Responses of Irregularly Discharging Chinchilla Semicircular Canal Vestibular-Nerve Afferents During High-Frequency Head Rotations

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Hullar, Timothy E., Charles C. Della Santina, Timo Hirvonen, David M. Lasker, John P. Carey, and Lloyd B. Minor. Responses of irregularly discharging chinchilla semicircular canal vestibular-nerve afferents during high-frequency head rotations. J Neurophysiol 93: 2777–2786, 2005. First published December 15, 2004; doi:10.1152/jn.01002.2004. Mammalian vestibular-nerve afferents innervating the semicircular canals have been divided into groups according to their discharge regularity, gain at 2-Hz rotational stimulation, and morphology. Low-gain irregular afferents terminate in calyx endings in the central crista, high-gain irregular afferents synapse more peripherally in dimorphic (bouton and calyx) endings, and regular afferents terminate in the peripheral zone as bouton-only and dimorphic endings. The response dynamics of these three groups have been described only up to 4 Hz in previous studies. Reported here are responses of chinchilla semicircular canal vestibular-nerve afferents to rotational stimuli at frequencies up to 16 Hz. The sensitivity of all afferents increased with increasing frequency with the sensitivity of low-gain irregular afferents increasing the most and matching the high-gain irregular afferents at 16 Hz. All afferents increased their phase lead with respect to stimulus velocity at higher frequencies with the highest leads in low-gain irregular afferents and the lowest in regular afferents. No attenuation of sensitivity or shift in phase consistent with the presence of a high-frequency pole over the range tested was noted. Responses were best fit with a torsion-pendulum model combined with a lead operator \((\tau_{HF} S + 1)(\tau_{HF} S + 1)\). The discharge regularity of individual afferents was correlated to the value of each afferent’s lead operator time constants. These findings suggest that low-gain irregular afferents are well suited for encoding the onset of rapid head movements, a property that would be advantageous for initiation of reflexes with short latency such as the vestibulo-ocular reflex.

INTRODUCTION

Vestibular-nerve afferents innervating the semicircular canals encode information about angular motion of the head. Afferents terminating exclusively as calyx endings onto type I hair cells in the central zone of the crista are irregularly discharging and have relatively low sensitivity to sinusoidal rotations at 2 Hz (“low-gain” irregular afferents). Dimorphic afferents have both calyx endings onto type I hair cells and bouton endings onto type II hair cells. Those dimorphic afferents terminating more centrally are irregularly discharging and have a high sensitivity to sinusoidal rotations at 2 Hz (“high-gain” irregular afferents), whereas those terminating in the peripheral zone of the crista are regularly discharging and have low rotational sensitivities. “Regular” afferents ending solely in boutons terminate in the peripheral zone of the sensory neuroepithelium also have a low sensitivity and discharge regularly (Baird et al. 1988).

The possible contribution of separate types of vestibular-nerve afferent fibers to downstream reflexes has been studied in the past. The contribution of irregular fibers to the angular vestibuloocular reflex (AVOR) has been examined by taking advantage of their sensitivity to electrical currents (Ezure et al. 1983; Goldberg et al. 1982, 1984, 1990). Inhibition of irregular-nerve input using electrical currents failed to cause a change in the AVOR of squirrel monkeys undergoing sinusoidal rotations over the frequency range of 0.5–4 Hz with a peak velocity at 4 Hz of ±20°/s (Minor and Goldberg 1991) and failed to change the average responses of secondary position-vestibular-pause (PVP) interneurons in alert monkeys subjected to 2-Hz sinusoidal head rotations (Chen-Huang et al. 1997). Some studies, however, have found a contribution of irregular afferents to the AVOR. Although inhibition of irregular afferents using electrical currents had no effect on the AVOR during low-frequency sinusoidal testing, it did reduce nystagmus induced by steps of angular velocity (Angelaki and Perachio 1993). Electrical inhibition of afferent input has also been shown to affect distance-related adjustments of the angular VOR during sinusoidal rotations at 2 Hz (Chen-Huang and McCrea 1998).

More recent experiments have suggested that irregular fibers may provide a particular contribution to the VOR at higher frequencies and velocities. The frequency content of head movements in humans (Grossman et al. 1988) and in squirrel monkeys (Armand and Minor 2001) extends at least as high as 15 Hz with peak angular accelerations up to 3,000–15,000°/s2, a range over which the AVOR is thought to be effective (Grossman et al. 1989; Huterer and Cullen 2002; Keller 1978; Minor et al. 1999). Angelaki et al. (2000) studied the translational VOR (TVOR) in rhesus monkeys at frequencies from 4 to 12 Hz, measuring eye movements in compensation for naso-occipital as well as interauricular motion of the head and finding that electrical inhibition of irregular fibers reduced the sensitivity of the TVOR to viewing distance while simultaneously increasing its sensitivity (Angelaki et al. 2000). During high-frequency (>4 Hz), high-velocity sinusoidal rotations, Minor et al. (1999) have shown that the AVOR of squirrel monkeys shows a nonlinear velocity sensitivity with an in-
crease in sensitivity at higher velocities during stimulation at frequencies $\geq 2$ Hz. Merwin et al. (1989) found a velocity-dependent nonlinearity in the chinchilla at much lower frequencies, showing that the gain of their horizontal angular VOR in response to 0.1-Hz sinusoidal whole-body rotations was lower in response to stimuli at 50°/s peak velocity than for rotations at 100 or 200°/s peak velocity. The neuronal pathways underlying this nonlinear component of the VOR remain to be determined, but because regularly discharging fibers innervating the semicircular canals of chinchillas have been shown to respond linearly to head rotations even at frequencies up to 20 Hz, these nonlinearities have been hypothesized to depend on irregular afferent input (Hullar and Minor 1999).

