Pursuit-Related Neurons in the Supplementary Eye Fields: Discharge During Pursuit and Passive Whole Body Rotation

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Fukushima, Junko, Teppei Akao, Norihito Takeichi, Sergei Kurkin, Chris R. S. Kaneko, and Kikuro Fukushima. Pursuit-related neurons in the supplementary eye fields: discharge during pursuit and passive whole body rotation. J Neurophysiol 91: 2809–2825, 2004. First published January 7, 2004; 10.1152/jn.01128.2003. The primate frontal cortex contains two areas related to smooth-pursuit: the frontal eye fields (FEFs) and supplementary eye fields (SEFs). To distinguish the specific role of the SEFs in pursuit, we examined discharge of a total of 89 pursuit-related neurons that showed consistent modulation when head-stabilized Japanese monkeys pursued a spot moving sinusoidally in fronto-parallel planes and/or in depth and with or without passive whole body rotation. During smooth-pursuit at different frequencies, 43% of the neurons tested (17/40) exhibited discharge amplitude of modulation linearly correlated with eye velocity. During cancellation of the vestibulo-ocular reflex and/or chair rotation in complete darkness, the majority of neurons tested (91% = 30/33) responded. However, only 17% of the responding neurons (4/30) were modulated in proportion to gaze (eye-in-space) velocity during pursuit-vestibular interactions. When the monkeys fixated a stationary spot, 20% of neurons tested (7/34) responded to motion of a second spot. Among the neurons tested for both smooth-pursuit and vergence tracking (n = 56), 27% (15/56) discharged during both, 62% (35/56) responded during smooth-pursuit only, and 11% (6/56) during vergence tracking only. Phase shifts (relative to stimulus velocity) of responding neurons during pursuit in frontal and depth planes and during chair rotation remained virtually constant (±1 Hz). These results, together with the robust vestibular-related discharge of most SEF neurons, show that the discharge of the majority of SEF pursuit-related neurons is quite distinct from that of caudal FEF neurons in identical task conditions, suggesting that the two areas are involved in different aspects of pursuit-vestibular interactions including predictive pursuit.

INTRODUCTION

With the development of overlapping visual fields and high acuity fovea, it became necessary for frontal-eyed primates to coordinate eye movements binocularly to aim the foveae of both eyes at objects of interest. For small objects moving slowly and smoothly in fronto-parallel planes, smooth-pursuit eye movements are used. This system precisely tracks targets of interest, and it maintains their images on the fovea by moving both eyes in the same direction. Smooth-pursuit has been extensively studied to understand the spatial and temporal processing of visual, target-motion signals that converts that information into motor commands to move the eyes. The pursuit system has also been studied to understand the neural basis of predictive pursuit and pursuit adaptation that maintain efficient tracking performance (see reviews by Barnes 1993; Keller and Heinen 1991; Leigh and Zee 1999; Lisberger et al. 1987).

The primate frontal cortex contains two areas related to smooth-pursuit: the caudal parts of the frontal eye fields (FEFs) in the fundus and posterior bank of the arcuate sulcus and the supplementary eye fields (SEFs) in the dorso-medial cortex (Leigh and Zee 1999; Tehovnik et al. 2000). Although potential differences in the roles of the two cortical areas in smooth-pursuit have been suggested (Tehovnik et al. 2000), published reports do not address certain questions regarding the unique discharge characteristics of SEF pursuit neurons (Heinen 1995; Heinen and Liu 1997). This is in contrast to the detailed studies on discharge characteristics of pursuit neurons in the caudal FEFs (Fukushima et al. 2000, 2002a,b; Gottlieb et al. 1994; MacAvoy et al. 1991; Tanaka and Fukushima 1998; Tanaka and Lisberger 2002a,b; Tian and Lynch 1996). Examples of such questions are as follows. First, do pursuit-related SEF neurons code parameters of tracking eye movements such as eye velocity? Second, because the pursuit system must maintain target images near the foveae during head or whole body movement, this system must interact with the vestibular system, which also has an important role in stabilizing visual images on the retina. It is still unknown whether the SEF participates in this interaction to match the eye-velocity-in-space (i.e., gaze velocity) to target velocity and/or codes gaze velocity during whole body movement. Because the vestibulo-ocular reflex (VOR) is referenced to the eye and not to the head and because the vestibular organs and eyeballs are separated in the head, the precise control of the gain of the VOR depends on the viewing distance of the target and is essential in primates for maintaining clear foveal images of targets close to the observer during movement (Paige and Tomko 1991; Wilson and Melvill Jones 1979). Third, although motor performance during predictive pursuit has been well documented (see review by Barnes 1993), efficient performance of smooth-pursuit requires prediction of target velocity. Indeed, the majority of caudal FEF pursuit neurons respond during tasks requiring visual prediction (Fukushima et al. 2002a), but it is not known whether SEF pursuit-related neurons carry similarly predictive information into motor commands to move the eyes. The pursuit system has also been studied to understand the neural basis of predictive pursuit and pursuit adaptation that maintain efficient tracking performance (see reviews by Barnes 1993; Keller and Heinen 1991; Leigh and Zee 1999; Lisberger et al. 1987).

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target velocity information. Fourth, in daily life, targets move not only in fronto-parallel planes but also in depth. To track such target motion in the three-dimensional (3D) visual world, we must use not only conjugate smooth-pursuit to move both eyes in the same direction but also disconjugate vergence eye movements to move the two eyes in opposite directions. Although the vergence and smooth-pursuit systems are thought to have separate neural substrates (see Leigh and Zee 1999), recent studies indicate that the majority of caudal FEF pursuit neurons respond during vergence tracking as well as fronto-parallel pursuit, thus coding smooth eye movements in 3D space (Fukushima et al. 2002b). It is unknown whether SEF pursuit-related neurons respond during vergence tracking as well. Answers to these questions are necessary to understand the specific role of the SEF in pursuit eye movements. In this study, we therefore examined discharge properties of pursuit-related SEF neurons using identical tasks to those that we had used previously for caudal FEF pursuit neurons (Fukushima et al. 2000, 2002a,b). Although some neurons showing similar discharge characteristics are present in each area, the majority of pursuit-related neurons discharged differently. Some of these results have been presented in preliminary form (Fukushima et al. 2003a).

