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Transformation of Vestibular Signals Into Motor Commands in the Vestibuloocular Reflex Pathways of Monkeys

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Abstract

Parallel pathways mediate the rotatory vestibuloocular reflex (VOR). If the VOR undergoes adaptive modification with spectacles that change the magnification of the visual scene, signals in one neural pathway are modified, whereas those in another are not. By recording the responses of vestibular afferents and abducens neurons for vestibular oscillations at frequencies from 0.5 to 50 Hz, we have elucidated how vestibular signals are processed in the modified versus unmodified VOR pathways. For the small stimuli we used ($\pm 15^\circ/\text{s}$), the afferents with the most regular spontaneous discharge fired throughout the cycle of oscillation even at 50 Hz, whereas afferents with more irregular discharge showed phase locking. For all afferents, the firing rate was in phase with stimulus head velocity at low frequencies and showed progressive phase lead as frequency increased. Sensitivity to head velocity increased steadily as a function of frequency. Abducens neurons showed highly regular spontaneous discharge and very little evidence of phase locking. Their sensitivity to head velocity during the VOR was relatively flat across frequencies; firing rate lagged head velocity at low frequencies and shifted to large phase leads as stimulus frequency increased. When afferent responses were provided as inputs to a two-pathway model of the VOR, the output of the model reproduced the responses of abducens neurons if the unmodified and modified VOR pathways had frequency-dependent internal gains and included fixed time delays of 1.5 and 9 ms. The phase shifts predicted by the model provide fingerprints for identifying brain stem neurons that participate in the modified versus unmodified VOR pathways.

INTRODUCTION

The rotatory vestibuloocular reflex (VOR) is an excellent system for understanding how neural circuits generate simple behaviors. VOR performance has been studied thoroughly and the neural circuit is localized in the brain stem and cerebellum, where it can be studied in awake animals. The inputs to the horizontal rotatory VOR arise from vestibular primary afferents of the horizontal semicircular canal, which discharge in relationship to head velocity and acceleration. At the outputs, motoneurons in the abducens nucleus discharge in relation to eye velocity and position. Afferents and motoneurons are connected by a series of parallel pathways, the shortest of which include only one interneuron.

Most of what we know about the operation of the neural circuits for the VOR comes from recordings made during the VOR induced by either low-frequency sinusoidal head oscillation or brief pulses of head velocity. In general, these stimuli have statistics that fall within the range found in head turns (Armand and Minor 2001; Grossman et al. 1988); they provide excellent, natural stimuli with which to analyze system performance (e.g., Felsen and Dan 2005; Rieke

et al. 1995; Simoncelli and Olshausen 2001). Three largely compatible concepts of parallel processing have dominated thinking about the central processing for the VOR. First, Skavenski and Robinson (1973) showed that signal transformations for the VOR can be modeled as two parallel pathways: 1) a velocity pathway that transmits afferent signals with little modification to provide the eye velocity component of motoneuron firing and 2) a position pathway that integrates the vestibular inputs to provide the eye position component of motoneuron firing. Second, Lisberger (1984, 1994) provided evidence that the velocity pathway itself is composed of two parallel pathways: one is modified when the VOR undergoes adaptive gain changes, whereas the other is not. Finally, a number of features of the VOR for stimuli that include large amplitudes of head velocity can be explained with a model in which parallel pathways provide linear versus nonlinear transformations of vestibular signals (Clendaniel et al. 2001; Lasker et al. 1999, 2000; Minor et al. 1999).

In an effort to understand parallel-pathway models of the VOR in terms of the discharge of neurons in the brain's VOR pathways, we have been using stimuli that test the limits of VOR performance. Our previous paper (Ramachandran and Lisberger 2005) studied the VOR with head motion stimuli at frequencies ≤ 50 Hz, revealing high gains and large phase lags at frequencies > 25 Hz. Analysis of the effects of motor learning induced by magnified or miniaturized vision (e.g., Miles and Fuller 1974) suggested a model in which the modified and unmodified VOR pathways have rather different dynamics.

We now report the responses of semicircular canal afferents and abducens neurons to the same head oscillations used in our previous behavioral study (Ramachandran and Lisberger 2005). Our results add neural reality to the model suggested by our behavioral data. With quantitative measurements of the responses of afferents and abducens neurons, we have been able to refine the model and make concrete predictions about the dynamic responses of interneurons in the VOR pathways. The performance of the VOR and the responses of abducens neurons can be reproduced if the unmodified and modified VOR pathways receive inputs from similar sets of vestibular afferents, but introduce phase shifts that can be modeled by different time delays of about 1.5 and 9 ms, respectively.

METHODS

Data were collected from three male rhesus macaque monkeys (*Macaca mulatta*) that had been prepared for chronic experiments using techniques previously described in detail (Lisberger and Pavelko 1986; Lisberger et al. 1994a; Ramachandran and Lisberger 2005). The monkeys had the VOR performance described in our previous paper (Ramachandran and Lisberger 2005). One monkey (W) was subject to physiological recordings from both vestibular afferents and abducens neurons, whereas the other two monkeys contributed data from either afferents (Z) or abducens neurons (U). All procedures were approved by the Institutional Animal Care and Use Committee at UCSF, and were in strict compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Briefly, three surgical procedures were required to prepare monkeys for physiological experiments. All surgeries were performed using sterile procedures with isoflurane anesthesia. First, a stainless steel head post was secured to the monkey's skull using materials developed for human orthopedic surgery: 5-mm-wide orthopedic strips and 8-mm-long screws, both made of titanium, as well as dental acrylic. The head post was used to fix the monkey's head to the chair during experiments, so that sinusoidal head velocity inputs could be imposed. Second, a 16-mm-diameter coil of extremely lightweight, Teflon-coated, stainless steel wire was sutured to the sclera (Ramachandran and Lisberger 2005) using techniques derived from human ophthalmologic surgery. Finally, we trephined a hole in the skull and implanted a recording cylinder aimed at the vestibulocochlear nerve (Lisberger and Pavelko 1986) or the

abducens nucleus (Broussard et al. 1995). Postsurgically, analgesics were administered and the monkey was monitored carefully to ensure that he was not experiencing pain or distress. Training and behavioral data collection started no sooner than 1 wk after the second surgical procedure, when monkeys were conditioned to head restraint and trained to fixate 0.1° spots of light for fluid reinforcement.

Vestibular stimulation

The physical setup for vestibular stimulation was previously described in detail (Ramachandran and Lisberger 2005). Briefly, monkeys were seated comfortably in a primate chair that was specially reinforced to faithfully transmit high frequencies. The monkeys' heads were fixed to the chair by means of the post implanted on their head, and were positioned so that the axis of oscillation of the chair matched the position of the head post. The monkeys' heads were positioned in a stereotaxic plane, so oscillations would activate horizontal canals less than optimally, but the anterior and posterior canals only weakly (Estes et al. 1975). The chair was bolted rigidly to a servo-controlled turntable (Contraves Goertz). The field coils were secured to the floor so that they did not impose a load for the turntable. Care was taken that the couplings between the turntable and the primate chair and between the field coil and the floor were as rigid as possible, and that the head was fixed as tightly as possible to the primate chair. Motion of the leads from the coils was minimized by stabilizing all wires at multiple places on the coil and the chair, and also at the connector on the monkey's implant.