To define the response dynamics of irregularly discharging vestibular-nerve afferents to high-frequency sinusoidal stimulation, we made single-unit extracellular afferent recordings in barbiturate-anesthetized chinchillas. The sensitivities of low-gain irregular afferents rose more rapidly with increasing frequency than did the sensitivities of other groups of afferents. The sensitivities of low-gain irregular afferents overlapped those of high-gain afferents at these higher frequencies. The phase lead of velocity of each type of afferents increased until, at the highest frequencies studied, some low-gain irregular afferents were nearly in phase with head acceleration. No evidence for a high-frequency pole located within the frequency range studied here was found. Time constants characterizing the lead operator of each afferent were found to vary with the CV of across all types of afferents, thus suggesting that the dynamic responses of low-gain afferents form a continuum with other afferents. Their response dynamics at high frequencies make them well suited for encoding the rapid onset of head motion.

**METHODS**

**Surgical procedures and recording techniques**

All surgical and recording procedures were in compliance with a protocol approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine (the institution at which the experiments were performed) and the Animal Studies Committee of Washington University Medical School. Data were obtained from 55 adult chinchillas. The surgical procedure and recording setup were identical to those described previously (Hullar and Minor 1999) except that the vestibular nerve was approached entirely through the mastoid bulla without opening the cranial cavity to reduce mortality from intracranial bleeding. An otologic drill was used to access the eighth nerve complex anteromedial to the ampullae of the horizontal and superior semicircular canals. Glass micropipettes filled with 3 M NaCl and having an impedance of 20–40 M$\Omega$ were used. Animal core temperature was maintained between 34 and 36°C.

**Rotational stimulation**

The stereotaxic frame holding the animal was mounted on a gimbaled superstructure, allowing the animal to be tilted to bring any semicircular canal into the plane of rotation. In the neutral position, the horizontal canal was in the earth-horizontal plane of rotation. The superstructure was bolted to a servo-controlled rate table (Acutronic USA, Model No. 130–80/ACT2000) programmed to supply rotations in the range of 2–16 Hz.

Once a unit was found, it was identified as a horizontal, superior, or posterior canal afferent by rotating the animal with each canal in the earth-horizontal plane. The superstructure was then secured in place.

<table>
<thead>
<tr>
<th>Table 1. Power-law constants relating CV at stated mean interspike interval (ISI) to CVs in the chinchilla</th>
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<tbody>
<tr>
<td>ISI &amp; a(ISI) &amp; b(ISI)</td>
</tr>
<tr>
<td>0.011 &amp; 0.51 &amp; 0.82</td>
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<tr>
<td>0.014 &amp; 0.87 &amp; 0.97</td>
</tr>
<tr>
<td>0.017 &amp; 1.28 &amp; 1.06</td>
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<tr>
<td>0.021 &amp; 1.94 &amp; 1.13</td>
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<tr>
<td>0.025 &amp; 2.09 &amp; 1.05</td>
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<tr>
<td>0.031 &amp; 2.14 &amp; 0.98</td>
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<tr>
<td>0.037 &amp; 2.14 &amp; 0.98</td>
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<tr>
<td>0.043 &amp; 1.95 &amp; 0.81</td>
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<tr>
<td>0.049 &amp; 1.57 &amp; 0.67</td>
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<tr>
<td>0.055 &amp; 1.07 &amp; 0.39</td>
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<tr>
<td>0.061 &amp; 1.13 &amp; 0.39</td>
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<tr>
<td>0.067 &amp; 1.28 &amp; 0.47</td>
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<tr>
<td>0.075 &amp; 1.25 &amp; 0.44</td>
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<tr>
<td>0.083 &amp; 1.06 &amp; 0.37</td>
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<tr>
<td>0.091 &amp; 1.50 &amp; 0.51</td>
</tr>
<tr>
<td>0.099 &amp; 1.37 &amp; 0.45</td>
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</table>

Formula is CV(ISI) = a(ISI)(CVs)$^b$(ISI) where ISI = mean interspike interval, in seconds. Data collected from chinchilla otolith afferents in response to static tilts.

It was not always possible to secure the animal exactly in the plane of rotation that yielded the maximum response; for these animals, a correction to the gain of the animal’s response was made as previously described (Hullar and Minor 1999).

