Vertical Purkinje Cells of the Monkey Floccular Lobe: Simple-Spike Activity During Pursuit and Passive Whole Body Rotation

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INTRODUCTION

With the development of the high acuity fovea in primates, smooth-pursuit eye movements have evolved to track small moving objects. During head movement, the smooth pursuit system does not work independently but interacts with the vestibular system to match the velocity of the eyes in space (i.e., gaze) with target velocity (Robinson 1981). Single-cell recording and lesion studies have implicated the cerebellar flocculus and ventral paraflocculus (which we refer to as the floccular lobe) in the control of gaze velocity. This control has been revealed both during horizontal pursuit-vestibular interactions (Lisberger and Fuchs 1978) and optokinetic eye movement (ocular following), which shares many pathways with smooth pursuit (Kawano et al. 1992, 1994; Shidara and Kawano 1993).

Gaze velocity (G) Purkinje (P) cells constitute the majority of horizontal P cells in the simian floccular lobe (Miles et al. 1980; Stone and Lisberger 1990). They respond during smooth pursuit and pursuit-vestibular interactions but little, if at all, during the vestibuloocular reflex (VOR) when gaze is stable (Krauzlis and Lisberger 1996; Lisberger and Fuchs 1978; Libberger et al. 1994; Miles and Fuller 1975; Miles et al. 1980; Shidara and Kawano 1993; Stone and Lisberger 1990). Removal of the floccular lobe results in an impairment of slow gaze movement during visual-vestibular interactions in many animal species and substantial impairment of smooth pursuit in monkeys. The horizontal VOR itself is not consistently affected (Flandrin et al. 1983; Hassul et al. 1976; Honrubia et al. 1982; Ito et al. 1982; Zee et al. 1981).

The simian floccular lobe contains another class of P cells that respond during downward smooth pursuit and ocular following (Krauzlis and Lisberger 1996; Miles et al. 1980; Shidara and Kawano 1993; Stone and Lisberger 1990). Although their activity has not been recorded during vestibular stimulation or visual-vestibular interactions, lesion studies have implicated them in the control of slow vertical eye movements. Chemical inactivation of the floccular lobe results in impairment of suppression of the vertical VOR but, as in the yaw plane, does not affect the VOR itself in monkeys tested while lying on their sides (Zhang et al. 1995). Inconsistent with this observation, many vertical P cells in the floccular lobe in alert cats tested in the upright position, respond not only during visual-vestibular interactions but also during the vertical VOR itself (Fukushima et al. 1993, 1996a). The majority respond to upward pitch rotation when the vertical VOR drives the eyes downward and to downward eye movements induced by optokinetic stimuli. Their firing is in-phase with eye velocity and these cells therefore were classified as vertical eye-velocity (VE) P cells. They also respond during vertical whole body oscillations in several planes and therefore have a vestibular sensitivity. Although they respond best during vertical eye movements, their preferred directions for vestibular activation...
Recoding procedures and behavioral paradigms

All stimuli were applied sinusoidally. As our search stimulus, the target was moved either obliquely or vertically (at 0.5 Hz ± 5° or ±10°) in association with vestibular rotation (0.25 Hz ± 10 or 0.5 Hz ± 5° or ±10°) in the pitch plane. The floccular lobe was identified physiologically by its characteristic saccade-related burst discharge (e.g., Noda and Suzuki 1979). Unit activity was recorded extracellularly with tungsten microelectrodes. P cells were identified by the existence of complex spikes as illustrated in Fig. 1B ( ). Once responding cells were encountered, we assigned the monkeys each of three standard tasks (Fig. 1A) that used various combinations of whole body rotation and target movement at 0.5 Hz (± 5° or ±10°). Pursuit responses were tested in two directions (vertical and horizontal) to determine the preferred direction for activation of each neuron (Fig. 1A, top). Because the great majority of neurons showed either a horizontal or vertical referred smooth pursuit direction, chair rotation was applied in either the horizontal (yaw) or vertical (pitch) plane, respectively.

To dissociate eye movement in the orbit from that in space (i.e., gaze), we employed two tracking conditions (Fig. 1A). In the first, the monkeys tracked a target that moved in space with the same amplitude and phase as the chair and in the same plane. This condition requires the monkeys to suppress the VOR so that the eyes remain virtually motionless in the orbit and gaze therefore moves with the chair (VOR X1, 2nd row). In the second condition, the target stayed stationary in space during chair rotation, which requires perfect VOR and no gaze movement (target fixed in space, VOR X1, 3rd row).

To assess possible eye-position sensitivity, we required stationary animals to fixate a target placed at various target eccentricities along the vertical or horizontal meridian.

To examine the vestibular direction tuning of vertical P cells, we determined the maximum activation directions (MADs) induced by vertical rotation, according to the method described by Baker et al. (1984). The horizontal orientation of the animal was selected by positioning the chair at different angles (see Figs. 9A and 10A). With the animal pointed straight ahead (i.e., 0°), vertical rotation about a horizontal interaural axis stimulated all four vertical canals. The animal then was oscillated around the same earth-horizontal axis while it was oriented in several different horizontal directions. At least five, and typically seven, horizontal orientations were tested to determine MADs for individual cell responses. For example, if the monkey was oriented to 45° toward the animal’s right, the vertical rotation would now maximally stimulate the left anterior and right posterior canals and not stimulate the other pair at all (see Figs. 9A and 10A). If, in addition, the monkey was required to fixate a target spot that rotated with it, the vertical VOR would be suppressed and unit modulation was attributed to a vestibular sensitivity. The MAD was the horizontal orientation that produced the maximum modulation in firing rate.