METHODS

General procedures

Three Japanese male monkeys (Macaca fuscata; C, M, and K; 4.5–6.0 kg) were used. One of them (monkey C) was used for caudal FEF recording in our previous study (Fukushima et al. 2002a). All experiments were performed in strict compliance with the Guide for the Care and Use of Laboratory Animals (DHHS Publication, NIH85–23, 1985). Specific protocols were approved by the Animal Care and Use Committee of Hokkaido University School of Medicine. Our methods for animal preparation, training, and recording were described in detail previously (Fukushima et al. 2000, 2002a,b). Briefly, each monkey was sedated with ketamine hydrochloride (5 mg/kg, im) and anesthetized with pentobarbital sodium (25 mg/kg, ip), and additional anesthesia (0.5–1.0% halothane mixed with 50% nitrous oxide and 50% oxygen) was administered as necessary. Under aseptic conditions, two head-holders were installed over the skull. A scleral search coil was implanted on one eye in one monkey (monkey C) and on both eyes in the other two (monkeys M and K) to record vertical and horizontal components of eye movement (Fuchs and Robinson 1966; Judge et al. 1980). Analgesics (pentazocine, 0.2 mg/kg) and antibiotics (penicillin G sodium, 20,000 U) were administered post-surgically to reduce pain and prevent infection.

Training procedures

Monkeys’ heads were firmly restrained in the primate chair in the stereotaxic plane. The monkeys were trained using two different training booths; in one, they learned to track a target spot with smooth-pursuit eye movements during chair rotation, and in the other, they learned both smooth-pursuit and vergence (depth) tracking using a stereo spot. Unfortunately, the latter was not equipped for vestibular stimulation. Monkey C was trained only in the vestibular booth, and the other two (monkeys M and K) were trained in both booths. In the vestibular booth, the monkey chair was fixed to a turntable that had two degrees of freedom of motion. The interaural axis of the animals’ head and its midpoint were brought close to the axis of pitch and yaw rotation, respectively. The chair was rotated sinusoidally in the pitch or yaw plane and also along oblique planes by combining pitch and yaw rotations. A tangent screen was positioned 75 cm in front of the animals’ eyes and subtended 60 by 80° of visual angle. The monkeys were trained to track, in darkness, a laser spot (0.2° diam) back-projected onto the tangent screen for apple juice reward. The target moved sinusoidally in either vertical, horizontal, or two oblique directions at 45 and 135° polar angles (Fig. 1, bottom). Target position signals were first calibrated before a recording session by placing the target at known horizontal and vertical locations. Eye position signals were calibrated to the target by requiring the animal to fixate the stationary target or pursue a slowly moving one. In the monkeys with binoculars, points were brought close to both eyes, each eye was calibrated separately.

In the “stereo target booth,” animals were seated in darkness, facing a 22-inch computer display placed 65 cm away from their eyes. A stereo, 0.2° diam red target spot was presented for smooth-pursuit and vergence tracking using a time multiplexed display. Liquid crystal shutters were used to create independent, alternating images for each eye at 120 Hz (Fukushima et al. 2002b; Kurkin et al. 2003). The target moved either in the frontal plane at 0.5 Hz (±5°) or depth from 65 to 10 cm apparent distance from the eyes in the midsagittal plane, requiring vergence eye movements of 10°.

After the animals were trained, a recording chamber was installed over a hole cut in the skull at anterior 21–25 and lateral 1–5 to allow single cell recording in the dorsomedial cortical areas.

Recording procedures and behavioral paradigms

To identify the SEFs, we applied microstimulation (50–100 μA, 20–30 cathodal pulses, 0.2-μs duration, 333 Hz) to the dorso-medial frontal cortex while the monkeys fixated a stationary spot or performed smooth-pursuit. Low-threshold areas (~50 μA) for evoking eye movements were located, and we started searching for responsive neurons in those areas. In the vestibular booth, the target was moved obliquely (at 0.5 Hz, ±5 or 10°) in the frontal plane in association with chair rotation at the same frequency either in the yaw or pitch planes to search for neurons responding to target and/or vestibular stimulation. Once responsive single neurons were encountered, smooth-pursuit responses were tested in four planes (vertical, horizontal, and 2 oblique planes at 45° angles) to determine the preferred direction for pursuit activation without chair rotation. For many neurons, responses to a variety of frequencies were examined (0.1–1.0 Hz) to assess velocity sensitivity.

To examine the importance of a visual target during pursuit, the target was briefly (500–800 ms) extinguished (“blanked”). In particular, we blanked the visual target shortly before it changed direction during its sinusoidal movement. The monkeys were required to continue smooth-pursuit by reversing tracking direction in the absence of a visual target.

To dissociate eye movement in the orbit from that in space (i.e., gaze), we employed two tracking conditions (Lisberger and Fuchs 1978; Miles and Fuller 1975). In the VOR cancellation task, the monkeys tracked a target that moved in space with the same amplitude, direction, and phase as the chair. This condition required the monkeys to cancel the VOR so that the eyes remained virtually motionless in the orbit and gaze therefore moved with the chair. The driving signal for the laser spot on the tangent screen during VOR cancellation was obtained from a position signal derived from the chair motion. We calibrated target amplitudes during chair rotation by matching back-projected target motion to motion of another laser spot fixed to the chair at the monkey’s eye level. The error between the two was <0.25°. In the second condition (VOR × 1), the target stayed stationary in space during chair rotation, and the monkeys were required to fixate the stationary spot, which required a perfect VOR and no gaze movement. Chair rotation was applied first in the plane closest to the smooth-pursuit preferred direction for individual neurons. For many neurons, yaw, pitch, or rotation in an oblique axis was tested. For some neurons, responses to a variety of frequencies were examined (0.1–1.0 Hz) for each of the tracking conditions. To examine vestibular responses, chair rotation was also applied in complete
darkness without a target. The monkeys were not required to perform any particular task during this condition but were kept alert by occasional random drops of apple juice.