Each unit was recorded at rest for 10–20 s before sinusoidal stimulation was begun and for an approximately equal amount of time at each frequency and velocity tested. It was recorded at 2 Hz, 20°/s before testing higher frequencies in increments of 2 Hz to a maximum of 16 Hz; in some cases, multiple velocities of several frequencies were tested. The range of velocities tested were as follows: 2 Hz, 6–64°/s; 4 Hz, 5–130°/s; 6 Hz, 8–88°/s; 8 Hz, 6–44°/s; 10 Hz, 4–58°/s; 12 Hz, 5–74°/s; 14 Hz, 4–28°/s; 16 Hz, 7–27°/s. Not every afferent provided data across the entire range of velocities shown, as many were lost during recording or did not meet inclusion criteria (see following text) at higher frequencies and velocities. In most cases, 20 s of response were recorded at each frequency and amplitude.

**Data acquisition and analysis**

Goldberg et al. (1984) divided squirrel monkey vestibular-nerve afferent fibers into groups depending on their normalized coefficient of variation, or CVs. This was determined by measuring the interspike interval (ISI) and coefficient of variation (CV) of otolith afferents at various positions of static tilt. The CVs was defined as the coefficient of variation of interspike intervals in an afferent’s spike train when its mean interspike interval was adjusted to 15 ms by tilting the animal. The curves relating interspike interval, coefficient of variation, and CVs were described as following the relationship CV(ISI) = a(ISI)·(CVs)$^b$(ISI). We followed a similar procedure, recording the varying rates of chinchilla otolith afferents subjected to static tilts to determine the coefficients a and b as shown in Table 1. These values were used to construct curves relating ISI, CV, and CVs as shown in Fig. 1. The curves shown are comparable to those published previously for the chinchilla (Baird et al. 1988) and were identical to those used to determine the normalized coefficient of variation in a previous study of regularly discharging chinchilla afferents (Hullar and Minor 1999).

Each cycle of a particular frequency and velocity stimulus paradigm was divided into bins each lasting ~2 ms (2 Hz, 250 bins; 4 Hz, 125 bins; 6 Hz, 83 bins; 8 Hz, 63 bins; 10 Hz, 50 bins; 12 Hz, 42 bins; 14 Hz, 36 bins; 16 Hz, 31 bins). A cycle histogram was created from average spike rates in each bin.
Frequency were recorded. For all afferents except one, responses meeting inclusion criteria were obtained for at least one sinusoidal paradigm (see Methods). Of the 153 afferents responding with at least one analyzable stimulus paradigm, the mean ± SD for CV* of the population was 0.21 ± 0.15; the resting rate was 42.2 ± 21.5 spikes/s, and the \( G_{2Hz} \) was 0.95 ± 0.66 spikes/s per °/s. The afferents were classified as follows: 60 units were low-gain irregular afferents (CV* = 0.34 ± 0.13 (SD); resting rate = 35.1 ± 20.9 spikes/s; \( G_{2Hz} \) = 0.83 ± 0.43 spikes/s per °/s), 55 units were high-gain irregular afferents (CV* = 0.18 ± 0.07; resting rate = 46.4 ± 22.9 spikes/s; \( G_{2Hz} \) = 1.37 ± 0.69 spikes/s per °/s), and 38 were regular afferents (CV* = 0.05 ± 0.02; resting rate = 50.2 ± 18.6 spikes/s; \( G_{2Hz} \) = 0.48 ± 0.52 spikes/s per °/s). One low-gain irregular afferent (CV* = 0.24; resting rate = 12.0 spikes/s; \( G_{2Hz} \) = 1.31 spikes/s per °/s) did not provide data that met the inclusion criteria. The resting rate of low-gain irregular fibers was significantly lower than that of high-gain irregular and regular fibers (\( P < 0.05 \)), but the rate of the latter two were not statistically different from one another. The resting rates for chinchilla afferents described here are somewhat lower than in a previous report (Baird et al. 1988) but match our previously published results (Hullar and Minor 1999).

Chinchilla afferent fibers responding to sinusoidal rotations at 0.1 Hz (Baird et al. 1988), and regularly discharging chinchilla fibers over a range of frequencies (Hullar and Minor 1999) have been reported to show essentially no velocity dependence. A sample of the units included in the present study (9 high-gain and 9 low-gain irregular afferents) were recorded while undergoing sinusoidal stimulation at various frequencies (range: 2–16 Hz) with at least three peak velocities (range: 6–60°/s) recorded at each frequency tested. Regression of sensitivity versus peak amplitude of stimulation was performed and confidence intervals determined. In all but one afferent/stimulus pair the confidence intervals included slope = 0, indicating no dependence of sensitivity on velocity. The remaining single low-gain irregular afferent showed a decrement in sensitivity with increasing velocity at 2-Hz stimulation, dropping from 0.49 spikes/s per °/s at 20°/s to 0.30 spikes/s per °/s at 40°/s.

**Inclusion criteria**

Particularly at high frequencies and velocities, many afferents cut off for some portion of their cycle, as reported previously for other animals (Dickman and Correia 1989b; Hartmann and Klinke 1980; Rabbit et al. 1996) and as shown in Fig. 2.