To assess the exact head stimulus applied to the vestibular apparatus, and the real eye movement response, we measured the position of two carefully calibrated search coils that resided within the magnetic field provided by the field coils bolted to the floor. The coil implanted on the monkey's eye measured its position with respect to the magnetic field (E_F ; also known as gaze position). A coil cemented to the head implant, parallel and as close as possible to the eye coil, measured the position of the head relative to the magnetic field (H_F). The output of the eye coil electronics was calibrated by rewarding the monkey for fixating targets at known positions along the horizontal meridian. The head coil was calibrated by imposing known angular displacements of the monkey's head. We differentiated the position output from each coil with the same analog circuits and then used the following equations to compute the two parameters we needed to measure: eye velocity with respect to the head ($\dot{E}_H$) and head velocity with respect to the world ($\dot{H}_W$)

$$\dot{H}_W = \dot{H}_F \quad (1)$$

$$\dot{E}_W = \dot{E}_F \quad (2)$$

$$\dot{E}_H = \dot{E}_W - \dot{H}_W = \dot{E}_F - \dot{H}_F \quad (3)$$

The dots over the main symbols in Eqs. 1–3 indicate the derivatives of the position signals defined in the previous paragraph. We verified the appropriate calibration of the coils by determining that the results of Eqs. 1–3 for low-frequency head oscillations agreed with our direct measurements of head and eye velocities using a tachometer attached to the shaft of the turntable and the coil implanted on the monkey's eye.

Data acquisition

In monkeys with normal VOR gains, glass-coated platinum-iridium electrodes were lowered into the brain to record from axons in the vestibular nerve or from neurons in the abducens nucleus. Voltage waveforms from the electrode were amplified conventionally and band-pass filtered. For most recordings, the filtering passed frequencies between 400 Hz and 8 kHz,

although both ends of the range were adjusted frequently to optimize isolation of the action potentials from single units. For the approach to the vestibulocochlear nerve, the electrode trajectory traversed the cerebellar floccular complex, which could be distinguished based on eye movement-related background activity (Lisberger and Fuchs 1978). A brief silence followed the electrode's exit from the vestibulocerebellum, followed by positive-negative waveforms with high spontaneous rates, and no modulation related to eye movements. The approach to the abducens nucleus included passage through the cerebellum, followed by silence as the electrode passed through the fourth ventricle. The right abducens nucleus was encountered soon after entry into the brain stem and was distinguished by the characteristic singing activity associated with eye movements toward the right (Fuchs and Luschei 1970).

Units were distinguished from background with the help of a dual-window discriminator (BAK Electronics), and the timing of the discriminator's recognition pulse was recorded at a 10- μ s resolution. The voltage waveforms from the electrode also were recorded continuously to allow off-line verification of the isolation of single units and, when deemed necessary, retriggering with a software time-window discriminator. Afferents were identified as originating from the horizontal canal based on acoustic monitoring of increased firing in response to ipsiversive head motion during sinusoidal oscillation in the yaw plane at 0.5 Hz. Once a horizontal canal afferent was identified, its spontaneous activity was recorded with the head stationary for 10 to 20 s. Abducens neurons were identified by their responses during smooth pursuit with the head stationary and the VOR in the light, and by the lack of modulation of firing rate during VOR cancellation, when the monkey tracked a target that moved exactly with the chair. They were characterized initially by recording their activity during fixations at a range of eye positions along the horizontal axis.

After initial characterization, both groups of neurons were studied during passive sinusoidal head oscillation at frequencies ranging from 0.5 to 50 Hz and head velocities of about $\pm 15^\circ/\text{s}$. For recordings from abducens neurons, monkeys were rewarded for keeping their eyes within 2° of a stationary target. For recordings from vestibular afferents the monkey's behavior did not matter; monkey W was rewarded for keeping his eyes close to straight-ahead gaze but monkey Z did not have an eye coil at the time of these recordings and was rewarded simply for remaining still. An eye coil was implanted after recordings from the vestibular nerve had been completed, to verify that his VOR showed normal behavior as a function of frequency (Ramachandran and Lisberger 2005). Afferent samples recorded in monkeys W and Z were indistinguishable.

The behavioral paradigms and data acquisition and stimulus triggering were controlled by a custom real-time data acquisition program that ran under Windows NT using the real-time kernel RTX (Ardence, Waltham MA). The signals from the two coils were differentiated in real time by an analog differentiator with an upper-frequency cutoff at 100 Hz. Signals related to coil position and velocity, as well as the head velocity signal from a tachometer on the shaft of the turntable, were sampled at 500 Hz per channel. Spike waveforms were sampled at 25 or 50 kHz. All data were stored on hard disk for later analysis. As a side effect of its filtering properties, the analog differentiator also changed the gain and phase of the underlying signal at higher frequencies. We were able to assess these changes with pure sine-wave inputs, and we then compensated for them by correcting the signals during data analysis.

Data analysis

The initial analysis of neural data involved reducing the data for each stimulus frequency and afferent to estimates of the sensitivity to head velocity and the phase shift between head velocity and firing rate. We used one of several methods. *Method 1* provided an estimate of firing rate as a function of head velocity that allows every interspike interval (ISI) to contribute to the analysis and eliminates all averaging. For each frequency of head oscillation, we divided the

data into individual cycles by marking the start of each cycle. Then, we calculated firing rate as the inverse of ISI and plotted the firing rate for each interval as a function of the time of the center of the ISI relative to the start of the cycle (Angelaki and Dickman 2000). The resulting envelope, labeled “instantaneous firing rate” in Fig. 1, superimposes the responses to all stimulus cycles. We then fitted the envelope of firing rate for each stimulus frequency and each afferent with the equation

$$fr(t) = A \sin(\omega t + \varphi) \quad (4)$$

where t is time in seconds, ω is the frequency of the stimulus in radians, A is the amplitude of modulation of firing rate in spikes/s, and φ is the phase difference between firing rate and the time used to mark the start of the head velocity stimulus. We performed the same fit for an average of the head velocity stimulus and computed the sensitivity to head velocity as the ratio of the amplitudes of the sine waves, and the phase difference between firing rate and head velocity. This procedure worked only when the neuron emitted spikes over the entire range of the sinusoidal head velocity stimulus: at low frequencies for all neurons (Fig. 1, *A* and *B*) and at higher frequencies only when the spikes of the neuron did not phase lock with the stimulus (Fig. 1*C*).

Method 2 relied on averages of firing rate accumulated in binned histograms. We divided each cycle of the stimulus into 16 equal-duration bins, counted the number of spikes in each bin, averaged across many cycles, and divided by the bin width to obtain estimates of firing rate. The resulting histogram and the associated average of head velocity were subjected to fast Fourier transform (FFT) to estimate the amplitude and phase of the fundamental components of firing rate and head velocity. This procedure worked only for lower frequencies of head velocity (Fig. 1, *A* and *B*). For higher frequencies, either the number of spikes available was simply too small to provide smooth histograms (Fig. 1*C*) or phase locking prevented the FFT from giving a meaningful answer (Fig. 1*D*).

Method 3 provided a way to estimate phase but not amplitude of the neural response. We estimated the phase difference between firing rate and head velocity by computing the value of phase that represented the center of mass of the spikes that occurred during the full set of stimulus cycles. When applied to data at low frequencies, this method yielded the same values of phase as did the first two analyses. However, it was the only method that could be used in instances where afferent responses showed phase-locking behavior. When the responses consisted of one spike per cycle, the method simply provides the phase at which that spike occurred. When the phase-locked response consisted of two or more spikes, the method finds the average phase relative to head velocity across all spikes.

Note that all analyses were done on pairs of consecutive cycles, advancing the analysis one cycle at a time to obtain averages. This procedure is only subtly different from analyzing single cycles and presenting the same cycle twice because the first cycle of the average lacks the last cycle of the raw data and the second cycle of the average lacks the first cycle of the raw data. However, the graphs for the two cycles still can differ because we have subsampled the graphs of firing rate versus phase shift by a factor of 4 for *Method 1*, to keep the graphics files of a manageable size. Also note that the graphs of firing rate versus phase at high frequencies have clumps of points because they are built up from multiple repetitions of cycles in which phase locking caused the action potentials to have stereotyped timing.