To examine whether SEF pursuit-related neurons receive retinal information about target movement, the stationary monkeys were rewarded for fixating a stationary laser spot (1st target, 0.2° diam) while a second laser spot (0.6° diam) moved sinusoidally along one of the four directions (Fukushima et al. 2000, 2002a). The first target was occasionally extinguished, whereas the second laser spot was presented continuously, and the monkeys were required to track the second spot. This procedure was used to insure that the monkeys attended to the second spot so that the second spot would not become behaviorally meaningless.

For our search stimulus in the stereo target booth, the target moved in oblique trajectories in virtual 3D space that were generated by combinations of the frontal and depth target motion at 0.5 Hz. Once responsive single neurons were encountered, responses were tested during smooth-pursuit in four frontal planes (vertical, horizontal, and 2 oblique planes along the 45° angles) to determine the preferred direction for pursuit activation and also during vergence tracking in the midsagittal plane. As before, visual responses were tested while the monkeys fixated a stationary target at the center of the screen. After 1–2 s, the target jumped to a new position 5 or 10° away from the center, and the monkeys made a saccade to the visible target.
Data analysis

The data were analyzed off-line as previously described (Fukushima et al. 2000, 2002a,b). Cell discharge was discriminated with a dual time-amplitude window discriminator and digitized together with eye position, chair position, and target position signals at 500 Hz using a 16-bit A/D board. Eye position signals were differentiated by analog circuits (DC, 100 Hz; −12 dB/octave) to obtain eye velocity. All position signals except for eye positions were differentiated by software to obtain velocity. Gaze velocity was calculated as the sum of eye velocity and chair velocity. During smooth-pursuit and chair rotation in monkeys M and K, after confirming that the signals from the two eyes were virtually identical, we analyzed eye movement signals only from the left eye. Vergence eye movements were calculated as the difference between the horizontal components of the left and right eyes. The traces were displayed, and saccades were marked with a cursor on eye and gaze velocity traces and were removed using our interactive computer program (Fukushima et al. 2000). None of the SEF neurons analyzed exhibited clear bursts associated with saccades. Although two neurons exhibited pauses during saccades (see RESULTS), the pause was apparent in histograms but not in single trials. Therefore we did not manipulate spike data for purposes of analysis in any of the SEF neurons analyzed in this study.

Rasters and histograms were constructed by averaging between 10 and 30 cycles. Each cycle was divided into 64 equal bins together with averaged velocity. To quantify responses, a sine function was fitted to the cycle histograms of cell discharge, exclusive of the bins with zero spike rate, by means of a least-squared error algorithm. Responses that had a harmonic distortion (HD) of more than 50% or a signal-to-noise ratio (S/N) of <1.0 were discarded. S/N was defined as the amplitude of the fundamental frequency component divided by the amplitudes of the third through eighth harmonic, and HD was defined as the amplitude of the second harmonic divided by that of the fundamental (Wilson et al. 1984). The phase shift of the peak of the fitted function relative to (re) upward or rightward stimulus velocity or convergent target velocity was calculated as a difference in degrees. Sensitivity (re stimulus velocity) was calculated as the peak amplitude of the fundamental component fitted to the cycle histogram divided by the peak amplitude of the fitted stimulus velocity (i.e., target velocity for pursuit in the frontal and depth tracking and chair velocity for other tasks during chair rotation). Sensitivity ≥0.10 spikes/s°/s was taken as significant modulation. For responses with oblique stimulus directions, radial stimulus velocity was first calculated by the Pythagorean theorem—the square root of the sum of the squares of the vertical and horizontal components. Radial eye and gaze velocities were similarly calculated, and sensitivity (re stimulus velocity) was calculated by dividing amplitude of modulation of cell activity by the radial stimulus velocity. The phase shift of cell response with oblique preferred directions was calculated relative to the rightward component of eye, gaze, or stimulus velocity. Eye, gaze, and vergence velocity responses were calculated similarly using fitted functions after deleting saccades. Sensitivity (re eye velocity) of neuron responses was also calculated by dividing peak discharge modulation by peak eye velocity during pursuit or peak vergence eye velocity.

Preferred direction of a cell’s response was estimated by the method of Krauzlis and Lisberger (1996) using a Gaussian function. Responses to eight polar directions along the four stimulus planes were examined. We estimated the Gaussian fit by plotting sensitivities (re stimulus velocity). Sensitivity values were plotted as positive for the increasing discharge and as negative for the direction to which discharge rate decreased, as previously described (Fukushima et al. 2000).

To analyze retinal image motion responses in the frontal plane, all traces were aligned on the motion of the second target. Traces that contained saccades or slow eye movement were removed since they were indicative of the monkeys’ failure to fixate the stationary primary target, and only those traces with eye position changes of <1° during each cycle were analyzed, as previously described for FEFs (Fukushima et al. 2000, 2002a).

Histological procedures

Near the conclusion of the recording period in monkey C, the sites of pursuit-related cell activity were marked by iron deposits produced by passing positive current (10–15 μA for 60–100 s; 800–1,200 μCoulombs). This monkey had previously been used for recording in the caudal FEFs (Fukushima et al. 2002a). After recording was completed, the monkey was deeply anesthetized by pentobarbital sodium (50 mg/kg, ip). After histological fixation, the brain was cut in the coronal plane at 100 μm thickness on a freezing microtome. The sections were stained for cell bodies and fibers, and the locations of recording sites were verified microscopically. The two other monkeys are still being used for recording in the dorso-medial frontal cortex and caudal FEFs.