An afferent was considered suitable for analysis if it responded to a sinusoidal head rotation for more than half of the cycle (i.e., at least half the bins in the cycle histogram were not empty) as in previous studies of vestibular-nerve afferent responses (Boyle et al. 2002; Highstein et al. 1996). Only afferents meeting this criterion were examined further. The stimulus velocity in each bin was determined by averaging the rotational table velocity, sampled at 2 kHz, during the bin. Cycle histograms were formed by tabulating the number of action potentials per cycle in each bin. Discrete Fourier transforms of the stimulus and response tracings were computed and compared with determine the gain and phase lead of the response cycle histogram with respect to the stimulus, evaluating at the known stimulus frequency. When the response cycle histogram contained empty bins, these were treated as regions void of data as described previously (Highstein et al. 1996). Response gain (at each frequency and velocity tested) was defined as the ratio of peak firing rate (in spikes/s) of the response sinusoid to stimulus peak velocity (in °/s); response phase was defined as the phase angle in degrees by which the response was lead of the response cycle histogram compared with the phase of the stimulus, evaluating at the known stimulus frequency. When the response cycle histogram contained empty bins, these were treated as regions void of data as described previously (Highstein et al. 1996). Response gain (at each frequency and velocity tested) was defined as the ratio of peak firing rate (in spikes/s) of the response sinusoid to stimulus peak velocity (in °/s); response phase was defined as the phase angle in degrees by which the response was lead of the response cycle histogram compared with the phase of the stimulus, evaluating at the known stimulus frequency.

Afferents with a CV* ≥ 0.20 and sensitivity at 2 Hz, 20°/s stimulation (\( G_{2Hz} \)) of <1.5 spikes/s per °/s were assigned to the low-gain irregular group as suggested by the morphophysiological results of Baird et al. in the chinchilla (Baird et al. 1988). Regular afferents were identified as having a CV* ≤ 0.1. All other afferents were identified as high-gain irregular. No distinction was made in this study between intermediate fibers (0.1 < CV* < 0.2) and high-gain irregular fibers.

**Results**

Responses from 154 afferents undergoing stimulation at 2 Hz (with a peak velocity of 20°/s) and at least one other frequency were recorded. For all afferents except one, responses meeting inclusion criteria were obtained for at least one sinusoidal paradigm (see Methods). Of the 153 afferents responding with at least one analyzable stimulus paradigm, the mean ± SD for CV* of the population was 0.21 ± 0.15; the resting rate was 42.2 ± 21.5 spikes/s, and the \( G_{2Hz} \) was 0.95 ± 0.66 spikes/s per °/s. The afferents were classified as follows: 60 units were low-gain irregular afferents (CV* = 0.34 ± 0.13 (SD); resting rate = 35.1 ± 20.9 spikes/s; \( G_{2Hz} \) = 0.83 ± 0.43 spikes/s per °/s), 55 units were high-gain irregular afferents (CV* = 0.18 ± 0.07; resting rate = 46.4 ± 22.9 spikes/s; \( G_{2Hz} \) = 1.37 ± 0.69 spikes/s per °/s), and 38 were regular afferents (CV* = 0.05 ± 0.02; resting rate = 50.2 ± 18.6 spikes/s; \( G_{2Hz} \) = 0.48 ± 0.52 spikes/s per °/s). One low-gain irregular afferent (CV* = 0.24; resting rate = 12.0 spikes/s; \( G_{2Hz} \) = 1.31 spikes/s per °/s) did not provide data that met the inclusion criteria. The resting rate of low-gain irregular fibers was significantly lower than that of high-gain irregular and regular fibers (\( P < 0.05 \)), but the rate of the latter two were not statistically different from one another. The resting rates for chinchilla afferents described here are somewhat lower than in a previous report (Baird et al. 1988) but match our previously published results (Hullar and Minor 1999).

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Afferents with a CV* ≥ 0.20 and sensitivity at 2 Hz, 20°/s stimulation (\( G_{2Hz} \)) of <1.5 spikes/s per °/s were assigned to the low-gain irregular group as suggested by the morphophysiological results of Baird et al. in the chinchilla (Baird et al. 1988). Regular afferents were identified as having a CV* ≤ 0.1. All other afferents were identified as high-gain irregular. No distinction was made in this study between intermediate fibers (0.1 < CV* < 0.2) and high-gain irregular fibers.
Analyzable responses were obtained at high frequencies (≥10 Hz) in 54 (15 low gain, 20 high gain, and 19 regular) of the 153 total afferents studied. The remainder were either lost before testing could be accomplished up to 10 Hz (40 low gain, 34 high gain, and 16 regular fibers were lost in this way) or were not analyzable because the afferent failed to meet inclusion criteria due to cutoff at frequencies of ≤10 Hz (5 low gain, 1 high gain, and 3 regular fibers). The baseline firing rate, CV*, and $G_{2Hz}$ of each group of afferents within this subset were not significantly different from each corresponding group in the entire population of 153 afferents ($P > 0.1$). The relationships of CV* versus $G_{2Hz}$ and CV* versus phase at 2 Hz, ±20°/s for each afferent in this subset are plotted in Fig. 3.