Data analysis

The data were analyzed off-line. Isolated single cell activity was digitized together with eye-, chair-, and target-position signals at 303 or 500 Hz using a 16-bit A/D board (Fuchs et al. 1994; Fukushima et al. 1995). These signals were differentiated by software to obtain velocity. Gaze velocity was calculated as the sum of eye velocity and chair velocity. Saccades were marked with a cursor on eye- and gaze-velocity traces and removed using an interactive computer program (Fukushima et al. 1995). Those occasional bursts or pauses in cell discharge associated with saccades were marked manually and removed from the analysis. Rasters and histograms were constructed by averaging between 10 and 30 cycles. Each cycle was divided into 64 equal bins ( see Figs. 9A and 10A). In the second condition, the target stayed stationary in space during chair rotation, which requires perfect VOR and no gaze movement (target fixed in space, VOR X1, 3rd row).

A sine function was fit to all velocity traces and to the cycle rasters.

METH ODS

General procedures

A total of five male macaques (3 Macaca fuscata and 2 M. mulatta) were used. Experiments on the three Japanese macaques (M. fuscata) were performed at Hokkaido University School of Medicine, Sapporo, Japan, and experiments on the two rhesus macaques (M. mulatta) were performed in the Regional Primate Research Center, University of Washington, Seattle, WA. The first author participated in all of the experiments, and he digitized and analyzed all of the data with the same computer programs. All experiments were performed in strict compliance with the Guide for the Care and Use of Laboratory Animals (DH E W Publication NIH85-23, 1985) and recommendations from the Institute of Laboratory Animal Resources and the American Association for Accreditation of Laboratory Animal Care International. Specific protocols were approved by the Institutional Animal Care and Use Committee at the University of Washington (ACC 2602-01) and by the Animal Care and Use Committee of Hokkaido University School of Medicine (00421 and 9290).

Our methods for animal preparation and training are described in detail elsewhere (Fuchs et al. 1994; Fukushima et al. 1996c) and therefore are summarized here only briefly. The monkeys were prepared surgically under aseptic conditions with a pair of head holders or head stabilization lugs, and a scleral search coil was implanted on the right eye to record horizontal and vertical eye movements (Fuchs et al. 1994; Fukushima et al. 1996c) and detail elsewhere (Fuchs et al. 1994; Fukushima et al. 1996c).

Neurophysiological recordings were made in the Regional Primate Research Center, University of Washington, Seattle, WA. The first author participated in all of the experiments, and he digitized and analyzed all of the data with the same computer programs. All experiments were performed in strict compliance with the Guide for the Care and Use of Laboratory Animals (DH E W Publication NIH85-23, 1985) and recommendations from the Institute of Laboratory Animal Resources and the American Association for Accreditation of Laboratory Animal Care International. Specific protocols were approved by the Institutional Animal Care and Use Committee at the University of Washington (ACC 2602-01) and by the Animal Care and Use Committee of Hokkaido University School of Medicine (00421 and 9290).

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(i.e., maximal activation directions) (Baker et al. 1984) lay near the posterior canal (not the pitch) plane. Finally, chemical inactivation of the floccular areas where up-pitch P cells were recorded affected the downward VOR exclusively in cats tested in the upright position (Fukushima et al. 1993, 1996a,b). Therefore several lines of evidence in different species implicate the floccular lobe in the control of slow vertical eye movements generated during smooth pursuit and vestibular stimulation.

The finding in the cat that the majority of vertical floccular P cells discharge with eye rather than gaze movement contrasts with data on the monkey where the majority of horizontal floccular P cells discharge with gaze rather than eye movement. To determine whether these observations indicate a fundamental difference in the role of the floccular lobe during vertical and horizontal pursuit and pursuit-vestibular interactions or simply reflect a species difference, we recorded simple-spike activity of vertical P cells in trained monkeys. Preferred directions during vestibular rotation (i.e., vestibular direction tuning) of these cells also were examined by applying vertical whole body rotation in several planes while the monkeys were required to suppress the VOR. Some of these results have been presented in abstract form (Fukushima et al. 1996d).

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histograms of cell discharge exclusive of the bins with zero spike rate by means of a least-squares error algorithm. Responses that had a harmonic distortion (HD) of >50% or a signal-to-noise ratio (S/N) of <1.0 were discarded. S/N was defined as the ratio of the amplitude of the fitted fundamental frequency to the root mean square amplitude of the third through eighth harmonics. HD was taken as the ratio of the amplitude of the second harmonic to that of the fundamental. The phase shift of the peak in the fitted-function relative to upward eye velocity or upward stimulus-velocity was calculated as a difference in degrees. Sensitivity was calculated as the peak amplitude of the fundamental component fitted to the cycle histogram divided by the peak amplitude of stimulus velocity (i.e., target velocity for pursuit and chair velocity for other tasks during vestibular rotation, Fig. 1A). A sensitivity >0.10 spikes/s per °/s was taken as significant modulation.