Results

In the dorsomedial frontal cortex of three monkeys, we tested activity of a total of 135 neurons (n = 32 from monkey C, n = 95 from monkey M, n = 8 from monkey K) that showed discharge modulation during combined smooth-pursuit + vestibular stimulation in the vestibular booth or smooth-pursuit + vergence-pursuit in the stereo target booth (see METHODS). Table 1 summarizes the number of neurons tested in the various conditions. Briefly, we tested a total of 60 neurons in the vestibular booth and 75 neurons in the stereo target booth. Consistent with previous observations (Heinen 1995; Heinen and Liu 1997), the responses of many of them varied even during the same task conditions. For example, they might be modulated during the initial two or three cycles and become almost completely unmodulated in the following cycles. Other neurons did not show any clear modulation initially, and variable amplitude modulation appeared during pursuit after several cycles. Of the 135 neurons, 25 showed some form of this type of inconsistent response during smooth-pursuit and 5 neurons during vergence tracking (Table 1, Inconsistent response). In our apparatus, we were unable to find the appropriate condition to evoke consistent responses in such neurons, and we did not analyze them any further. Sixteen neurons in the vestibular booth responded only to chair rotation but not during smooth pursuit (Table 1, Vestibular-only neurons). The remaining 89 neurons responded during smooth-pursuit and/or vergence tracking at least for 10 consecutive cycles at a search frequency of 0.5 Hz. Their discharge characteristics were analyzed further below during different task conditions, although the number of neurons tested varied between tasks due to the limitations of each apparatus and occasional degradation or loss of neural recordings.

Smooth-pursuit eye movements of our monkeys using the two target presentation conditions were similar and had eye velocity gains >0.83 to those reported previously (Takeichi et al. 2003). The discharge characteristics of neurons during fronto-parallel smooth-pursuit were also similar in all monkeys irrespective of whether we used an actual target or a stereo target, so we combined the data for analysis.

Response during smooth-pursuit

Consistent with previous studies (Heinen 1995; Heinen and Liu 1997), all pursuit directions are represented in the SEFs.
A total of 135 SEF neurons were tested (60 neurons in the vestibular booth, 75 neurons in the stereo target booth). Of these, neurons that responded to chair rotation but not during pursuit (vestibular only neurons, n = 11) and neurons that showed inconsistent response to smooth-pursuit and/or vergence tracking (n = 30) were not examined further. The remaining 89 pursuit-related SEF neurons were examined: 33 neurons in the vestibular booth and 56 neurons in the stereo target booth. Their discharge characteristics were analyzed during different task conditions, although the number of neurons tested varied between tasks. For eye velocity coding, neurons that were examined at more than 4 different target frequencies are included. Data for caudal FEF were taken from previous studies (Fukushima et al. 2000, 2002b).

Table 1 summarizes preferred discharge and sensitivity for each neuron during pursuit at 0.5 Hz along the axis closest to the preferred direction of each neuron. Although neurons with horizontal preferred directions predominated, preferred directions for individual neurons were distributed across all directions.

To understand how SEF pursuit-related neurons discharge during smooth-pursuit, Fig. 2, B and C, plots phase shift distributions (re eye velocity; B) and sensitivity (re eye velocity; C) for each neuron during pursuit at 0.5 Hz along the axis closest to the preferred direction of each neuron. Although many neurons showed phase leads of a quarter of a cycle, the distribution was broad, flat, and had a median of 5° lag (mean 6° lag; Fig. 2B). Sensitivities (re eye velocity; Fig. 2C) also varied widely with a skewed distribution that had a median of 0.43 spikes/s/°s (mean, 0.51 spikes/s/°s). Some neurons were in phase with eye position (near +90° or −90°; Fig. 2B). Indeed, 3 of 10 neurons examined exhibited weak eye position–related discharge during steady fixation at different eye positions during the saccade task (Fig. 2, B and C). Figure 3A shows an example of such a neuron with a leftward preferred direction during smooth-pursuit. It increased activity during steady fixation at 10° left (−10°; Fig. 3, B and C), but not at straight-ahead (0°) or 10° right (Fig. 3D).

To study whether pursuit-related SEF neurons code eye velocity, a total of 40 neurons (18 neurons in the vestibular booth and 22 neurons in a stereo booth; Table 1) were examined using different target frequencies at a constant amplitude along the axis closest to the preferred direction of each neuron. Of the 40, 26 neurons were examined at more than four different frequencies, whereas the remaining 14 neurons were examined only at two different frequencies. Figure 4 summarizes phase (Fig. 4A) and sensitivity (re target velocity; Fig. 4B) as a function of stimulus frequency for the 26 neurons. Since discharge did not vary systematically across preferred direction for the population, data were combined. Phases of most neurons were fairly constant over the wide frequency range. Many neurons showed phase near 0° and nearly constant sensitivity (Fig. 4, A and B). This suggests that velocity sensitivity of many neurons is fairly constant. We therefore plotted the amplitude of modulation against peak eye velocity for the 40 neurons examined and performed linear regression analysis on the data for each cell. Seventeen of the 40 (43%) showed a significant relationship between amplitude of modulation and peak eye velocity (Table 1). Figure 4C plots these neurons together with fitted linear regressions for four representative neurons. The slopes (i.e., eye velocity sensitivity) of the linear regression for each neuron ranged from 0.10 to 1.36 spikes/s/°/s, with a mean of 0.56 spikes/s/°/s, which is similar to the mean sensitivity (re eye velocity) calculated by dividing amplitude of modulation by peak eye velocity at 0.5 Hz (0.51 spikes/s/°/s; Fig. 2C). Thus the discharge rate of these 17 neurons (Fig. 4C) coded pursuit eye velocity. Amplitudes of discharge modulation of other neurons did not show a significant relationship with peak eye velocity.

Response during whole body rotation
A striking result in this study is the robust vestibular response seen in the majority of pursuit-related SEF neurons.