We found excellent agreement between the numbers provided by the three analysis methods whenever more than one could be applied. When the afferent did not show phase locking, so that the ISIs evenly tiled the stimulus cycle (e.g., Fig. 1, *A–C*), we used the numbers from the firing rate method to estimate the sensitivity to head velocity and phase difference between firing rate and head velocity. When the afferent showed phase locking, we estimated phase by

the center of mass method and we did not attempt to estimate sensitivity to head velocity because the occurrence of one or two spikes at the same time on each cycle made this measure meaningless. We used the analysis based on binned histograms only to validate the other methods and as a way of screening the data visually to determine which analysis method was most valid. In a subset ($n = 18$) of our irregular afferents, we found that the distribution of ISIs was not entirely smooth, either during sinusoidal stimulation at low frequencies or during spontaneous firing. This led us to discover a tiny amount of 60-Hz vibration in the head velocity provided by the chair. Remarkably this subset of afferents showed evidence that $\pm 0.25^\circ/\text{s}$ of 60-Hz vibration could alter the details of the ISI distribution, presumably because they were high-sensitivity, irregular afferents that became phase locked to sinusoidal head velocity stimuli at frequencies well below 50 Hz. However, we could not find any evidence that this minor noise source confounded our measures of phase or sensitivity to head velocity, so we retained the data.

Method 1 works well for the analysis of data obtained with high-frequency sinusoidal stimuli because it requires relatively few cycles of stimulation. However, it brings the legitimate concern of whether the inverse of each ISI should be plotted as a function of the time at the midpoint of that interval, or perhaps closer to the start or end of that interval. At low frequencies, this choice does not have a large impact on estimates of phase shift, but at 50 Hz, a 5-ms error in where the points are plotted on the time axis would alter phase shift by 90° . To obviate this class of concerns, we conducted computer simulations of conductance-based integrate-and-fire units (Troyer and Miller 1997) with large steady-input currents to create resting rates comparable to those seen in afferents and abducens neurons. A small-amplitude noise current was injected into the neurons to obtain spontaneous spike trains with the same degree of ISI regularity seen in abducens neurons and the more regular vestibular afferents. We then injected many cycles of sinusoidal current at 50 Hz into the model neurons and then subjected the resulting spike trains to analysis by *Methods 1* and *2*. The simulations provided enough spikes to allow a statistically believable comparison of different analysis methods, revealing that the phases generated by *Method 1* and *Method 2* differ by $<1\%$ over the full range of stimulus frequencies we had used.

RESULTS

Response characteristics of vestibular afferents

We characterized the responses of 71 vestibular afferent fibers that showed increased firing for ipsiversive head oscillation ($n = 37$ and $n = 34$ in monkeys W and Z, respectively). All neurons in our sample were recorded during oscillation at least at 0.5, 4, 10, and 50 Hz, and most remained isolated through the entire set of frequencies. Initially, afferents were categorized according to the normalized coefficient of variation of the ISIs recorded with the head stationary (CV^* , after Goldberg et al. 1984) and the responses to sinusoidal head oscillation at 0.5 Hz. In Fig. 2, each symbol summarizes the responses of an individual afferent by plotting sensitivity to head oscillation at 0.5 Hz as a function of CV^* . The distribution of responses in our sample of afferents looks similar to that in other studies (e.g., Bronte-Stewart and Lisberger 1994; Goldberg et al. 1984), allowing us to use the standard classification scheme according to morphological correlates (Lysakowski et al. 1995). Putative calyceal afferents (filled symbols) were identified as the cluster with values of $CV^* > 0.35$ and relatively low sensitivities to head velocity at 0.5 Hz. Putative bouton/dimorphic afferents (open symbols) lay along a fairly linear relationship between sensitivity and CV^* and had relatively high values of sensitivity to head velocity if CV^* was > 0.35 . Bouton/dimorphic afferents were further subdivided into “regular” and “irregular” according to whether CV^* was < 0.1 or > 0.1 (vertical dashed line in Fig. 2). We chose to assign afferents to morphological classes on the basis of their responses at 0.5 Hz because all afferents were recorded at that frequency. About 75% of

afferents also were studied at 2 Hz, the frequency used in prior studies (Lysakowski et al. 1995), and none changed morphological classes when assigned on the basis of their responses at that frequency. Our assignments of afferents to different groups should be viewed as tentative, given the absence of morphophysiological data for macaque monkeys. At the same time, it seems unlikely that our assignments are grossly wrong given that we have based our assignments on data from another primate species, i.e., the squirrel monkey.

In response to vestibular oscillation at 50 Hz, many afferents continued to respond by modulating the firing rate in their ongoing ISIs (Fig. 1C), but some showed phase locking and emitted action potentials consistently at the same phase of the head velocity stimulus (Fig. 1D). We developed the analysis outlined in Fig. 3 to quantify phase-locking behavior and characterize each afferent according to the frequency where it transitioned from firing rate modulation to phase locking. For each cycle of the 50-Hz stimulus, we created rasters of action potential timing. We used two cycles in each line to allow better visualization of the periodic events in the neural response, but each individual cycle triggered one line of the raster; thus each cycle appears in two lines of the raster, as a first and a second cycle. To make phase locking or its absence clear visually, the lines of the raster were ordered according to the time from a fixed point denoting the start of each cycle to the time of the first action potential emitted in that cycle of the stimulus.

For a 0.5-Hz vestibular stimulus, the timing of the first action potential drifted consistently across the periodic stimulus in all afferents, so that the latest occurrence of the first action potential in the *top line* of the raster (e.g., circled point in Fig. 3B) corresponded to the time of the first occurrence of the second action potential in the *bottom line* of the raster (Fig. 3, A and B). The same was true even at 50 Hz in afferents that continued to signal by modulation of firing rate even at 50 Hz (e.g., Fig. 3C). As a consequence, action potentials were distributed almost evenly across the stimulus cycle and the firing rate analysis could be used for quantitative estimates of afferent responses. In afferents that underwent phase locking at 50 Hz (e.g., Fig. 3D), the first action potential in each cycle lined up nearly vertically and there were periods in the stimulus cycle where no action potentials ever occurred. The phase locking could be associated with as few as one or two spikes per cycle of the stimulus, or (at lower frequencies) up to five or six spikes. All bouton/dimorphic afferents with regular baseline firing ($CV^* < 0.1$) showed tonic firing without phase locking at all frequencies ≤ 50 Hz; most irregular bouton/dimorphic afferents ($CV^* > 0.1$) and all calyceal afferents showed phase-locked responses at 50 Hz.

To quantify the phase-locking behavior of afferents, we computed the phase-locking index (PLI) at each frequency as

$$PLI = 1 - \frac{m \cdot N}{ISI} \quad (5)$$

where m is the slope of the relationship between the time of the first spike and the line number in the ordered rasters at the *top* of Fig. 3, A–D; N is the number of lines in the raster; and ISI is the mean interspike interval for spontaneous firing. In effect, this analysis asks what fraction of an average ISI is covered by the range of times of the first spikes in all lines of the rasters of Fig. 3. If the range of spike times covers an average ISI (Fig. 3, A–C), then the response is not phase locked, and the value of PLI is about zero. If a neuron shows phase locking so that the time of the first spike is stereotyped and the range covers only a tiny fraction of an average ISI (Fig. 3D), then the value of PLI is ≈ 1 . We then plotted PLI as a function of frequency. The afferent presented in the *left column* of Fig. 3 did not show phase locking at any frequency (Fig. 3E), whereas the one in the *right column* showed an abrupt transition to phase-locking behavior between 10 and 20 Hz (Fig. 3F). For each afferent, we characterized the phase-locking

behavior by fitting a sigmoid function to the relationship between phase-locking index and stimulus frequency

$$PLI(freq) = \frac{freq^n}{freq^n + (K_m)^n} \quad (6)$$

where *freq* is the frequency of stimulation, *n* represents the steepness of the sigmoid's rise from zero to one, and *K_m* represents the frequency at which the phase-locking index attains a value of 0.5, which we will call the "phase-locking threshold." For the afferent in Fig. 3*F*, the phase-locking threshold was 16.75 Hz. For afferents like the one illustrated in Fig. 3*E*, the phase-locking threshold was not assigned a numerical value.