Eye or gaze gain was calculated by dividing peak eye or gaze velocity by stimulus velocity. We calculated eye position sensitivity (k) during sinusoidal smooth pursuit using the equation \( k = \frac{A_u \cos(p)}{A_e} \) of Chubb and Fuchs (1982), where \( A_u \) was the amplitude of the modulation in cell activity, \( A_e \) was the amplitude of the eye movement, and \( p \) was the phase lead of the modulation in cell activity with respect to eye position. Eye-position sensitivity also was examined for the periods when monkeys fixated the stationary target for >1 s. Discharge rates of individual cells were plotted against the corresponding horizontal or vertical eye position, and a linear regression analysis was performed to examine eye-position sensitivity.

We also calculated eye-velocity sensitivity (r) during smooth pursuit using the equation \( r = \frac{A_u \sin(p)}{2\pi f A_e} \) of Chubb and Fuchs (1982), where \( A_u, A_e, \) and \( p \) are the same as for the previous equation and \( f \) is the frequency of the stimulus.

The MAD was calculated from responses to sinusoidal vertical rotation at different horizontal orientations while the monkeys performed the VOR suppression task (see Figs. 9A and 10A). We used the convention of Baker et al. (1984), who plotted sensitivity and phase (re: chair position) of neural responses against horizontal orientation relative to the recording side; sensitivity values were plotted as positive when the phase values lagged chair position and as negative when they led. Horizontal orientations were plotted as positive for those toward the side contralateral to the recording site and as negative for those toward the side ipsilateral to the recording. Responses were considered to be evoked primarily by canal inputs when their modulation varied sinusoidally with the orientation of the rotation plane but phase was mostly constant except when rotation was near the null orientation where there was no modulation (see Figs. 9 and 10). The null orientation was calculated from response reversal by fitting a least-squares error sinusoid to each sensitivity curve (Baker et al. 1984). The amount of error (in degrees) in fitting a sine curve also was estimated by calculating the mean square root of errors divided by the square root of the number of horizontal orientation angles examined. The null orientation was used to estimate the MAD by assuming that the latter should be perpendicular to the former (Blanks et al. 1978; Estes et al. 1975). Paired or unpaired Student’s t-tests were used for statistical analysis.

**Histological procedures**

Near the conclusion of the recording period in each monkey, the sites of vertical P cell activity were marked by iron deposits produced by passing positive current (10 –15 µA for 60 –100 s; 800 –1,200 mCoulombs) or by electrolytic lesions produced by passing negative current through the microelectrode (20 mA for 30 s). After floccular recording was completed, the three Japanese monkeys were anesthetized deeply by pentobarbital sodium (50 mg/kg ip) and perfused by physiological saline followed by 3.5% formalin for reconstruction of electrolytic lesions and by both formalin and 2% ferrocyanide (Suzuki and Azuma 1976) for iron marking. After histological fixation, transverse sections of the brain stem and cerebellum were cut at 100-µm thickness on a
freezing microtome in the plane of recording electrode tracks with the aid of two pins left during perfusion. The sections were then stained using the Klüver-Barrera method (Klüver and Barrera 1953). The flocculus and ventral paraflocculus were identified according to the description of Gerrits and Voogd (1989), and the locations of recording sites were verified. The two rhesus monkeys are still being used for other experiments. However, because of our extensive experience with the characteristic discharge properties of P cells of the floccular lobe (e.g., Lisberger and Fuchs 1978), we are certain that recordings in these monkeys also were from the floccular lobe.

RESULTS

In the floccular lobes of five monkeys, we examined the discharge characteristics of 102 cells that responded to vertical or horizontal smooth pursuit and/or chair rotation. Of these, 70 cells were identified as P cells by the existence of complex spikes (e.g., Fig. 1B, ○). Of the 70, 58 cells responded during vertical pursuit and/or vertical whole-body rotation; the remaining 12 responded to horizontal pursuit and horizontal rotation. The small number of cells with horizontal sensitivities does not reflect the true percentage of horizontal P cells because we documented them only occasionally as a basis for comparison with vertical P cells. Among the 32 of 102 cells in which complex spikes were not discernable, 25 responded to vertical rotation and vertical pursuit; the remaining 7 responded mainly to horizontal rotation and horizontal pursuit.

Of the 58 vertical P cells, 30 were recorded in the Japanese macaques and 28 in the rhesus macaques. Because the response properties of cells recorded in the two types of macaque were similar, we considered the cells as one population and their data were combined. All 58 cells were recorded during vertical smooth pursuit and VOR suppression in the pitch plane. For 54 of these, the target-fixed-in-space condition during pitch rotation (pitch X1) in which gaze was largely stable (Fig. 2C, G), the modulation was much less than during either smooth pursuit (Fig. 2A) or VOR suppression (Fig. 2B). This neuron showed essentially no modulation during either horizontal smooth pursuit (Fig. 2D) or suppression of the VOR during yaw rotation (Fig. 2E).

Figure 3A compares the responses of all 11 neurons in this group during the three task conditions at 0.5 Hz (A, 1, 2, and 4); the responses are displayed in polar coordinates with sensitivity plotted as a radius and the phase as an angle (reset: stimulus velocity). Most of these neurons (8/11) had downward on directions for pursuit and VOR suppression, whereas three had upward on directions. The average sensitivity is 0.91 ± 0.56 (SD) spikes/s per °/s for vertical smooth pursuit and 0.99 ± 0.59 (SD) spikes/s per °/s for pitch VOR suppression. Although 9 of the 11 were modulated when gaze was roughly constant in the pitch X1 condition (A4, mean sensitivity = 0.49 ± 0.42 SD spikes/s per °/s, n = 11), their values scattered around zero except for one outlying cell (Fig. 3A4). Gaze gain during recording of these cells ranged from 0 to 0.23 with the mean of 0.10 ± 0.08.