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A total of 49 neurons were tested during both smooth-pursuit and whole body rotation. Of these, 33 neurons were modulated during smooth-pursuit, and the great majority (30/33) of them also responded during whole body rotation and VOR cancellation and/or VOR in complete darkness (Table 1). The remaining 16 neurons responded only during whole body rotation.

We classified pursuit-related neurons as “gaze velocity” if they met the following criteria that characterized the horizontal gaze velocity Purkinje cells of Lisberger and Fuchs (1978) (also Fukushima et al. 1999a, 2000; Miles and Fuller 1975; Shinmei et al. 2002): 1) modulation occurred for movements of the eye (smooth-pursuit) and the head (VOR cancellation) in the same direction (see METHODS), 2) modulation during one of these two tasks was less than twice that during the other, and 3) modulation during the VOR × 1 was less than that during VOR cancellation. An example of such a response is shown in Fig. 5. This neuron responded during leftward pursuit (Fig. 5A) and leftward whole body rotation during VOR cancellation, but showed little modulation during VOR × 1 (Fig. 5, B and C, respectively). Thus it satisfies the gaze velocity criteria. The existence of vestibular inputs is also supported by clear modulation during chair rotation in complete darkness (Fig. 5D).

In contrast to the FEFs, gaze velocity signals were rarely represented in SEF neuron discharge. A more typical example of SEF discharge is illustrated in Fig. 6 for a single SEF neuron that responded during horizontal pursuit with a leftward preferred direction (Fig. 6A). During VOR cancellation, its modulation was rightward and almost two times larger than that during horizontal pursuit (Fig. 6, B vs. A). Its activity during VOR × 1, which required no gaze movement, was even larger than that during VOR cancellation (Fig. 6C). Thus this neuron does not code gaze velocity. In the caudal FEFs, pursuit responding neurons could be classified either as gaze velocity or eye velocity (Fukushima et al. 2000). However, the neuron shown in Fig. 6 cannot be classified simply as eye velocity, because its activity during VOR × 1 is almost two times larger than the response during smooth-pursuit, despite only a small difference in the accompanying eye velocity (eye velocity gains 1.0 and 0.88 during VOR × 1 and smooth-pursuit, respectively; Fig. 6, A vs. C). The clear discharge modulation during VOR in complete darkness further supports the existence of vestibular inputs (Fig. 6D). We therefore call these neurons pursuit plus vestibular neurons.

Table 1 summarizes the percentage of gaze velocity neurons among neurons that responded to both smooth-pursuit and VOR cancellation; only 17% (4/30) of pursuit-related SEF neurons could be classified as gaze velocity. This percentage is significantly smaller than that of gaze velocity neurons in the caudal FEF in our previous study (66/100 = 66%, $\chi^2$ test, $P < 0.01$, Table 1) (Fukushima et al. 2000).

The majority of pursuit-related neurons in the SEFs responded during VOR cancellation during whole body rotation in several planes (15 of 21 neurons tested; 71%). For example, the neuron shown in Fig. 6 was modulated during VOR cancellation in the yaw (Fig. 6B), pitch (Fig. 6E), and oblique planes (data not shown), and also during whole body rotation in complete darkness in various planes (Fig. 6D), suggesting that these neurons receive vestibular inputs from more than one semicircular canal.

Most SEF pursuit-related neurons discharged during brief (500–800 ms) blanking of a tracking target. As illustrated in Fig. 6F, we extinguished the visual target shortly before it changed direction during sinusoidal movement. The monkeys were required to continue tracking by reversing tracking direc-
tion. Their responses during blanking were similar or even slightly increased compared with those without blanking (cf. Fig. 6, A and F). Ten neurons were examined; for 7 neurons, the target was blanked for 500 ms, and in 3 neurons, for 800 ms. The results for the two blanking periods were similar. Modulation during target blanking ranged from 61 to 119% (mean, 81.4%) of the modulation without blanking. Activity during predictive pursuit has been noted in the SEFs (Heinen and Liu 1997). The activity during target blanking may reflect such predictive activity (see DISCUSSION).

Comparison of discharge characteristics during pursuit-vestibular interactions

Figure 7 summarizes discharge characteristics of SEF neurons during smooth-pursuit, VOR cancellation, and ×1 at 0.5 Hz (Fig. 7, A, C, and E). For comparison, Fig. 7 also shows discharge characteristics of caudal FEF neurons (Fig. 7, B, D, and F) during the same task conditions from our previous study (Fukushima et al. 2000). By definition, the gaze velocity response requires similar preferred directions and similar response magnitudes for smooth-pursuit and VOR cancellation. Although the majority of caudal FEF pursuit neurons show such responses (Fig. 7, B and D; points cluster near the dashed line of slope = 1.0), the great majority of SEF neurons did not (Fig. 7, A and C). If neurons coded eye velocity irrespective of vestibular inputs, the modulation during smooth-pursuit should have been correlated with modulation during VOR × 1, because both required eye movements with the identical magnitude. In caudal FEF eye velocity neurons (filled squares), significant correlation was observed between the two (Fig. 7F), but there was no clear correlation between the two for SEF pursuit plus vestibular neurons (Fig. 7E, filled circles). These comparisons suggest that the majority of SEF neurons do not code parameters of eye or gaze movement during pursuit-vestibular interactions.

Response similarities between VOR cancellation and VOR in complete darkness are shown in Fig. 8, which summarizes the distributions of phase and sensitivity values (re chair velocity) during VOR cancellation (Fig. 8, A and B) and during chair rotation in complete darkness (Fig. 8, C and D) at 0.5 Hz. Although phase values are widely distributed, during VOR cancellation, 21/30 neurons showed phase near 0° (±45 to 45°) or ±180° (−135 to −180 and 135 to 180°) with a median sensitivity of 0.40 spikes/s°/s (mean, 0.45 spikes/s°/s). Even in complete darkness, a median sensitivity of 0.35 spikes/s°/s was obtained (mean, 0.35 spikes/s°/s). These results suggest that SEF pursuit-related neurons receive substantial vestibular inputs (see DISCUSSION).