All irregularly discharging afferents in the high-frequency subset (≥10 Hz) showed an increase in sensitivity and phase lead with increasing frequency. Responses for individual afferents in this subset, divided by group, are shown in Fig. 4.

The average responses of those afferents with responses meeting the inclusion criteria over the entire range 2–16 Hz (7 low gain, 9 high gain, and 14 regular) are shown in Fig. 5. Within each of the three groups, individual afferents having a higher gain at 2 Hz increased more in sensitivity with increasing frequency than those having a lower gain at 2 Hz. Phase relationships among individual afferents remained relatively constant across the same range. The average sensitivity of low-gain irregular afferents grew sharply compared with high-gain afferents and regular afferents. The average sensitivity of high-gain irregular afferents at 16 Hz was 2.3 times higher than that at 2 Hz, whereas low-gain afferents increased by 4.5 times over the same range. This sharp increase in gain by low-gain afferents at higher frequencies calls into question the terminology that has been used to describe them, an issue that is discussed in more detail in the following text. Average phases increased monotonically and were roughly parallel for all three groups of afferents.

**FIG. 3.** Sensitivity (top) and phase (bottom) vs. CV* for 2 Hz, 20°/s sinusoidal head rotations for the subset of afferents recorded at ≥10 Hz. X, regular afferents; ◇, high-gain irregular afferents; ●, low-gain irregular afferents. Afferents with a CV* ≥0.20 and sensitivity at 2 Hz, 20°/s stimulation ($G_{2Hz}$) <1.5 spikes/s per °/s were assigned to the low-gain irregular group as suggested by results described by Baird et al. in the chinchilla (Baird et al. 1988).

**FIG. 4.** Frequency responses of the subset of afferents tested at ≥10 Hz (shown in Fig. 3). Top: sensitivity (spikes/s per °/s); bottom: phase (deg lead relative to head velocity). Left: regular afferents; middle: high-gain irregular afferents; and right: low-gain irregular afferents.
corresponds to a high-frequency pole at 22 Hz for chinchillas and 19 Hz for squirrel monkeys that would manifest as a high-frequency response attenuation. No evidence for a pole at these frequencies was seen, however, in a study of regularly discharging chinchilla afferents responding to stimuli up to 20 Hz (Hullar and Minor 1999) or in squirrel monkey afferents responding to stimuli up to 8 Hz (Fernandez and Goldberg 1971). Among the afferents tested here, six fibers (2 of each type) recorded at stimulus frequencies up to 20 Hz also failed to show any evidence of a high-frequency pole such as a phase shift or gain decrement. Therefore a smaller value such as 0.002 s may be more accurate, resulting in a chinchilla torsion-pendulum model of the form \((4.25 s)/(4.25 s + 1)(0.002 s + 1)\).

In conjunction with the torsion pendulum model, an additional lead operator is necessary to explain the high-frequency gain enhancement and phase lead in the average responses of each afferent group shown in Figs. 4 and 5. The physiologic basis for this lead effect is hypothesized to be a combination of mechanical and transduction effects in the neuroepithelium (Rabbitt et al. 1995). Goldberg and Fernandez (1971) used a single zero of the form \((\tau_{HF1} s + 1)\) as a lead operator to fit squirrel monkey semicircular canal afferents responding to sinusoidal stimuli up to 8 Hz. Fractional-exponent lead operators have also been used extensively to model vestibular afferent responses (Anastasio et al. 1985; Baird et al. 1988; Boyle and Hightson 1990; Brichita and Goldberg 1998; Dickman and Correia 1989a,b; Hartmann and Klinke 1980; Landolt and Correia 1980; Schneider and Anderson 1976; Segal and Outerbridge 1982; Tomko et al. 1981). In chinchilla, Baird et al. (1988) used a fractional-exponent lead operator of the form \(g(\tau_{HF1} s + 1)^k\) to fit afferents responding to sinusoidal rotations \(\pm 4\) Hz, with \(k\) having a value of 0.056 for regular afferents and 0.49 for irregular afferents and \(\tau_{HF1}\) set to 0.2. In monkeys, Haque et al. (2004), following a construction proposed by Correia et al. (1992), used a lead term of the form \(g(s^4)(\tau_{HF1} s + 1)^k\). Each of these lead operators was used to fit the data presented here. In addition, as it was clear that a single lead-operator zero was insufficient to model the phase lead and gain enhancement seen here, another formulation of the lead operator, \(g(\tau_{HF1} s + 1)(\tau_{HF2} s + 1)\), was also tested.

The fit using each lead operator was evaluated by comparing fitted curves to mean afferent sensitivities as shown in Fig. 6 and by calculating each fit’s mean squared error (MSE) with respect to the complex-valued sensitivity at that frequency. Calculation of the Bayesian Information Criterion (BIC) for each fit correlated with its MSE, with lower values indicating a superior fit (Cullen et al. 1996; Galiana et al. 1995; Minor et al. 1999). Numerical results of the fitting routine are shown in Table 2 and graphical fits are shown in Fig. 6.