Our findings about phase-locking responses of afferents agree with previous reports (Dickman and Correia 1989; Hartmann and Klinke 1980; Rabbitt et al. 1996). However, we note that the degree of phase locking in any group of afferents should depend on the amplitude of the vestibular oscillation, and that more afferents should show phase-locking behavior if we could deliver larger-amplitude oscillations at 50 Hz.

Frequency responses of vestibular afferents

We have summarized the frequency responses separately for three groups of afferents: regular bouton/dimorphic, irregular bouton/dimorphic, and calyceal (Fig. 4). Even though there was considerable diversity within each group, especially in the relationship between sensitivity to head velocity and stimulus frequency, comparison across groups reveals a number of consistent features of the frequency responses. At low frequencies of vestibular oscillation, each group's behavior followed prior reports. The regular bouton/dimorphic afferents (Fig. 4, *A* and *E*) showed low sensitivities and firing rate roughly in phase with head velocity at low frequencies. As a population, the irregular bouton/dimorphic afferents (Fig. 4, *B* and *F*) showed higher sensitivities and more phase lead over the entire range of stimulus frequencies, whereas the calyceal afferents (Fig. 4, *C* and *G*) had low sensitivities to head velocity at low frequencies along with the greatest phase lead of the three populations across the frequency range (compare the means in Fig. 4, *D* and *H*). Consideration of the responses of individual afferents, however, reveals as much overlap between the groups as separation of their means.

As the frequency of sinusoidal head oscillation increased, all groups of afferents showed the same general feature of continuous increases in both sensitivity to head velocity and phase lead with respect to head velocity. Comparison of the means for the three subgroups of afferents (Fig. 4*H*) shows that the high-frequency increases in phase lead were greatest in the calyceal afferents, less in the irregular bouton/dimorphic afferents, and least in the regular bouton/dimorphic afferents. On average, the irregular bouton/dimorphic afferents showed the greatest sensitivity to head velocity, whereas the calyceal afferents showed the steepest increase as a function of frequency. Note that sensitivity to head velocity could not be measured over the entire frequency range for the irregular bouton/dimorphic afferents or the calyceal afferents because they broke into phase-locking behavior at some point. Thus the mean sensitivities cover different ranges of stimulus frequencies for the three groups (Fig. 4*D*).

Across our population of afferents, phase-locking threshold was relatively poorly related to the other parameters of afferent responses. For example, aside from the absence of phase locking in the most regular afferents, there was no clear relationship between the phase-locking threshold and the normalized coefficient of variation (Fig. 5*A*). The same absence of relationship was seen when plotting the phase-locking threshold versus measures of the sensitivity to head velocity or the phase shift. As others have shown before, there was a reasonable correlation between the phase shifts of individual afferents at 0.5 and 4 Hz (Fig. 5*B*, *r* = 0.50). The correlation was stronger when comparing the phase shifts at 4 and 20 Hz (Fig. 5*C*, *r* = 0.79), and scatter reappeared in the relationship when comparing phase shift at 4

and 50 Hz (Fig. 5D, $r = 0.56$). Thus the phase shift of an afferent at intermediate values of stimulus frequency such as 4 Hz has excellent predictive value for phase shift at stimulus frequencies ≤ 20 Hz and reasonable predictive value even for 50 Hz.

Our recordings from vestibular primary afferents agree with a considerable number of prior studies of their responses to sinusoidal head oscillation at low frequencies and with a smaller number of recordings at frequencies ≤ 20 Hz (Hullar and Minor 1999; Hullar et al. 2005). Our recordings from even the regular bouton/dimorphic afferents contradict the predictions of the traditional torsion-pendulum model of cupular displacement (Wilson and Melvill Jones 1979), which is represented by the dashed curves in Fig. 4, D and G. The deviation, which is most pronounced at high frequencies, does not require that we discard the torsion-pendulum model. Rather, it requires that caution be used in applying the classical model across a wide range of stimulus frequencies. Perhaps the best approach would be to use the frequency responses of selected groups of afferents as inputs to future models, as we will do here.

Response characteristics of abducens neurons

We recorded from 90 abducens neurons in two monkeys (U and W). Responses were sampled for sinusoidal vestibular oscillation only ≤ 25 Hz in 65 units (28 and 37 units in monkeys U and W) and ≤ 50 Hz in the other 25 units, all in monkey W. As previously described by others, discharge regularity is much more uniform across abducens neurons than across vestibular primary afferents. For example, Fig. 6A plots a version of the standard firing rate versus eye position graphs, where each point plots the inverse of one ISI as a function of the mean eye position in that interval. Although the repeatability of the responses in this individual abducens neuron is clear, so is the presence of a finite amount of variation in the duration of ISIs at each eye position. We quantified the ISI variability of abducens neurons in terms of the CV. When we assembled data from many 600-ms intervals of steady fixation across all our abducens neurons (Fig. 6B), the logarithm of CV was linearly related to the logarithm of the mean ISI (regression slope = 0.49; $r = 0.78$) with more variation for longer ISIs. As long as the ISI was < 20 ms (firing rate > 50 Hz), the value of CV was < 0.1 . At straight-ahead gaze, most abducens neurons fired very regularly: CV was < 0.1 in all but four of the 75 neurons that were active with the eyes at this position (Fig. 6C).

The responses of abducens neurons during the VOR evoked by low-frequency sinusoidal head oscillation were documented extensively before, and our data at low frequencies (e.g., Fig. 7A) agree with prior reports (e.g., Fuchs et al. 1988; Skavenski and Robinson 1973). When firing rate was analyzed by plotting the inverse of each ISI in the response as a function of the phase of the midpoint of the ISI (Fig. 7A, *fourth trace*), the resulting envelope of firing rate is sinusoidal and is described well by fitting it with a sine wave (Eq. 4). We further analyzed the responses of each neuron at each frequency by using the static relationship between firing rate and eye position to compute the expected firing rate for the eye position in each ISI and subtracting that value from the actual firing rate. We then estimated the response amplitude and phase separately from the sine-wave fits to both the raw firing rate and the residual firing rate after the eye position component had been removed.

One way to think of the firing rate of abducens neurons during the VOR is as the sum of a vestibular input that depends on stimulus frequency and the output of a velocity-to-position integrator that provides a signal related to eye position. Within the framework of this model, subtracting the eye position component from the firing rate of abducens neurons provides a useful estimate of the vestibular input to abducens neurons by eliminating the component that is thought to arise from the integrator (Skavenski and Robinson 1973). The residual “extrapositional” component of firing rate (Fig. 7A, *bottom trace*) was in phase with eye velocity for lower frequencies of stimulation and we will call it the “velocity component” of firing rate with the caveat that it will not be in phase with eye velocity at all frequencies.

Subtraction of the eye position component of firing rate has the advantage that it preserves information about the phase shift of vestibular inputs to abducens neurons, whereas the regression analysis used by Fuchs et al. (1988) does not.