To study whether pursuit-related SEF neurons code chair velocity, each neuron was examined at different frequencies.
of chair rotation at a constant amplitude. Figure 9 summarizes phase (Fig. 9A) and sensitivity (re chair velocity; Fig. 9B) as a function of chair rotation frequency for 11 neurons during VOR cancellation. Phases are mostly constant over the range of frequencies, although sensitivity varied (Fig. 9B). By plotting amplitude of modulation against peak chair velocity, about one-half of them (5/11) exhibited significant correlation between the two as shown by the example linear regressions in Fig. 9C. The mean slope (i.e., chair velocity sensitivity) was 0.34 spikes/s°/s. Thus these neurons code chair velocity.

Visual response

To examine whether SEF pursuit-related neurons respond to retinal image velocity of a target, we tested the responses of 34 neurons to a second spot moving sinusoidally while the monkeys fixated a stationary spot. Only 7 neurons (7/34 = 20%) showed visual responses (4/19 in the vestibular booth and 3/15 in the stereo booth; Table 1; Fig. 10C). Representative discharge is illustrated in Fig. 10A for a neuron with a leftward preferred direction (Fig. 10B). This neuron showed a visual response with a sensitivity of 0.38 (re velocity of 2nd spot) and peak modulation near peak target velocity of the second spot. The monkey fixated the stationary spot well (Fig. 10A: HE and
VE), as indicated by the eye gain (re velocity of 2nd spot), which was only 0.03 during this task. During smooth-pursuit, pursuit eye gain was 0.84 and discharge sensitivity was 0.82 spikes/s/° (Fig. 10B). If the visual response to the second target during the fixation (Fig. 10A) had been induced by the small residual pursuit sensitivity, the modulation should have been 0.82 × (0.03/0.84) = 0.03 spikes/s/°. The fact that we actually observed modulation of 0.38 spikes/s/° suggests that the modulation of cell activity during this task cannot reflect residual eye velocity. Visual preferred directions of five of the seven neurons were similar to their pursuit preferred directions, while two neurons showed opposite visual preferred directions (Fig. 10D). The magnitude of the visual response was correlated with the magnitude of the smooth-pursuit response (n = 7, Fig. 10C). Table 1 compares the percentage of visual motion responding neurons in the SEFs and caudal FEFs from previous studies. The percentage is much smaller in the SEFs (7/34 = 20% vs. 21/40 = 53%, χ² test, P < 0.05, Table 1) (Fukushima et al. 2000).

Response during vergence tracking

The SEF also contained vergence-related neurons. A total of 56 neurons was tested during smooth-pursuit in the frontal plane and vergence tracking in the midsagittal plane (see Methods). Figure 11 illustrates the discharge of three representative neurons. The neuron shown in Fig. 11, A1 and A2, responded during horizontal pursuit but not during vergence tracking, whereas the neurons shown in Fig. 11, B1 and B2 and C1 and C2, responded during convergence and vertical pursuit and divergence and vertical pursuit, respectively. Of the 56 neurons examined, 35 responded only during smooth-pursuit (35/56 = 62%), 15 neurons responded both during smooth-pursuit and vergence tracking (15/56 = 27%), and the remaining 6 neurons responded only during vergence tracking (6/56 = 11%). Table 1 summarizes the percentage of vergence-related neurons. The percentage of smooth-pursuit + vergence-related neurons is significantly smaller than that in the caudal FEFs in our previous study (80/122 = 66%, χ² test, P < 0.05, Table 1) (Fukushima et al. 2000).

To understand how SEF neurons discharge during vergence tracking, Fig. 12 plots the distributions of phase shifts (re convergence eye velocity at 0.5 Hz; Fig. 12A) and sensitivity (re vergence eye velocity; Fig. 12B) for vergence responses of the above 21 vergence-responding neurons and 2 other neurons in which smooth-pursuit was not tested. Phase shift values were distributed mostly around −45° (convergent neurons) or +45, 135° (divergent neurons), which seem to correspond to intermediate phase values lying between vergence position (+90 or −90°), and velocity (+180, −180, or 0°). The mean sensitivity was 0.68 spikes/s/°/s (median, 0.69 spikes/s/°/s).

To examine whether smooth-pursuit + vergence-responding neurons have specific preferred directions, Fig. 12C plots preferred smooth-pursuit directions of neurons that responded during both smooth-pursuit and vergence tracking as a function of sensitivity (re vergence eye velocity) of convergence + smooth pursuit neurons (open circles) and divergence + smooth-pursuit neurons (filled circles). Although the sample is small, preferred smooth-pursuit directions of these neurons were distributed widely, with a tendency of convergence neu-
rons to have upward components and divergence neurons to have downward components. They also showed nearly evenly distributed vergence velocity sensitivity. Also plotted are preferred directions of smooth-pursuit only neurons (squares outside the circle, Fig. 12 C). Preferred directions were distributed widely. Preferred directions of six vergence-only neurons (data not shown) were either convergence (n = 4) or divergence (n = 2).

To examine whether SEF neurons code vergence velocity, each neuron was tested at different frequencies of vergence target motion at a constant amplitude. Figure 13 plots phase (Fig. 13A) and sensitivity (re stimulus velocity) during smooth-pursuit and VOR cancellation. Phases are nearly constant over the range of frequencies. Sensitivity at the higher frequencies of 0.5 and 1.0 Hz was nearly constant (Fig. 13B, right). Indeed, amplitude of modulation plotted against peak vergence eye velocity indicates that many neurons increased discharge modulation as vergence eye velocity increased. This is confirmed in Fig. 13C, which also shows linear regressions for representative neurons.

The mean vergence velocity sensitivity for 10 neurons was 0.34 spikes/s/°/s. These neurons code vergence eye velocity.