The results shown in Table 2 and Fig. 6 indicate no distinct advantage of one lead term over the others. However, the lead term with the lowest MSE and BIC, both overall as well as within each afferent group, was of the form \(g(\tau_{HF1} s + 1)(\tau_{HF2} s + 1)\). When fit to regular afferent responses, one factor of the lead operator contained a negative time constant, indicating a nonminimum-phase solution. A zero with a negative time constant has the same effect as the corresponding positive time constant zero on frequency response magnitude but contributes a phase lag instead of a lead (Lathi 1974).

Previous work has shown that the response dynamics of afferents vary with their discharge regularity (Baird et al. 1988;}

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**FIG. 5.** Average sensitivities and phases of regular afferents \((n = 14, \times)\), high-gain irregular afferents \((n = 9, o)\), and low-gain irregular afferents \((n = 7, *);\) included are only fibers recorded over the entire range 2–16 Hz. **Top:** sensitivity (spikes/s per °/s); **bottom:** phase (deg lead relative to head velocity). Error bars represent mean gain ± 1 SD. Bars are displaced slightly along frequency axis and only one bar is shown for clarity. Note that the average responses of low-gain irregular afferents rise to match those of high-gain irregular afferents at high frequencies.

**Calculation of lead operator and transfer function fits**

The contributions of afferent types to vestibular reflexes depend on the response dynamics of the afferents themselves. The response dynamics of vestibular afferents have been described using a torsion-pendulum model combined with a lead operator (Dickman and Correia 1989b; Fernandez and Goldberg 1971). The torsion-pendulum model uses the physical characteristics of the semicircular canal system to describe the displacement of the cupula in terms of head velocity in the form \((\tau_1 s + 1)(\tau_2 s + 1)\), where the “long time constant” \(\tau_1\) depends mainly on the elastic restoring force of the cupular membrane, the “short time constant” \(\tau_2\) depends mainly on the time constant of viscous flow of the endolymph, and \(s\) is an operator denoting time differentiation in the Laplace domain (Steinhausen 1933). In chinchillas, \(\tau_1\) has been calculated directly from afferent responses as 4.37 s for regular units and 3.96 s for irregular units, with an average value of 4.25 s (Baird et al. 1988).

In mammals, the torsion pendulum’s short time constant \(\tau_2\) has been estimated based on biophysical principles but has not been directly observed in afferent responses. In chinchillas, \(\tau_2\) has been calculated as 0.0072 s using the technique of Ramprashad et al. (Baird et al. 1988; Ramprashad et al. 1984) while an alternative technique results in a figure of 0.0019 s (Jones and Spells 1963). In squirrel monkeys, \(\tau_2\) has been calculated as 0.003 s (Fernandez and Goldberg 1971; Jones and Spells 1963) or 0.0084 s (Ramprashad et al. 1984). If the larger value is used, the short time constant of the torsion pendulum is an advantage of one lead term over the others. However, the lead term with the lowest MSE and BIC, both overall as well as within each afferent group, was of the form \(g(\tau_{HF1} s + 1)(\tau_{HF2} s + 1)\). When fit to regular afferent responses, one factor of the lead operator contained a negative time constant, indicating a nonminimum-phase solution. A zero with a negative time constant has the same effect as the corresponding positive time constant zero on frequency response magnitude but contributes a phase lag instead of a lead (Lathi 1974).

Previous work has shown that the response dynamics of afferents vary with their discharge regularity (Baird et al. 1988;

A regression between each afferent’s CV* and each of the two time constants making up the two-zero lead operator best fitting its responses was performed. A linear relationship exists between CV* and the lower-frequency time constant ($r^2 = 0.69$), as shown in Fig. 7, while the relationship is less well defined for the higher-frequency time constant ($r^2 = 0.38$). The poorer regression of the higher-frequency time constant versus CV* may be due in part to the fact that the values of this time constant in the lead term correspond to frequencies outside the range of stimuli employed in this study.

DISCUSSION

The results presented here extend our understanding of vestibular-nerve afferent responses to high frequencies of stimulation. Our data provide new insights into the distinctive physiology of low- and high-gain irregular afferents. The sensitivities of low-gain afferents rise to equal those of high-gain irregular afferents at 16 Hz. These low-gain irregular afferents also have higher phase leads at these higher frequencies, making them well suited for encoding the onset of rotary motion. A two-zero lead operator, in conjunction with the torsion-pendulum model, fits the data well. The lower frequency time constant of this lead operator varies with CV* across all types of afferents, suggesting that the dynamic responses of low-gain afferents form a continuum with other afferents. The dynamic characteristics of low-gain irregular afferents make them candidates for carrying short-latency information regarding rapid head movements to downstream vestibular reflexes.

Classification of afferent types

Afferents with a CV* $\leq 0.1$ in this study were termed regularly discharging, as defined previously (Baird et al. 1988).