We next examined whether the modulation due to vertical pursuit cancels that due to head rotation (assessed during pitch VOR suppression) during the pitch × 1 condition when the eyes and head move in opposite directions and gaze movement is nearly zero. Figure 3A3 plots predicted modulation of individual cells during the VOR (pitch X1) in polar coordinates by adding spike histograms of each cell during vertical pursuit and pitch VOR suppression. Their values scattered around zero (Fig. 3A3), suggesting that their response as a whole is consistent with a gaze velocity response.

Vertical gaze velocity (VG) P cells

The P cell shown in Fig. 2 was deeply modulated for downward smooth pursuit (A) and downward head rotation during suppression of the pitch VOR (B). Both responses were essentially in phase with eye or head velocity, consistent with the lack of eye position sensitivity of this cell during fixation of a stationary target at different vertical eye positions (Fig. 2F). During the target-fixed-in-space condition (pitch X1) in which gaze was largely stable (Fig. 2C, G), the modulation was much less than during either smooth pursuit (Fig. 2A) or VOR suppression (Fig. 2B). This neuron showed essentially no modulation during either horizontal smooth pursuit (Fig. 2D) or suppression of the VOR during yaw rotation (Fig. 2E).

The remaining 19 of the 58 (~33%) did not respond during pitch suppression. However, when eight cells of this type were tested during the suppression condition in a rotation plane other than pitch (see Fig. 10), seven had head-motion modulation. So we call these cells off-pitch VE/H P cells.

We now will examine the behavior of representative neurons of each of the three cell types in some detail.

Vertical eye and head velocity (VE/H) P cells

The P cell shown in Fig. 4 was modulated during downward smooth pursuit (A) and the peak discharge occurring between the peaks in eye velocity, indicating that the cell had both eye velocity and position sensitivity. Indeed, when the animal looked at fixed positions, firing rate was weakly related to eye position (Fig. 4D). Although this cell also was modulated during downward head rotation during suppression of the pitch VOR (Fig. 4B), its modulation was less than half of its modulation during vertical pursuit (Fig. 4A). Moreover, its modu-
lation during the target-fixed-in-space condition in which gaze was roughly constant (pitch X1, Fig. 4C, G˙) was larger than the modulation during pitch VOR suppression (Fig. 4B). This neuron showed essentially no modulation during either horizontal smooth pursuit (Fig. 4E) or suppression of the VOR during yaw rotation (Fig. 4F).

Figure 3B plots responses of VE/H P cells during the three diagnostic task conditions at 0.5 Hz. (B, 1, 2, and 4). Their average response sensitivity is 0.75 ± 0.51 spikes/s per °/s for vertical smooth pursuit and 0.52 ± 0.31 spikes/s per °/s for pitch VOR suppression. Twenty of the 24 tested were modulated when gaze was roughly constant in the pitch X1 condition (Fig. 3B4, mean sensitivity = 0.67 ± 0.51 SD spikes/s per °/s).

### Off-pitch VE/H P cells

In contrast to the cells in Figs. 2 and 4, the P cell illustrated in Fig. 5 responded to vertical smooth pursuit and the target-fixed-in-space (pitch X1) condition with similar magnitude (Fig. 5, A and C), but there was no modulation (Fig. 5B) during VOR suppression in the pitch plane. Slightly more than half of such cells (11/19) had downward ON directions during pursuit; the remaining 8 had upward ON directions. The peak discharge of this cell during smooth pursuit and the target-fixed-in-space (X1) condition lay between peak eye and target/head velocity (Fig. 5, A and C), indicating that the cell had both eye velocity and position sensitivity. Indeed, when the animal looked at fixed positions, firing rate was related to eye position (Fig. 5D). This neuron did not respond during either horizontal smooth pursuit or yaw suppression (Fig. 5, E and F). Although cells of this type did not respond during pitch VOR suppression, all but one of the eight tested were modulated when the chair was oscillated in other directions.

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**FIG. 2.** Activity of a representative VGP cell recorded in the floccular lobe. Top to bottom in A and E are averaged target or chair velocity, “desaccaded” vertical (VE) or horizontal (HE) eye velocity, gaze velocity (G) with the overlaid fitted sine curve, spike rasters, and histograms of cell discharge with superimposed fitted sine wave. Top to bottom in F are vertical target position, vertical (VE) and horizontal (HE) eye position, and instantaneous firing rate. See figure for calibrations.
vertical planes (see Fig. 10). This vestibular sensitivity and its preferred plane of modulation is examined later.

Figure 3C plots responses of off-pitch VE/H P cells during the three diagnostic task conditions at 0.5 Hz (C, 1, 2, and 4). The average sensitivity for vertical smooth pursuit is $1.15 \pm 0.79$ spikes/s [per] deg/s. All but one of the 18 responded during the target-fixed-in-space (x1) condition with the mean sensitivity of $0.99 \pm 0.0.59$ SD spikes/s[per] deg/s ($n = 19$). Their predicted modulations during the VOR (pitch X1) obtained by adding spike histograms of individual P cells during vertical pursuit and pitch suppression conditions according to Lisberger and Fuchs (1978). For further explanation, see text.