Our decision to subtract the eye position component of abducens neuron firing to estimate their vestibular inputs makes the assumption that the output of the velocity-to-position integrator is in phase with eye position across the range of frequencies we used. We would prefer to be able to represent the phase shift of the integrator on the basis of knowledge of the neural mechanisms of integration, but this knowledge is not available. Therefore the assumption of a perfect integrator is our only recourse for estimating the gain and phase of the vestibular input to abducens neurons. Because the excursion of eye position is quite large at lower frequencies, this is where any errors in our assumption of a perfect integrator will have the greatest impact; however, the assumption of a perfect integrator is probably quite good for lower frequencies. Because the excursion of eye position is very small at high frequencies, the impact of our assumption of a perfect integrator will be smaller and our estimates of the eye velocity component of firing should be quite accurate. At 20 Hz, for example, the peak-to-peak excursion of eye position was about 0.25° , which would contribute on average just over 1 spike/s of firing rate, or $<5\%$ of the peak-to-peak excursion of abducens firing. Thus the potential error, if present, is likely to be quite small.

The exact responses of abducens neurons at high frequencies of vestibular oscillation depended on the baseline firing rate, which is controlled by the eye position in the orbit. Consider, for example, the responses of one abducens neuron to vestibular oscillation at 50 Hz while the monkey fixated at different eye positions. For fixation at straight-ahead gaze (Fig. 7B, *second* and *third traces*), the firing rate was low and the raster shows that the neuron emitted only one or two spikes per cycle of stimulation. However, unlike vestibular afferents, the resulting response neither was truly phase locking nor provided spikes that were distributed uniformly across the stimulus cycle. Indeed, we never observed phase locking in abducens neurons like that seen in some vestibular afferents, even when the neurons emitted only one to two spikes per cycle at 50 Hz. When fixation was further in the on-direction, in this instance at $+10^\circ$ (Fig. 7B, *fourth* and *fifth traces*), the raster shows that the neuron emitted three to four spikes per cycle. The plot of instantaneous firing rate (*fifth panel*) shows that spikes were distributed uniformly enough across the cycle so that the plot of firing rate as a function of phase during the stimulus cycle yielded a modulation that was fitted well by a sine wave (Eq. 4). For all but three of our abducens neurons, we were able to take the eyes far enough in the on-direction to obtain plots like that shown in the *bottom* of Fig. 5B; the remaining three neurons were not included in further analyses.

Frequency responses of abducens neurons

Figure 8 summarizes the gain and phase of the responses of abducens neurons as a function of frequency. As indicated by the broad similarity of the fine lines representing the data for individual neurons, the responses across the sample of neurons was remarkably uniform and each individual neuron lay close to the means (bold lines in Fig. 8). To fully summarize the responses of abducens neurons, we will consider first the relationship between eye movement and abducens firing (Fig. 8, *left column*). This analysis evaluates the transformation of the sinusoidal modulation of abducens firing into eye movement. Then, we will replot the data considering the relationship between abducens firing and head velocity, to evaluate how vestibular inputs are transformed in the VOR pathways. Because the gain and phase shift of the VOR depend on frequency, the transformation from the *left* to the *right column* of Fig. 8 is not something that can be done easily in one's head and thus warrants graphical presentation.

The gain of the relationship between eye velocity and the firing of abducens neurons showed a gradual increase as the frequency of head and eye oscillation was increased ≤ 2 Hz, and a

relatively flat profile as a function of further increases in frequency. We do not have an explanation for the increase in the gain of eye velocity with respect to abducens responses at 40 and 50 Hz, which suggests that the conversion of neural signals into muscle force may be more efficient at the highest frequencies. The phase relationship started with eye velocity leading firing rate at low frequencies, as expected, given the strong eye position component of abducens firing at low frequencies. Eye velocity and firing rate were approximately in phase at 5 Hz, after which further increases in frequency caused a progressive increase in the phase lag between abducens firing rate and eye velocity.

Our graphs of the transfer function from abducens firing to eye velocity provide an assessment of the transformation done by the oculomotor “plant,” which includes the time delays of neural transmission and the dynamics of the orbital tissues, the eyeball, and the muscles themselves. Fuchs et al. (1988) demonstrated reasonable agreement between a third- or fourth-order model of the oculomotor plant and the responses of abducens neurons over frequency ranges ≤ 2 Hz. Our data allow us to assess the accuracy of similar models over a much wider frequency range. We consider two sets of parameters for the third-order model described in terms of Laplace transforms as

$$\frac{\dot{E}}{fr} = \frac{sT_4(1+sT_4)e^{-0.004s}}{4.51 \times 0.1496(1+sT_1)(1+sT_2)(1+sT_3)} \quad (7)$$

where $\dot{E}/fr$ is the frequency response defining the gain and phase of the relationship between eye velocity and firing rate, the value 4.51 in the denominator is the average static sensitivity to eye position of our sample of abducens neurons, s is the Laplace operator, $e^{-0.004s}$ introduces a time delay of 4 ms, and T_x denotes the time constants. The first s term in the numerator allows the model to describe the relationship between eye velocity (rather than eye position) and firing rate. The dot-dashed line in Fig. 8A shows the predictions of the model used by Fuchs et al. (1988) and Minor and Goldberg (1991): $T_1 = 0.003$ s, $T_2 = 0.0356$ s, $T_3 = 0.28$ s, and $T_4 = 0.14$ s. It was possible to improve the fit somewhat by optimizing the values of the time constants. The dashed line in Fig. 8A shows the best fit we were able to obtain; values of the time constants were: $T_1 = 0.003$ s, $T_2 = 0.15$ s, $T_3 = 0.28$ s, and $T_4 = 0.20$ s. Our data differ slightly from those of Fuchs et al. (1988), so the need to alter the time constants to obtain the model that best predicts our abducens data could represent a sampling bias in our data (or theirs). We also note that the exact parameters that best fitted the data depended to some degree on whether we biased the optimization algorithm toward a better fit for the gain or phase data. The fit we have provided was biased somewhat toward fitting the phase data; parameters were a bit further from those used by Fuchs et al. (1988) if we aimed for a better fit to the gain data. Finally, the “saccade” model of Sylvestre and Cullen (1999) reproduced the responses of abducens neurons fairly well if we added a 4-ms time delay to their formulation, although our optimized parameters provided a better fit to our data.

Fuchs et al. (1988) found better agreement between the model and the responses of motoneurons versus internuclear neurons in the abducens nucleus. Using their plot of sensitivity to eye velocity (r) versus eye position threshold (T) to identify our neurons as putative motoneurons or interneurons, all but two of the abducens neurons we studied over the full frequency range were putative motoneurons. We have simply left the two putative internuclear neurons in our sample, on the basis that they would have little impact on the average effect of frequency on the gain and phase of abducens responses. Comparison of the responses of 59 putative motoneurons and 31 putative internuclear neurons studied at frequencies ≤ 25 Hz revealed no differences in the gain or phase of their responses.

The relationships between eye movement and abducens responses became somewhat simpler when we considered the residual modulation of firing rate after the eye position component had been removed (Fig. 8C). Now, the gain of the relationship between eye velocity and

abducens firing was relatively flat across the frequency spectrum with a slight increase at the highest frequencies. At low frequencies, eye velocity was in phase with the velocity component of abducens firing, whereas phase lag developed progressively as stimulus frequency was increased toward 50 Hz.