<table>
<thead>
<tr>
<th>Table 2. Parameters for four lead operators fitting afferent data in combination with torsion pendulum model of the form $(4.25s)/(4.25s/H^{1.00}) + 0.002s/(1.00)$</th>
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The lowest total mean squared error (MSE) and Bayesian Information Criterion (BIC) were associated with the lead operator of the form $g(t_{HF1}s + 1)(t_{HF2}s + 1)$. $g$, gain term; $t_{HF1}$, longer time constant of lead operator; $t_{HF2}$, shorter time constant of lead operator (in two-zero model); $k$, exponent, in models with fractional exponents.
However, no intracellular labeling was performed in this study to morphologically separate low-gain irregular afferents, which end exclusively in calyx endings, and high-gain irregular endings, which end in both calyx and bouton endings (Baird et al. 1988). We nonetheless believe that the physiologic criteria proposed in METHODS are sufficient to differentiate these two groups of afferents for further analysis. Identical criteria applied to a group of physiologically identified and intracellularly labeled afferents (Fig. 11A in Baird et al. 1988) identified 15 afferents as calyx-only fibers. This included every proven calyx fiber in the original set, for a sensitivity of 100%, and incorrectly included a single dimorphic afferent, for a specificity of 14/15, or 93%.

Sensitivity of afferents to high-frequency rotations

Previous work divided irregularly discharging vestibular-nerve afferents into two groups based on morphologic and physiologic grounds (Baird et al. 1988). High-gain afferents were shown to have higher values of CV*, to terminate as dimorphic endings more centrally in the cristae than regular afferents, and to have a high rotational sensitivity measured at 2 Hz, ±20°/s. Low-gain irregular afferents were shown to have the highest values of CV*, to synapse in the central region of the cristae exclusively as calyx endings onto type I hair cells, and to have a lower rotational sensitivity at 2 Hz, ±20°/s than high-gain irregular fibers that terminate as dimorphic endings. The data presented here suggest that although describing afferent groups as low gain and high gain appears reasonable at 2 Hz, it is not accurate at higher frequencies, where the sensitivity of low-gain afferents rises to match that of high-gain afferents. Therefore instead of grouping afferents based on their regularity of discharge and sensitivity to stimulation at 2 Hz (Baird et al. 1988), it may be preferable to distinguish them based on their presumed anatomic structure in the neuroepithelium, as suggested previously (Lysakowski et al. 1995), or on their relative phase relationships. Relative phase between afferent types is constant across frequencies, while their relative gain changes (as shown in Fig. 6) suggesting that low-gain irregular afferents might better be termed “phase-led” irregular afferents in a manner analogous to the “acceleration” units described by Highstein et al. (1996) in toadfish.

In a previous report (Hullar and Minor 1999), no significant change was noted in the sensitivity of regularly firing afferents over the range of 2–20 Hz, whereas in this study they increased by an average factor of 1.8 over the range of 2–16 Hz. The phase of regular afferents increased from 0 to 30° relative to head velocity in the earlier study and from 4 to 43° in the results shown here. The difference may be explained in terms of the population of regular afferents included in each study. Five of eight regular afferents tested over the range 2–16 Hz for this study had a CV* ≥ 0.05, compared with only 5 of 51 linearly responding horizontal-canal regular afferents in the previous study; 3 of the 8 had a G0.5 Hz ≥ 0.5 spikes/s per °/s versus only 4 of the 51 in the previous study (Hullar and Minor 1999). An increased CV* corresponds to longer lead operator time constants (Fig. 7) and decreased low-frequency inflection point, thereby increasing the phase lead and change in sensitivity of the afferent over the frequency range studied.

Change in sensitivity with respect to velocity

The finding that there is little dependence of sensitivity on velocity among regular and high-gain irregular afferents is consistent with previous studies in chinchilla, chicken, and toadfish. Baird et al. (1988) reported that the sensitivity of chinchilla vestibular-nerve afferents responding to sinusoidal stimulation at 0.1 Hz did not depend on the velocity of stimulation when tested over the range between 10 and 160°/s. Natural head movements in mammals extend to higher velocities, and we cannot rule out the possibility that changes in velocity sensitivity will be evident at higher stimulus velocities. Similar velocity independence has been noted in chicken afferents responding to sinusoidal microindentation at peak amplitudes equivalent to an estimated 70°/s at frequencies ≤ 10 Hz (Boyle et al. 2002). In the toadfish, afferent response gains over the frequency range 0.01–10 Hz were shown to be independent of velocity at relatively low stimulus amplitudes (<25°/s) for high-gain afferents and up to somewhat higher stimulus amplitudes (<100°/s) for low-gain afferents (Boyle and Hightstein 1990).