**Figure 3.** Polar plots of phase and sensitivity of the response of representative vertical P cells of each type (VG, VE/H, and off-pitch VE/H) during different task conditions (1, 2, and 4) and the predicted response for the target-fixed-in-space (pitch X1) condition (3). Predicted responses were calculated by adding averaged histograms of individual P cells during vertical pursuit and pitch suppression conditions according to Lisberger and Fuchs (1978). For further explanation, see text.

**Comparison of discharge characteristics of the three cell types**

**EYE MOVEMENT SENSITIVITY.** During smooth pursuit, the phase of the modulation of some vertical P cells (e.g., Fig. 5A) lay intermediate to eye position and velocity, suggesting that their discharge was sensitive to both. To examine P-cell activity related to smooth pursuit further, we calculated the phase shifts of individual P cells relative to upward eye velocity (Fig. 6, A1, B1, and
Compared with VG P cells (Fig. 6A1), VE/H P (Fig. 6B1), and off-pitch VE/H P cells (Fig. 6C1) showed a wide range of phases. We calculated eye-velocity and eye-position sensitivities during sinusoidal vertical pursuit by decomposing the modulation into separate components related to eye velocity and position (see METHODS) (Chubb and Fuchs 1982). The distribution of eye-velocity sensitivities for the three cell types is similar (Fig. 5, A2, B2, and C2) with means ± SD of 0.24 ± 0.23, 0.21 ± 0.16, and 0.35 ± 0.38 spikes/s per °/s, for VG, VE/H, and off-pitch VE/H P cells, respectively.

The eye position sensitivity determined from smooth pursuit also showed a similar distribution for the three cell types (Fig. 6, A3, B3, and C3) with means ± SD of 0.53 ± 0.51, 0.76 ± 0.94, and 0.82 ± 0.65 spikes · s⁻¹ · deg⁻¹, for VG, VE/H, and
off-pitch VE/H P cells, respectively. For 31 vertical P cells (7 VG, 11 VE/H and 13 off-pitch VE/H), eye position sensitivity also was calculated during a variety of steady fixations (see METHODS). The majority (21/31 = 67.7%) did not show consistent eye-position-related activity during fixation. However, like the examples in Figs. 4G and 5G, the remainder (32.3%, 2/7 VG, 2/11 VE/H, and 6/13 off-pitch VE/H) showed an average eye-position sensitivity ranging from 0.7 to 3.1 spikes/s.
The values of phase shift (re: eye velocity) of these cells during sinusoidal pursuit are indicated in Fig. 6 (A1, B1, and C1, ■ and □ bars). Off-pitch VE/H P cells tended to show static eye position sensitivity more often.

**ADDITION OF EYE- AND HEAD-MOVEMENT SENSITIVITIES.** We asked whether the eye- and head-movement sensitivities accounted for the entire behavior of vertical P cells during the condition when gaze was stable in space (pitch X1). To do this, we compared actual cell modulation during the target-fixed-in-space (pitch X1) condition with the predicted modulation calculated by adding averaged histograms during pitch VOR suppression and vertical pursuit. In Fig. 7, this comparison is shown for a VG P cell (A) and for VE/H P cells with eye- and head-velocity sensitivity in the same direction (B), or the opposite direction (C). For the majority of vertical P cells, the modulation during the target-fixed-in-space condition (actual pitch X1) was not well predicted from the linear addition of the pursuit and suppression conditions (predicted pitch X1, Fig. 7A–C). For example, the actual modulation of the cell in Fig. 7A is 180° out of phase with the predicted response (predicted pitch X1, Fig. 7A), whereas the cell in Fig. 7B actually was unmodulated (actual pitch X1) although a fairly robust modulation was predicted (predicted pitch X1, Fig. 7B).

To quantify the difference between the predicted and actual modulation of individual P-cell responses, the absolute phase difference between actual and predicted modulation was plotted against the absolute sensitivity difference between actual modulation and predicted modulation for individual P cells (Fig. 7D). If actual modulation is well predicted by the linear addition of pursuit and suppression sensitivities, the data should fall at the origin. However, as can be seen, the majority of vertical P cells, regardless of the cell type, show actual responses that are poorly predicted by the linear addition of eye- and head-movement sensitivities. Only 13/54 had sensitivity differences $<0.25$ and phase differences less than $20°$. The overall mean sensitivity and phase differences (± SD) between actual and predicted modulation were 0.34 ±0.28 spikes/s per °/s and 70 ± 2°.

**COMPARISON WITH HORIZONTAL GAZE VELOCITY P CELLS.** The sensitivity of horizontal G P cells during pursuit is well correlated with their sensitivity during VOR suppression (Lisberger and Fuchs 1978). Although a significant correlation...
was observed between the two sensitivities for VG P cells (Fig. 8A), there was no significant correlation ($r = 0.24, P > 0.1$) between the two sensitivities for VE/H P cells (Fig. 8B).