**Activity during saccades**

We examined activity of 10 pursuit-related SEF neurons during saccades. The majority (8/10) exhibited no clear change in their discharge rate, and the remaining two neurons paused during saccades (Fig. 3 C). Although we did not search for saccade-related neurons, in the tracks that we recorded pursuit-related neurons, we also occasionally encountered neurons that discharged bursts during saccades but that did not show clear modulation during smooth-pursuit, suggesting that saccade neurons are separate from pursuit-related neurons in the SEFs.

**Recording location**

Figure 14 illustrates the reconstructed recording locations (monkey C). We tracked ~7 mm rostrocaudally and ~7 mm
Pursuit-related neurons were found ~1.5 mm rostrocaudally and ~2 mm medio-laterally near the caudal edge of the arcuate sulcus (Fig. 14, A and B). They were recorded typically between 2 and 4 mm from the surface. Two other monkeys have not yet provided histology (see METHODS). However, as shown in Fig. 14B, in all monkeys, the stereotaxic anterior-posterior location of “SEF pursuit area” is very similar to the location of the caudal FEF pursuit area of the same monkeys. Therefore we are certain that recordings in these two monkeys also were from similar areas in the dorsomedial frontal cortex.

**DISCUSSION**

To begin to understand the specific role SEFs play in smooth-pursuit, we examined the discharge characteristics of SEF pursuit-related neurons using tasks that are identical to those we had used previously for caudal FEF pursuit neurons. Although neurons are present in each area that show similar discharge characteristics in preferred directions, eye velocity sensitivity, vestibular sensitivity, visual response sensitivity to a second target’s velocity, and vergence velocity sensitivity (Table 1) (Fukushima et al. 2000, 2002a,b), they are in the minority; the discharge sensitivities of the majority of neurons in the two cortical areas are quite different.

**Smooth-pursuit areas in the SEFs**

Schall (1991) and Heinen and Liu (1997) reported the location in the SEF where smooth-pursuit and/or eye position–related neurons were recorded. These areas correspond to the locations in the SEF where saccadic or smooth eye movements were induced by electrical stimulation (Fujii et al. 2002; Missal and Heinen 2001; Schall et al. 1993; Tian and Lynch 1996). These locations are generally similar to the areas where we recorded our smooth-pursuit–related neurons (Fig. 14), and the majority of our pursuit-related neurons exhibited discharge characteristics similar to those reported previously (Heinen 1995; Heinen and Liu 1997; Schall 1991). Therefore we conclude that the area we recorded was the SEF. We did not search for saccade neurons in this study and only occasionally encountered saccade-related neurons in the pursuit-related areas. Histological identification of saccade-related neuron locations in previous studies are generally more rostral than our pursuit-related area (Chen and Wise 1995; Fujii et al. 2002; Isoda and Tanji 2003; Schall 1991; Schall et al. 1993). In the areas we recorded, pursuit-related SEF neurons seemed slightly caudal to the saccade-related SEF areas.

**Comparison of discharge characteristics of pursuit-related neurons between the SEFs and caudal FEFs**

To understand the specific role of each frontal cortical area in smooth-pursuit, it is necessary to compare its activity in identical task conditions. For pursuit-responding neurons, similar discharge characteristics were observed in the two areas. For example, eye velocity sensitivity during smooth-pursuit was similar for the SEF and caudal FEF (mean, 0.56 vs. 0.50–0.53 spies/s/°/s, respectively) (Fukushima et al. 2000). Although the high percentage of neurons with vestibular-related activity in both SEFs and caudal FEFs was strikingly similar (Table 1), the activity of the great majority of SEF pursuit-related neurons did not code gaze (Fig. 6). This is in a sharp contrast to the common, gaze velocity signals found in the caudal FEF in the same task conditions (Table 1). We do not exclude the possibility that gaze velocity signals in the SEFs may become important in different task conditions (see below). Nevertheless, the present results, together with the clear differences in the discharge characteristics between the two areas (Table 1), indicate that the population of pursuit-related neurons in each area is different. In contrast to the majority of caudal FEF neurons that code parameters of...
smooth-pursuit such as eye velocity, gaze velocity, retinal image motion for target velocity, and smooth eye movements in 3D (Table 1), the majority of SEF pursuit-related neurons did not, despite the fact that they coded eye velocity during smooth-pursuit without vestibular stimulation. These results suggest that the SEFs and caudal FEFs are involved in different aspects of pursuit-vestibular interactions and that eye velocity coding of SEF pursuit neurons is specific to the task conditions.

Heinen and Liu (1997) reported that smooth-pursuit neurons in the SEFs exhibit prediction-related activity before initiation of pursuit. Consistent with this observation, our results show that many pursuit-related neurons exhibited phase leads during sinusoidal target motion (Fig. 2B), and they discharged appropriately during blanking of the target before it changed direction (Fig. 6F). Moreover, individual SEF neurons exhibited virtually constant phase shifts (re target velocity), even at 1 Hz during pursuit in the frontal and depth planes (Figs. 4A and 13A). Such constancy requires accurate prediction to compensate for the long delays involved in processing visual motion information and/or eye velocity commands (Barnes 1993).

Prediction should occur not only on the motor side as preparation and perseveration of ongoing movements (Barnes 1993) but also on the sensory and/or perception side (Umeno and Goldberg 1997). For example, a visual response that anticipates the eventually renewed direction and speed of the target movement of a temporarily occluded visual input. In fact, in addition to vigorous discharge during pursuit of an invisible target, the majority of caudal FEF pursuit neurons receive visual inputs reflecting target motion in the absence of pursuit, and their activity reflects the direction and speed of the reconstructed target image—signals sufficient for estimating target motion (Fukushima et al. 2002a). Using identical tasks in the same animals, this study shows that the majority of SEF pursuit-related neurons do not exhibit visual responses to spot motion (Table 1). This suggests that in the present task conditions SEF neurons do not exhibit visual prediction, despite the fact that the phase invariance of SEF neurons during tracking described above is similar to the phase behavior of caudal FEF pursuit neurons (Fukushima et al. 2000, 2002a). These contrasting results between SEF and caudal FEF pursuit neurons in the present task condition suggest that the SEF involvement in pursuit prediction is more on the motor than sensory or perception side, different from the caudal FEF. Preliminary studies by Kim and Heinen (2001) reported that SEF pursuit neurons discharge vigorously during a task condition that requires prediction. It may well be that pursuit-related neurons in this study would discharge more vigorously to target motion in a predictive manner in the conditions used by Kim and Heinen (2001). Reciprocal connections between the SEF and FEF could transfer the necessary visual signals for task dependent performance including target velocity information (Schall et al. 1993; Stanton et al. 1993).