Determination of a lead term

The lead operator best fitting the data presented here consisted of two zeros in the form (τHF1s + 1)(τHF2s + 1). Although the two-zero lead operator proved to be the best fit using the MSE criteria, the general shape of the Bode plots of the average responses can also be fit with a lead operator containing a fractional exponent. Increasing the value of the exponent in a fractional-exponent lead term increases the slope of the gain plot and shifts the phase plot by a uniform amount across all frequencies, as observed in the average sensitivities of high-gain irregular and low-gain irregular afferents and in the phase plots of all three types of fibers (Fig. 6). Frequency responses described by similar fractional-exponent zero terms are common in sensory systems and may reflect the dynamics of distributed processes such as neurotransmitter release and reaccumulation (Thorson and Biederman-Thorson 1974) such
as might be expected to occur in the vestibular neuroepithelium. Further work is necessary to examine this hypothesis further.

The high-frequency response dynamics of chinchilla fibers mirror those seen in other organisms. In toadfish, three groups of afferents have been described: “A” (acceleration) afferents, “HG” (high-gain) afferents, and “LG” (low-gain) afferents. Although toadfish do not have calyces, the dynamics of their afferent fibers appear to correspond to the chinchilla’s low-gain irregular, high-gain irregular, and regularly firing fibers, respectively. The sensitivity of LG toadfish and regular chinchilla fibers climb slightly across the frequency range 2–16 Hz, whereas the sensitivities of toadfish HG fibers increase more and A afferents increase the most. Similar to the relationship between low-gain irregular and high-gain irregular afferents seen in Fig. 6, toadfish A afferents increase their sensitivity enough to exceed that of HG afferents at frequencies ~0.02 s. A high-frequency pole located at ~0.002 s has been observed in data collected using a microindenter in pigeons (Dickman and Correia 1989b) and a high-frequency pole at a similar location has been described in goldfish (Hartmann and Klinke 1980). The existence in chinchillas of one or more high-frequency poles is required to make the system physically realizable, but the location of a pole may be sufficiently above the bandwidth studied here that its position cannot be determined. Complicating this calculation is the fact that, in several of the lead operations proposed, there is a zero in approximately the same position as that expected for the torsion pendulum’s high-frequency pole at 0.002 s. Further study with higher frequency stimuli would be required to determine with certainty the location of the high-frequency pole(s).

Relationship of CV* and time constant

Earlier work has shown that general groups of afferents and even single fibers form a concentric gradient across the neuroepithelium according to their regularity of discharge and response dynamics (Baird et al. 1988; Brichta and Goldberg 2000; Honrubia et al. 1981; Lysakowski et al. 1995; O’Leary et al. 1974). The correlation of the larger time constant of the lead operator to discharge regularity shown in Fig. 7 suggests a similar, frequency-dependent gradient across the neuroepithelium, with lower-frequency lead-term time constants associated with more centrally derived fibers and higher-frequency lead-term time constants associated with more peripherally derived fibers. Instead of low-gain irregular fibers forming a wholly distinct group from other afferents, this apparent continuum suggests that a static gain term alone may be the most significant factor causing them to form a distinct group at 2 Hz as shown previously (Baird et al. 1988).

Function of afferent nerve types

The distinctive contributions of irregular and regular afferents to vestibular function have been considered previously. The lower gain associated with calyx-only afferents may expand the operating range of vestibular-nerve afferents (Goldberg 2000). A range of fiber types may serve to match the requirements of dynamic loads represented by downstream reflexes (Bilotto et al. 1982; Boyle et al. 1992; Fernandez and Goldberg 1971). It has been suggested that irregular afferents, because of their higher phase lead and sensitivity, provide more suitable inputs for vestibulocollic reflexes to better compensate for the high inertial load of the neck. Regular afferents, with lower phase leads and more tonic response dynamics, provide signals that are appropriate for the vestibulocollic reflex (VOR) at least at lower frequencies and velocities of stimulation.

Gating of inputs from irregular afferents to central VOR pathways has been suggested as a mechanism to provide rapid changes in VOR dynamics (Goldberg 2000; Minor and Goldberg 1991). Vergence-mediated modulation of VOR gain is one situation in which gating of inputs from irregular afferents appears to have a role in VOR responses (Chen Huang and McCrea 1998).

Location of a high-frequency pole in the torsion-pendulum transfer function

The location of the torsion pendulum’s high-frequency pole could not be determined from the data presented here. A previous study of regularly discharging chinchilla afferents (Hullar and Minor 1999) did not find evidence for a pole predicted to exist at 0.007 s (Ramprashad et al. 1984). Using the technique outlined by Jones and Spells (1963), however, this pole’s value was determined to lie closer to 0.002 s. A high-frequency pole located at ~0.002 s has been observed in data collected using a microindenter in pigeons (Dickman and Correia 1989b) and a high-frequency pole at a similar location has been described in goldfish (Hartmann and Klinke 1980).
Our findings indicate that, at high frequencies, the sensitivities of low-gain afferents rise into the range of high-gain afferents and achieve a phase lead corresponding to acceleration, making them potentially well-suited for encoding the onset of rotatory motion (Lewis and Parnas 1994). The latency of the VOR in primates is ≈ 7 ms (Huterer and Cullen 2002; Minor et al. 1999), and the ability of the VOR interneurons to detect the onset of head motion in such a short period of time would be enhanced by contributions from afferents with the high gains and phase leads demonstrated by low-gain afferents at high frequencies.

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