Because the majority of vertical P cells responded during the target-fixed-in-space condition (pitch X1, Fig. 3), we asked whether they are involved in both the VOR and pursuit. For this, we examined whether response sensitivities during vertical pursuit are correlated with sensitivities during the target-fixed-in-space condition (pitch X1). The results are plotted in Fig. 8, C–E for each cell type. All three types of vertical P cells showed a significant correlation between sensitivities during the two task conditions ($r = 0.81, 0.58$, and $0.75$ for VG, VE/H, and off-pitch VE/H P cells, respectively, $P < 0.001$).

**Vestibular direction tuning of three cell types**

**DIRECTION SELECTIVITY.** We examined the direction selectivity of our cell types during VOR suppression ($n = 41$, 6 VG, 11 VE/H, 12 off-pitch VE/H, and 12 H P cells). The majority of vertical P cells responded for either yaw or pitch rotations but not both ($27/41 = -66\%$). The remaining cells ($n = 11$, 3/6 VG, 8/11 VE/H, 0/12 off-pitch VE/H and 3/12 H P) responded to both (Fig. 8, F and G). In particular, for eight of these vertical P cells, the modulation during yaw suppression was >0.5 times that during pitch suppression; one H P cell also was equally sensitive during yaw and pitch suppression. These results suggest a convergence of vertical and horizontal canal inputs on these cells (see DISCUSSION). During smooth pursuit ($n = 41$), a majority of P cells (25/41 = 61\%) also responded to either vertical or horizontal pursuit. A minority ($n = 16$, 3/6 VG, 6/13 VE/H, 3/12 off-pitch VE/H, and...
4/12 H P) responded to both; six of these (6/41 = 15%), had almost equal sensitivities to both (not shown), suggesting that their preferred directions were oblique.

**MAD Analysis.** To examine the direction tuning of the vestibular response, we calculated MADs for seven VG, seven VE/H, and eight off-pitch VE/H P cells. Figure 9 shows examples of VG (B and C) and VE/H P cells (D and E). During vertical chair rotation, their activity depended on the horizontal orientation of the head (Fig. 9, top, cartoons). Modulation was almost maximal during pitch suppression (0°, C1 and E1) and almost zero (E3, and E5) or reversed (C3 vs. C5) during roll rotation (ipsilateral or contralateral 90°, C3, C5, E3, and E5). In Fig. 9, F and G, the sensitivity and phase of the modulation of these two cells are plotted against horizontal orientation relative to the recording side. The phase (●) remained fairly constant near either +90° or −90° except for abrupt shifts when sensitivity was ~0. The sensitivity curves (●) fit well with a least-squares sinusoid. The peak response calculated from the least-squares sinusoid yielded MADs for these two cells of 114 and 23° (Fig. 9, F and G) with errors for the fit sine functions (see METHODS) of 4.8 and 1.5°, respectively.

Figure 9, H and I, summarizes the MADs of seven VG and seven VE/H P cells on a polar plot, which also indicates the
approximate angles of the functional canal planes (— — —) (Blanks et al. 1978; Robinson 1982) and pitch/roll planes. The majority of MADs for VG P cells clustered around the pitch plane (0°), and all were distributed in a quadrant between the two anterior canals (Fig. 9H). VE/H P cells with eye-and head-movement sensitivity in the same direction had MADs distributed similarly in the same quadrant (Fig. 9I). The MADs of three VE/H P cells with oppositely directed eye- and head-movement sensitivities were distributed outside this quadrant (Fig. 9I, **, n = 4). The MADs of three VE/H P cells with oppositely directed eye- and head-movement sensitivities were distributed outside this quadrant (Fig. 9I, **, n = 4). Errors for the fit sine functions for the 14 total VG and VE/H P cells ranged from 1.5 to 7.3° with the mean of 5.0°.

Of eight off-pitch VE/H P cells, the modulation of seven to vertical rotation depended on the horizontal orientation of the head. The remaining P cell did not respond at all to any vertical rotation, suggesting that it carried a pure eye-movement signal. Representative examples of two off-pitch VE/H P cells responding during downward (Fig. 10, B and C) and upward (Fig. 10, D and E) smooth pursuit were recorded in the left (B and C) and right (D and E) floccular lobe, respectively. Although these cells showed no (E1) or weak (C1) biphasic responses during rotation in the pitch plane (0°), they were modulated during vertical rotation at other horizontal orientations (C, 2–5, and E, 2–5). For example, at 45° ipsilateral (C2 and E2), both cells showed peak activity during downward rotation. At this orientation (Fig. 10A, ipsi 45°), downward rotation increased the activity of the contralateral anterior canal and decreased the activity of the ipsilateral posterior canal. The MADs of these cells were estimated to be −103 ± 4.8° and −72 ± 4.9° (Fig. 10, F and G), respectively. The MADs of
three down off-pitch VE/H P cells (Fig. 10, •) were distributed between the contralateral posterior canal and roll planes, whereas the MADs of up off-pitch VE/H P cells (Fig. 10, ○) were distributed near the contralateral or ipsilateral roll plane.

**Horizontal G and E P cells**

Nine of 12 horizontal P cells in this study were identified as gaze velocity (HG) P cells. All HG P cells increased their activity during ipsilateral pursuit. Figure 8H shows that their sensitivity during horizontal pursuit is well correlated with their sensitivity during yaw VOR suppression. Although we tested only a small number of HG P cells (Fig. 8H, ▲), the correlation is significant with a slope near one. For the remaining three cells, sensitivities during yaw VOR suppression were only 31, 45, and 58% of the target-fixed-in-space (yaw X1) condition. Because their response phases in the yaw X1 condition were near eye velocity, we call these cells E P cells (Lisberger 1996). Two of the three E P cells exhibited an increased activity during ipsilateral pursuit and one during contralateral pursuit.