The *right column* of Fig. 8 assesses the transformation that occurs between the head velocity input and the firing of abducens neurons. The gain of abducens firing with respect to head velocity (Fig. 8B) decreased as a function of stimulus frequency over the range from 0.5 to 5 Hz and then increased as stimulus frequency approached 50 Hz. When the eye position component of abducens firing was removed (Fig. 8D), the decrease in gain at low frequencies largely disappeared. The phase shift between head velocity and the firing of abducens neurons showed a steady, almost log-linear increase as a function of frequency when we considered the raw firing (Fig. 8B). After the eye position component had been removed, however, the residual components of abducens firing were essentially in phase with head velocity (-180°) at low frequencies and showed a progressive increase in phase lead as stimulus frequency started to increase beyond 1 Hz.

Vestibulo–abducens transformations

Our previous and present papers provide the basis for a new description of the transformation done in the VOR pathways to convert the responses of vestibular afferents into those of abducens neurons. The description may be limited because of our use of small signals, but it will be useful for understanding the neural basis of the behavior evoked by those stimuli, and thus should advance us toward a model of the VOR that can account for all data in all stimulus regimes. In our earlier paper (Ramachandran and Lisberger 2005), we described the gain and phase shift between eye and head velocity for sinusoidal vestibular stimuli over a frequency range from 0.5 to 50 Hz, before and after adaptive increases or decreases in the gain of the VOR. We developed a model that converted head velocity into eye velocity. The model accounted for the frequency response of the VOR before and after adaptive modification by postulating parallel modified and unmodified pathways that had rather different gain and phase characteristics. The model had two critical features: 1) to account for the effect of changes in gain on the phase of the VOR (Ramachandran and Lisberger 2005), it had two parallel pathways with very different phase shifts; and 2) to account for the phase reversal of the interneurons in the modified pathways when the gain of the VOR was quite low (Lisberger et al. 1994b), it assigned the two pathways approximately equal gains at low frequencies (Lisberger 1994).

We now are in a position to refine that model by reevaluating the transformations done in the modified and unmodified pathways after replacing head velocity and eye velocity with the signals measured in the present paper, from vestibular afferents and abducens neurons. To keep our model compatible with existing data, we started with a model that retained the two basic features of the successful model in our previous paper. To avoid complicating our model by including the velocity-to-position integrator of Skavenski and Robinson (1973), we will attempt to explain the relationship between the firing of vestibular afferents and the velocity component of abducens neurons, which was obtained by removing the eye position contribution from their firing. Thus our model complements, rather than replaces, the previous model of Skavenski and Robinson (1973).

In the present report, we evaluated the responses of vestibular afferents and abducens neurons only during the normal VOR, and not after adaptive modification of the VOR. However, prior data showed that modification of the gain of the VOR has no effect on the responses of afferents (Miles and Braitman 1980) or motoneurons (SG Lisberger, unpublished observations). Even though the afferent recordings were limited to low frequencies of sinusoidal head rotation, there is no reason to believe that the results would be different for stimuli of higher frequencies. Further, the fact that adaptive modification of the VOR has minimal effect on other eye

movements such as pursuit (Lisberger 1994) argues that there should not be changes in the relationship between abducens firing and eye movement. Thus we can use the average frequency response of abducens neurons at normal VOR gains from the present study along with the effect of VOR adaptation on the gain and phase of eye velocity from our previous study to estimate the gain and phase of abducens responses as a function of stimulus frequency after adaptive modification of the VOR. Estimates were derived under the assumption of a linear relationship between abducens responses and eye movement, by scaling and phase shifting the velocity components of normal abducens responses according to the gain and phase of the eye movements measured after adaptive modification of the VOR.

The model we fitted to our data appears in the *inset* at the *top* of Fig. 9. Vestibular primary afferent responses provide the inputs to each of two pathways that are summed to obtain models of the responses of abducens neurons. The lower, unmodified, pathway introduces a fixed time delay T_u and performs a frequency-dependent scaling of its inputs (G_u) that is fixed during adaptive changes in the VOR. We used the same equation for G_u that had been successful in Ramachandran and Lisberger (2005)

$$G_u(f) = -0.0000386f^3 + 0.0033f^2 - 0.024f + 0.547 \quad (8)$$

The upper, modified pathway performs the same set of transformations as the unmodified pathway except that the value of G_m can be adjusted at each frequency and each VOR gain to reproduce the gain and phase of abducens firing. The fixed time delay in the modified pathway (T_m) takes on a single value that is the same across stimulus frequency and adaptive modification of the VOR.

We fitted the model to the abducens responses computed for different VOR gains using methods described in our previous paper (Ramachandran and Lisberger 2005). The goal of the fitting procedure was to find values of G_m , T_m , and T_u that minimized the mean squared error of the sensitivities and phase differences in all three conditions. Only G_m was allowed to vary as a function of the gain of the VOR. To afford gain and phase approximately equal impact on the values of the parameters in the model, we weighted the error in gain 100-fold compared with the error in phase, accounting for the difference in the scales of the values of gain and phase.

Figure 9 shows that the model ($\times$ symbols and black lines) did an excellent job of reproducing the gain and phase of the velocity component of abducens firing as a function of frequency (open circles and gray lines). The parameters of the best fitting model are illustrated graphically in Fig. 10. G_u (Fig. 10A) was steady across frequency ≤ 10 Hz and then increased steadily. G_m (Fig. 10B) varied as a function of the gain of the VOR, as expected. It was steady across frequency ≤ 10 Hz at each gain and then showed a dip to a minimum at 25 Hz. The best fits were provided by values T_m and T_u of 9.10 and 1.52 ms, respectively. Time delays cause phase shifts that depend on frequency. Figure 10C plots the predictions of the best-fitting time delays for the vestibular signals that emanate from the unmodified and modified VOR pathways. The prediction is that the phase of the interneurons in the unmodified pathway (filled circles) will increase steadily as a function of frequency. The interneurons in the modified pathway (open circles) are predicted to show phase lag relative to the unmodified pathway at all stimulus frequencies, with a progressive increase in phase lag as a function of frequency.

In our model, the phase of the signals that emanate from the unmodified and modified pathways are controlled by two factors: the phase lead of their afferent inputs and the phase lag caused by time delays. Because the time delay in the model's unmodified pathway is small, it provides signals with phase shifts that are dominated by the afferent responses, which show increasing phase lead as a function of frequency. Because the time delay within the model's modified

pathway is relatively large, it provides signals that turn to phase lag at high frequencies because of the large effect of the time delay. In the model illustrated in Figs. 9 and 10, we drew the afferent inputs to each of the two parallel pathways randomly from the sample of afferents we had recorded. However, the performance of the model did not depend more than incidentally on the blend of afferent responses to each pathway. In particular, it was not possible to substitute for the large difference in time delays between the unmodified and modified pathways by judicious selection of afferent inputs. The pronounced effect of adaptive modification on the phase of the VOR (and therefore the phase of abducens responses) could not be reproduced even when regular bouton/dimorphic afferents provided the input to the modified pathways and calyceal afferents provided the input to the unmodified pathways.

DISCUSSION

Much of our earlier research has suggested that vestibular inputs for the VOR are processed in two sets of parallel pathways, one that is and one that is not modified in association with adaptive modification of the VOR (Lisberger 1984, 1994; Lisberger and Pavelko 1986; Ramachandran and Lisberger 2005). In our most recent prior paper, we developed a model of the two parallel pathways on the basis of the VOR eye movements evoked by sinusoidal head oscillation at low amplitudes (about $\pm 15^\circ/\text{s}$) over the frequency range from 0.5 to 50 Hz. We argued that the effect of VOR modification on the phase of the VOR could be explained only if the two pathways had rather different relationships between phase shift and frequency. The data presented in the present paper allowed us to make the model more precise, by using the responses of vestibular primary afferents instead of head velocity as inputs to the model and using the responses of abducens neurons rather than eye movement as its output. Thus the internal workings of our successful model make testable predictions about how vestibular signals are processed in modified and unmodified VOR pathways.