Olson and his colleagues reported that SEF neurons encode an object-centered frame of reference (Olson and Gettner 1995; Tremblay et al. 2002). Although it is unknown whether SEF neurons could encode a frame of reference of a moving, rather than stationary target, despite a weak visual response to the target alone (Fig. 10, C and D), we do not exclude the possibility that the phase invariance we observed reflected SEF neurons’ encoding relative spatial position of the tracking target after training.

Possible relations of SEF pursuit-related neuronal activity to task-dependent pursuit eye movements

Although electrical stimulation of the SEF has been shown to facilitate smooth eye movements (Missal and Heinen 2001), SEF lesions are known to have minimum effects on pursuit (see review by Tehovnik et al. 2000). Consistent with these results, muscimol injection into the SEF pursuit area failed to induce clear effects on smooth-pursuit and VOR cancellation.
in the same task conditions in our monkeys (Fukushima et al. 2003b; also see review by Tehovnik et al. 2000). These results are in striking contrast to the deficits in smooth-pursuit and VOR cancellation induced by caudal FEF lesions or chemical inactivation (Fukushima et al. 1999b; Keating 1991, 1993; Lynch 1987; MacAvoy et al. 1991; Shi et al. 1998). Those observations and the present results taken together suggest that, with simple ocular tracking tasks, a specific role of the SEF could not be detected.

In addition to the well-known saccade-related activity (Schall 1991; Schlag and Schlag-Rey 1987), the SEF is reported to play an important role in more complex behaviors such as learning-related activity (Chen and Wise 1995; Nakamura et al. 1998), planning of saccades (Olson et al. 2000), decision-making processes (Coe et al. 2002), sequential performance of saccades (Isoda and Tanji 2002, 2003; Lu et al. 2002; Pierrot-Deseilligny et al. 1995; Schiller and Chou 1998), antisaccades (Schlag-Rey et al. 1997), and eye-hand reach coordination (Mushiake et al. 1996). Reward-predicting activity is also reported (Amador et al. 2000), and apparently, SEF neuron activity is task-dependent (Tanji 1996). Furthermore, in our task conditions, if we presented a tracking target that moved across a stationary structured background, after muscimol infusion into the SEF pursuit area our monkeys exhibited impairment of upward smooth-pursuit, despite the fact that the same monkeys did not show impairment in tracking across a homogeneous background after infusion (Fukushima et al. 2003b). Again, this is in contrast to the impairment induced by muscimol infusion into the caudal FEFs. FEF inactivation also impaired vertical pursuit across the textured background, but the effects were less selective, since a similar impairment was observed across the homogeneous background. These results indicate that, as Tanji (1996) clearly states, “the usage of the SEF is more dependent on the behavior or conditional state than the usage of the FEF.”

The possible importance of vestibular signals in SEF function has been suggested by clinical studies. De Waele et al. (2001) reported vestibular evoked potentials in the anterior portion of the supplementary motor area with latencies of ~6 ms induced by electrical stimulation of the vestibular nerve in patients. Israel et al. (1992, 1995) and Pierrot-Deseilligny et al. (1993) reported that vestibular contingent memory-guided saccades are impaired in patients with SEF lesions, although they did not exhibit abnormalities in memory-guided saccade tasks without vestibular stimulation (Pierrot-Deseilligny et al. 1993). These observations suggest the importance of vestibular information in self-centered spatial representation during the memory-guided saccade tasks. Olson and Gettner (1995) (see also Tremblay et al. 2002) reported that the SEF provides an object-centered frame of reference. Strong vestibular-related activity in the great majority of SEF pursuit-related neurons in this study (Fig. 6; Table 1) may provide that body-centered frame of reference for animal behavior in 3D space (Fukushima 1997). It is possible that vestibular signals may also be used for calculation of gaze velocity during demanding task conditions that require learning. Vestibular signals might facilitate
FIG. 11. Discharge of 3 representative SEF neurons during vergence tracking and smooth-pursuit. For vergence tracking (A1–C1), traces from top to bottom indicate superimposed vergence target position, left and right horizontal eye position (LHE and RHE), vergence eye velocity (LHE and RHE), spike rasters, and histograms of neuron discharge. For smooth-pursuit (A2–C2), traces are target velocity and superimposed eye velocity and cell discharge.

FIG. 12. Characteristics of vergence response. Distributions of phase shift (A) and sensitivity (re vergence eye velocity; B) for individual neurons. C: polar plot of preferred direction (angle) during smooth-pursuit and sensitivity (re vergence eye velocity, radius) of SEF neurons that responded to both smooth-pursuit in the frontal plane and vergence tracking. Open and filled circles indicate convergence + smooth-pursuit neurons and divergence + smooth-pursuit neurons, respectively. Preferred directions of smooth-pursuit only neurons (open squares) are also plotted outside the circle.
smooth-pursuit and vergence tracking (Fukushima et al. 2001; Sato et al. 2004). Vestibular signals can also be used for pursuit adaptation, and caudal FEF neurons exhibit adaptation-related activity (Fukushima et al. 2001). The SEF may provide adaptation-related vestibular signals to the FEF via their direct projections (Schall et al. 1993). Prediction-related activity of SEF pursuit neurons (Heinen and Liu 1997; Kim and Heinen 2001) should be tested further to examine a specific role of vestibular signals in the SEFs for task-dependent pursuit eye movements.

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