**Incompletely identified cells**

Among the 25 cells with vertical smooth pursuit sensitivity in which complex spikes were not discernable, 5 were classified as VG and 13 as VE/H. The remaining seven did not respond at all during pitch VOR suppression. However, two of the seven responded during vertical rotation in the roll plane, suggesting that they had a vestibular sensitivity like that of off-pitch VE/H P cells. Of seven cells with horizontal smooth
plane, we have shown that individual vertical P cells could be classified as either VG (19%, n = 11), VE/H (48%, n = 28), or off-pitch VE/H P cells (33%, n = 19). VG P cells constituted only a small portion of the total vertical P-cell population with vertical pursuit sensitivity. In contrast, but consistent with previous reports (Lisberger 1996; Lisberger and Fuchs 1978; Miles et al. 1980; Stone and Lisberger 1990), horizontal P cells that encoded gaze velocity outnumbered (9/12) horizontal P cells with predominantly eye-movement sensitivity (3/12) even in our small sample. In alert cats, VE P cells also are more numerous than P cells that seem to encode vertical gaze velocity (Fukushima et al. 1996a) (80 vs. 20%, n = 30). Only 3 of the 30 in the monkeys we have histology for lay in the dorsal paraflocculus. Therefore the two cell types we describe here have not been drawn from two separate anatomic locations.

During the target-fixed-in-space condition (VOR X1), vertical P cells and HG P cells not only preferred different smooth pursuit directions but also exhibited qualitative differences in their response. The great majority of vertical P cells (9/11 VG, 20/24 VE/H, 18/19 off-pitch VE/H P, 47/54 = 87%) were modulated during this condition when the mean VOR gain was 1.03 ± 0.13. In contrast, HG P cells exhibited virtually no modulation under the target-fixed-in-space condition (yaw X1) in monkeys with a VOR gain of 1.0 (Lisberger and Fuchs 1978) (also our HG P cells). Moreover, unlike HG P cells (Lisberger and Fuchs 1978), the modulation of many VG and VE/H P cells when the target was fixed in space (pitch X1) was not well predicted by the linear addition of their modulations during smooth pursuit and VOR suppression (Fig. 7).

Unlike horizontal P cells the responses of which were predominantly in phase with gaze or eye velocity (e.g., Lisberger and Fuchs 1978; Miles et al. 1980; Stone and Lisberger 1990), the responses of some VE/H and off-pitch VE/H P cells were in phase with eye position during smooth pursuit (Fig. 6, B1 and C1). Nevertheless, population eye-velocity and eye-position sensitivities of the three types of vertical P cells calculated from sinusoidal vertical pursuit were similar (Fig. 6, A, 2 and 3, B, 2 and 3, C, 2 and 3). In contrast, the three types of vertical P cells behaved very differently during pitch VOR suppression and the target-fixed-in-space condition (pitch X1, Figs. 2, 4, 5). The difference among the three cell types in the interaction conditions therefore can be attributed primarily to vestibular (ipsilateral or contralateral anterior or posterior canal) inputs to these cells, suggesting that the three types of vertical P cells might constitute extremes of a continuum reflecting different vestibular sensitivities. The difference in the vestibular direction tuning of the three types of P cells using the MAD analysis supports this interpretation.

**Vestibular direction tuning**

Although eye-movement tuning has been tested during smooth pursuit for monkey floccular P cells (Krauzlis and Lisberger 1996; Stone and Lisberger 1990), there is no previous data available for vestibular direction tuning, so we will compare our data with those in the cat. The MAD distribution of VGP cells was shifted considerably toward the pitch plane (Fig. 9H). This distribution is similar to the distribution of VE/H P cells with eye- and head-velocity sensitivity in the same direction (Fig. 9I, *). Because errors for calculating MADs were <10º, the majority of the MADs for these P cells
apparently are shifted considerably toward the pitch plane away from the MADs of the vertical rectus muscles (~25°) (Baker and Peterson 1991; Robinson 1982). In contrast, none of the off-pitch VE/H P cells and VE/H P cells with oppositely directed eye- and head-movement sensitivity had MADs in the quadrant near the pitch plane (Fig. 10H). Instead, they were more aligned with the roll plane.

The MAD distribution of the three types of vertical P cells in this study (Figs. 9, H and I, and 10H) is different from the distribution found in previous studies on alert cats (Fukushima et al. 1993) in two ways. First, in this study, the MADs are shifted either more toward the pitch plane (Fig. 9H) or toward the roll plane particularity on the contralateral side (Fig. 10H). The former distribution can be attributed to convergence of excitatory inputs from the bilateral anterior canals (Baker et al. 1984; cf. King and Leigh 1982). Second, some MADs are distributed in the quadrant of the ipsilateral posterior canal. Although previous studies in decerebrate cats showed more widely distributed MADs for vertical P cells (Powell et al. 1996), P cells with MADs in the ipsilateral posterior canal quadrant (Figs. 9H and 10H) rarely were observed, even in alert cats (Fukushima et al. 1993). A portion of the variability of MADs in our study may be attributable to otolith Influences on the possibility of modulation of VE/H P cells (e.g., Fig. 9G, near 0°) (Baker et al. 1984), but we did not examine their contribution.