Our new data and the model based on them have important implications for the organization of the VOR pathways. In the model presented herein, the modified and unmodified pathways have frequency responses that result from differences in the amount of delay they insert in vestibuloocular processing: the delay is 1.5 and 9 ms in the unmodified and modified pathways, respectively. In principle, the brain could use one or more of several mechanisms to create the phase differences between the modified and unmodified pathways that we have modeled as time delays: 1) different phase shifts might be present in the vestibular inputs to the two pathways; 2) different amounts of phase shift might be introduced by dynamical properties of neurons in the two pathways; or 3) different time delays might be interposed within the two pathways.

Sources of time delays in VOR pathways

Two plausible neural mechanisms could create a 7.5-ms difference in the time delays introduced within the modified and unmodified VOR pathways, even though the brain stem components of both pathways appear to be disynaptic. First, the modified pathway receives inputs from cerebellar circuits that may provide part of the altered signals to drive changes in the gain of the VOR. The combination of three extra synapses and slow conduction in cerebellar parallel fibers could provide enough delay in vestibular transmission through the floccular complex to account for 7.5 ms of delay in transmission of modified vestibular signals to the abducens nucleus. Second, even at the monosynaptic vestibular inputs to floccular target neurons in the brain stem (Broussard and Lisberger 1992), the effective delay of signal processing in those pathways could exceed the physical time delays of axonal conduction and synaptic transmission. For example, the time from the start of an excitatory postsynaptic potential (EPSP) to its center of mass defines a time delay that is seen when sinusoidally modulated inputs from a group of simulated vestibular afferents are integrated to create the

spikes of a model target neuron (SG Lisberger, unpublished observations). If the vestibular inputs to floccular target neurons had short- and long-duration components mediated by α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors, then the center of mass of the net EPSP would depend on the relative sizes of the two pharmacological inputs. The effective synaptic delay could be longer in the modified pathway than in the unmodified pathway, creating time delays of ≥ 7.5 ms in transmitting physiological signals across a single synapse.

The delays suggested in our model are compatible with the 5-ms latencies in the VOR that were obtained with very high head accelerations in some studies (e.g., Huterer and Cullen 2002; Maas et al. 1989; Minor et al. 1999). In our model, the unmodified VOR pathway inserts 1.5 ms of delay between afferents and abducens neurons and the transformation of abducens firing into eye movement adds an additional 4 ms of time delay. Thus the lower bound on the latency of the VOR seems to be 5.5 ms, in good agreement with the data obtained at high head accelerations under the assumption that afferent latencies are very short for these stimuli. In response to lower head accelerations ($600^\circ/\text{s}^2$), the latencies of vestibular afferents range from 5 to 20 ms (Lisberger and Pavelko 1986), potentially adding enough time delay to account for the 14-ms latency of the VOR for the same stimuli.

Time delays provide an effective way of modeling the modified and unmodified VOR pathways, but the brain could create phase shifts in a number of ways. Indeed, it seems unlikely that time delays provide the entire explanation: the delays in our model's modified and unmodified pathways differ by somewhat more than the 5.2-ms difference in the average latencies of identified brain stem interneurons in the modified and unmodified VOR pathways during the VOR evoked by brief pulses of head velocity (Lisberger et al. 1994b). Neural processing contains many dynamic processes that could contribute to phase shifts. For example, the frequency-dependent dynamics of floccular target neurons (Sekirnjak and du Lac 2002; Sekirnjak et al. 2003) could provide an alternative source of the phase shifts we have modeled as time delays.

Vestibular inputs to, and properties of, modified and unmodified VOR pathways

We previously argued that the vestibular inputs to the modified and unmodified VOR pathways arise primarily from the regular and irregular afferents, respectively (Lisberger and Pavelko 1986). This broad division was supported by differences in the latencies and dynamics of the responses of VOR interneurons and floccular target neurons during the VOR induced by brief pulses of head velocity (Lisberger et al. 1994b). However, other data, including our own, imply that the difference in vestibular inputs to the two pathways may not be so clear. It now appears that neither the afferents with the most regular spontaneous discharge and the least phase lead, nor the most irregular and phase-leading afferents contribute much to the VOR for the low head velocities used in our experiments (Bronte-Stewart and Lisberger 1994; Minor and Goldberg 1991). Therefore the afferents that contribute to the VOR arise from the middle of the distribution of afferent discharge regularity, probably constituting bouton/dimorphic afferents that have been called "regular" and "intermediate." Further, any given secondary vestibular neuron receives inputs from a diversity of primary afferents (Highstein et al. 1987), suggesting that both the modified and unmodified pathway receive diverse vestibular inputs.

Our model does not further constrain the identity of afferents that project into the modified versus unmodified VOR pathways. It shows only that the range of phase shifts in afferent responses was too small to allow different inputs to the modified and unmodified VOR pathways to make a major contribution to the effect of changing the gain of the VOR on the phase shifts of the VOR. At 20 Hz, where changes in the gain of the VOR have the greatest impact on the phase of the VOR, an 8-ms difference in time delays would cause a phase

difference of 57.6° between the modified and unmodified pathways. Much of this phase difference could be achieved if we endowed the modified and unmodified pathways with inputs only from the afferents with the most phase lag and lead, respectively, but not if we took any reasonable sample of afferents. Therefore differences in the afferent inputs to the modified and unmodified pathways can provide at best an incomplete explanation of the effect of VOR modification on the phase shift of the VOR.

Phase locking in the VOR pathways

The absence of phase-locking behavior in abducens neurons might be explained partly by the fact that the irregular afferents with the greatest prevalence of phase locking do not appear to make a contribution to the VOR (Bronté-Stewart and Lisberger 1994), at least over the range of stimulus parameters used in our studies. At high frequencies, however, most vestibular afferents show phase-locking behavior whereas abducens neurons still do not. One explanation for this paradox would be that the membrane properties of abducens neurons might mediate in favor of rate coding and resist phase-locking behavior even when it is in their inputs. Alternatively, phase-locking behavior may be diluted by the diversity of vestibular inputs to the last-order interneurons in the VOR pathways (Highstein et al. 1987), thereby minimizing the amount of phase locking in the inputs to abducens neurons, even at 50 Hz. Until we have made recordings from a large sample of VOR interneurons at high frequencies of head oscillation, we will not be able to make a clear statement about what is occurring with phase locking in the VOR pathways.

Multiple flavors of parallel VOR pathways

As neuroscientists have dug deeper into the behavior of the VOR and its neural underpinnings, the original three-neuron reflex arc (Lorente de N6 1933; Ramon y Cajal 1909) has been subdivided into parallel pathways with different functions. First, Skavenski and Robinson (1973) developed a two-pathway model using a *direct* pathway to provide a command for eye velocity and an *integrated* pathway through the velocity-to-position integrator to provide a command for eye position. Then, Gonshor and Melvill Jones (1973), Robinson (1976), and Miles and Fuller (1974) discovered adaptive modification of the VOR and many studies (Lisberger 1984, 1994; Lisberger et al. 1994a,b,c) provided evidence that there were separable *modified* and *unmodified* VOR pathways. To allow the correct balance of eye position and eye velocity components in the firing of abducens neurons, the modified and unmodified VOR pathways must be components of the direct pathway of Skavenski and Robinson (1973), and both must provide inputs to the integrated pathway. Finally, Minor et al. (1999) and Clendaniel et al. (2001) used vestibular stimuli of large amplitudes to argue that the VOR is mediated by separate *linear* and *nonlinear* pathways. Our experiments, using vestibular stimuli of low amplitudes, almost certainly bear only on the linear component of Minor's models. It is possible that our analysis is limited by the small size of the signals we have used. To us, however, it seems plausible that the separation proposed by Minor et al. (1999) is orthogonal to the approach we have used for subdividing the VOR into parallel neural pathways. Thus the application of diverse stimulus regimes to the same behavioral system has elaborated the appealing but unrealistic concept of a three-neuron reflex arc into a realistic and plausible set of interacting parallel pathways that endow even the simplest of behaviors with rich properties.

