPA. The second possibility is that signals relating to laminar and optic flow motion are important for the neural processing that occurs in the FPA. Some evidence implies that the firing rates of FPA neurons discharge in relation to image motion: their firing rates decrease slightly during pursuit when the target is blinked, removing all visual inputs (Tanaka and Fukushima 1998); their sensitivity to target/eye velocity is greater during the initiation of pursuit when there is more image motion than during the maintenance of steady-state pursuit (Tanaka and Lisberger 2002b); and they do have small responses to peripheral visual motion presented during fixation (Gottlieb et al. 1994). In the FPA, the visual signals might play a role in selecting between potential pursuit targets (Gardner and Lisberger 2002) or might work in tandem with signals related to eye velocity to modulate the gain of visual-motor transmission for pursuit (Tanaka and Lisberger 2001). Perhaps signals related to optic flow are used to create outputs related to smooth eye motion in three dimensions as recently reported by Fukushima et al. (2002).

When we began these experiments, we had expected to find that antidromically activated MST neurons would show a strong bias for ipsiversive image and/or eye motion because both MST (Dursteler et al. 1987) and the FPA (Bruce et al. 1985; Gottlieb et al. 1993; Keating 1991; Lynch 1987; MacAvoy et al. 1991; Tian and Lynch 1996b) seem to play roles that are somewhat selective for the generation of ipsiversive eye movements. We did not find the expected bias, which raised the question of how a structure can contribute selectively to ipsiversive pursuit while having neurons that discharge preferentially for either ipsiversive or contraversive eye and image motion. One possibility is based on the observation that FPA neurons show a fairly even distribution of preferred pursuit directions (Gottlieb et al. 1994; Tanaka and Fukushima 1998). Perhaps the bias seen during stimulation and lesions results from distinct outputs of FPA neurons that prefer ipsiversive and contraversive pursuit rather than from a biased cortico-cortical input to the FPA. We must now look to other neural loci to understand the fact that cortical lesions in MST and FPA cause deficits that are largest for ipsiversive pursuit. Alternatively, future experiments may explain the directional bias in the effects of lesions by determining the physiological response properties of MST neurons that project to subcortical pursuit areas such as the dorsolateral pontine nuclei (DLPN). Perhaps the expected ipsiversive bias is present only in subcortical projections from MST or the FPA, as found by Hoffman et al. (2002) for the projections from MST to the accessory optic system.

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