**Convergence of multiple canal inputs to three types of vertical P cells**

In addition to possible convergence of vertical canal and/or otolith inputs on the three types of vertical P cells, the responses of 22% (9/41) of our P cells during pitch and yaw suppression (Fig. 8, F and G) suggest convergence of inputs from the vertical and horizontal canals. The P-cell response during yaw suppression might have been contaminated from stimulation of the vertical canals because in our experiments the head was stabilized in the stereotaxic plane, where yaw rotation would have stimulated vertical canals, which are tilted backward by ~21° (Böhmer et al. 1985). However, if those P cells had been activated by vertical canal inputs alone, their response magnitudes during yaw suppression would have been only half of those during pitch [i.e., \( \cos(90° - 21°) \)] of 0.36/0.71 = 0.5]. In fact, the responses of these vertical P cells during yaw suppression were >0.5 times those during pitch suppression; five responded with equal magnitude to pitch and yaw (Fig. 8, F and G), indicating that their behavior cannot be explained by vertical canal input alone. Consequently, we conclude that some vertical P cells in the floccular lobe receive convergent inputs from the horizontal and vertical canals.

In alert and decerebrate cats, the existence of biphasic responses in vertical P cells of the floccular lobe during pitch rotation previously has been taken as evidence of convergent multiple vertical canal inputs (Powell et al. 1994, 1996). Nine VE/H P cells during pitch VOR suppression and three VE/H P cells during roll VOR suppression exhibited responses with a second harmonic ≥1.0 (e.g., Fig. 10C1, see METHODS). This distortion may have been the result of convergent input from the vertical canals. It is also possible that the lack of modulation during pitch VOR suppression in the off-pitch VE/H P cells may have been due to convergence of opposing anterior and posterior canal inputs.

Vertical rotation in the roll plane should elicit counterrolling eye movements, which may have contributed to unit modulation. Unfortunately, we were unable to record torsional eye movements in this study. Although the functional significance of convergence of multiple, even orthogonal, canal and otolith inputs onto single P cells is not clear, such convergence may provide the opportunity to produce adaptive plasticity in behavioral conditions such as cross axis VOR adaptation (e.g., Fukushima et al. 1990, 1996d; Schultheis and Robinson 1981).

**Differential role of the floccular lobe in vertical and horizontal eye movements**

To track a moving object with the head moving, gaze velocity signals must be calculated to match the velocity of the eyes in space to target velocity (Robinson 1981). Gaze movement, which can occur in any direction, might be driven by the omnidirectional signals represented in different cells in visual areas like the medial superior temporal (MST) cortex (Kawano et al. 1984; Thier and Erickson 1992) and the dorsolateral pontine nucleus, which receives direct projections from the MST and projects directly to the floccular lobe (Kawano et al. 1992). To drive ocular motoneurons, however, two stages of signal conversions are necessary; omnidirectional gaze velocity signals must be sorted into roughly horizontal and vertical components and such gaze velocity components must be converted into oculomotor signals. In floccular P cells, the first sorting has largely already taken place (Krauzlis and Lisberger 1996; Lisberger and Fuchs 1978; Miles et al. 1980; Shidara and Kawano 1993; Stone and Lisberger 1990) although a small portion of cells with oblique preferred directions are present (Krauzlis and Lisberger 1996) (Fig. 8, F and G).

The second stage of conversion may occur downstream of the floccular lobe for the horizontal component because the dominant type of horizontal floccular P cell is related to gaze velocity (Lisberger and Fuchs 1978 and others). However, our results showing that VG P cells are in the minority may suggest that the transformation of retinotopic visual signals into gaze velocity signals may be generated upstream of the floccular lobe and then converted to the eye-movement signals there. Thus vertical P cells may play some role in this conversion. A possible source of gaze velocity signals to the brain stem is the MST area (Kawano et al. 1984; Their and Erickson 1992) and the pursuit area of the frontal eye fields (Gottlieb et al. 1994; Tanaka and Fukushima 1998), where many neurons carry gaze velocity signals during smooth pursuit and VOR suppression in every direction (Fukushima 1997; Kawano et al. 1984; Their and Erickson 1992).

Our results showing that most vertical P cells are modulated during the VOR (pitch × 1) apparently are inconsistent with those of Zhang et al. (1995), who showed that chemical inactivation of the floccular lobe does not effect the vertical VOR itself. However, their monkeys were lying on their sides, and it is unknown how a static ocular counterrolling reflex, which should have been evoked by placing the animals on their sides, might have affected the vertical VOR. For example, it has been...
reported that the vertical VOR gain in alert cats tested with cats sitting normally is 14.5% higher even at 0.1–1.0 Hz than when they lie on their sides (Tomko et al. 1988). Although similar studies have not been performed in monkeys, these results suggest that interactions of gravity-sensitive signals and vertical canal signals are required for the vertical VOR to work properly, even at the relatively higher stimulus frequency of 0.5 Hz. Although further studies are needed to examine whether the simian floccular lobe plays an essential role in the vertical VOR itself in the normal upright position, it is clear that the vertical VOR, which relies on the normal activity of four vertical canals and otolith inputs, differs in a fundamental way from the horizontal VOR, which relies solely on signals from two semicircular canals (cf. Tomko et al. 1988).

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