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FIG. 1.

Responses of typical vestibular afferents to sinusoidal whole body oscillation at low and high frequencies. *A* and *C*: afferent with regular spontaneous discharge. *B* and *D*: afferent with irregular spontaneous discharge. *A* and *B*: oscillation at 0.5 Hz, $15^\circ/\text{s}$. *C* and *D*: oscillation at 50 Hz, $\pm 15^\circ/\text{s}$. In each panel, the *top trace* shows angular head velocity. In the *middle trace*, each symbol plots the inverse of one interspike interval (ISI) function of where the middle of the interval falls on the stimulus cycle. In *A* and *B*, we plotted data from only one out of each 4 ISIs to avoid an indistinguishable blob of points. Solid curve shows the best-fitting sine wave. *Bottom*: histograms created by dividing the stimulus cycle into 16 bins, counting the spikes within each bin, and converting the counts to averages of the firing rate in each bin. A single cycle is repeated in each panel to facilitate viewing of periodic events.

FIG. 2.

Division of vestibular afferents according to putative morphological origin. Each point plots data for one afferent, showing sensitivity to head velocity at 0.5 Hz as a function of the normalized CV with the head stationary. Open and filled symbols show putative bouton/dimorphic vs. calyceal afferents. Vertical dashed line is plotted at a coefficient of variation of 0.1 and divides the bouton/dimorphic afferents into those with regular and irregular spontaneous discharge.

FIG. 3.

Phase-locking behavior of vestibular afferents at low and high frequencies of sinusoidal oscillation. *A*, *C*, and *E*: afferent with regular spontaneous discharge. *B*, *D*, and *F*: afferent with irregular spontaneous discharge. *A* and *B*: rasters of spike responses on 10 cycles of whole body oscillation at 0.5 Hz. *C* and *D*: rasters of spike responses on many cycles of oscillation at 50 Hz. *Bottom trace*: angular head velocity. Lines of the rasters are ordered from *bottom* to *top* according to the time of occurrence of the first spike in each cycle. In *A* and *B*, only 40 ms of the 2,000-ms stimulus cycle is shown. In *C* and *D*, 2 cycles of stimulation at 50 Hz are shown to facilitate viewing of periodic events. To show the spike events on a temporal resolution that would be meaningful, it was necessary to use the same duration of stimulus in *A* and *B* vs. *C* and *D*, rather than presenting 2 full stimulus cycles in each panel. In *B*, the first spike in the *top* line of the raster is circled. *E* and *F*: symbols plot phase-locking index as a function of stimulus frequency. In *F*, the curve shows the sigmoid fit to the data and the 2 arrows indicate the stimulus frequency at which the phase locking index was 0.5.

FIG. 4.

Gain and phase of vestibular afferent responses for stimulus frequencies over the range from 0.5 to 50 Hz. *A–D*: gain vs. stimulus frequency. *E–H*: phase shift between firing rate and head velocity as a function of frequency, with positive values indicating the firing rate leads head velocity. From *left to right*: columns of graphs show the gain and phase for: bouton/dimorphic afferents with regular spontaneous discharge ($CV^* < 0.1$); bouton/dimorphic afferents with irregular spontaneous discharge ($CV^* > 0.1$); calyceal afferents; means for the 3 groups. In *A–C* and *E–G*, the *fine traces* show data from individual afferents and the *bold traces* show means. *D* and *H*: comparison of the means for the 3 groups with each other and with the predictions of the classical torsion-pendulum model of cupular displacement.

FIG. 5.

Correlations among different parameters used to characterize the response properties of vestibular afferents. In each graph, the individual symbols show data for different individual afferents. Filled and open symbols plot data from putative calyceal and bouton/dimorphic afferents. *A*: phase-locking threshold vs. CV^* . *B*: phase shift at 4 Hz vs. that at 0.5 Hz. *C*: phase shift at 20 Hz vs. that at 4 Hz. *D*: phase shift at 50 Hz vs. that at 4 Hz. Positive values of phase difference indicate that firing rate led head velocity.

FIG. 6.

Analysis of discharge regularity of abducens neurons. *A*: plot of firing rate vs. eye position where each symbol shows data for a single ISI from one neuron. *B*: plot of coefficient of variation vs. mean ISI, where each symbol shows data from a single 600-ms interval of fixation. Data are pooled across the full sample of abducens neurons. *C*: distribution of values of coefficient of variation at straight-ahead gaze for our full sample of abducens neurons.

FIG. 7.

Data analysis methods for abducens neurons. *A*: responses for vestibular oscillation at 0.5 Hz, $\pm 15^\circ/\text{s}$. *Traces, top to bottom*: angular head velocity; eye velocity; eye position; raw firing rate; and eye velocity component of firing rate. *Bottom traces*: each symbol shows the inverse of an ISI at the midpoint of the interval; solid curves show the best-fitting sine wave. Here, we plotted data from only one out of each 4 ISIs to avoid an indistinguishable blob of points. *B*: absence of phase-locking behavior in abducens neurons for vestibular oscillation at 50 Hz, $\pm 15^\circ/\text{s}$. *Top trace*: angular head velocity and the *2nd pair of traces* shows average eye velocity: *continuous* and *dashed traces* show data when the monkey was fixating at straight-ahead gaze or 10° in the on-direction of the abducens neuron under study. Next 2 *pairs of 2 traces* show rasters and instantaneous firing rate analysis for fixation at straight-ahead gaze and 10° to the right. Lines of the rasters are ordered from *bottom to top* according to the time of occurrence of the first spike in each cycle. In both *A* and *B*, 2 cycles are shown to facilitate viewing of periodic events.

FIG. 8.

Gain and phase of abducens neuron responses for stimulus frequencies over the range from 0.5 to 50 Hz. *A* and *C*: gain and phase of eye velocity relative to abducens firing. *B* and *D*: gain and phase of abducens firing relative to the head velocity stimulus. *A* and *B*: analysis based on the raw firing of abducens neurons. *C* and *D*: analysis based on the firing of abducens neurons after the component related to eye position had been removed. In each panel, the *fine traces* show data from individual neurons and the *bold continuous traces* show means. In *A*, the dot-dashed curve shows the predictions of the model used by Fuchs et al. (1988) and the dashed curve shows the predictions of a model of the same order but with the parameters adjusted to optimize the fit to the data.

FIG. 9. Performance of a 2-pathway model of the vestibuloocular reflex (VOR) that took the responses of vestibular afferents as its inputs and predicted the responses of abducens neurons as its output. *Inset, top:* model and the 2 rows of graphs show the gain and phase of the abducens responses as functions of frequency. Gray circles and black $\times$ symbols plot the predicted abducens responses for Fig. 9C and the output of the best model, respectively.

FIG. 10.

Predictions of the model for the responses of brain stem neurons that contribute to the modified and unmodified VOR pathways. *A* and *B*: gains for neurons in the unmodified and modified VOR pathways. In *B*, squares, circles, and triangles show predictions when the gain of the VOR is high, normal, and low, respectively. *C*: phase shift as a function of stimulus frequency. Filled and open symbols show predictions for neurons in the unmodified and modified pathways, respectively.