Electrical response properties of avian lagena type II hair cells: a model system for vestibular filtering

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Ricci, Anthony J., and Manning J. Correia. Electrical response properties of avian lagena type II hair cells: a model system for vestibular filtering. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R943–R953, 1999.—Data presented represent the first electrical recordings from avian lagena type II hair cells. The perforated-patch variant of the whole cell recording technique was used to investigate how the macroscopic currents shaped the voltage response of the hair cells. Voltage-clamp data separated cells into two broad classes on the basis of differences in activation rates, rates and degree of inactivation, and pharmacological sensitivity. Current-clamp recordings revealed low-quality membrane voltage oscillations (Qc < 1) during pulse current injections. Oscillation frequency correlated with activation rate of the macroscopic currents. The quality of membrane oscillations (Qc) varied linearly with frequency for cells with little inactivation. For cells with rapid inactivation, no relationship was found between Qc and frequency. Rapid inactivation may serve to extend the bandwidth of vestibular hair cells. The frequency measured from voltage responses to pulsed currents may reflect the corner frequency of the cell. The filtering properties of avian lagena hair cells are like those found in all other vestibular end organs, suggesting that the electrical membrane properties of these cells are not responsible for specializing them to a particular stimulus modality.

Inward and outward currents as well as by the magnitude of these currents (13, 17, 39).

Rapidly activating, rapidly inactivating A-type potassium conductances have been described in some auditory and most vestibular hair cells (19, 20, 22, 24, 25, 32, 35). The functional significance of inactivation is unknown. It has been suggested that the inactivating conductance acts as a transient buffer against depolarization induced by head rotation (1, 22, 32). The transient buffer would counter the adaptation process related to the mechanoelectrical transduction elements of hair cells, thereby leading to the tonic nonadaptive response observed in some afferents (1). Data presented here suggest that inactivation serves to limit the quality of membrane potential resonance in lagena type II hair cells at higher frequencies.

The function of the lagena has been reviewed (21). It senses gravitational forces or seismic or airborne vibrations (some teleosts and frogs). The function of the avian lagena is unknown. Its afferent fibers project to vestibular (4) and cochlear nuclei (5). Morphometric data (30) indicate that both type I and type II hair cells are present in the avian lagena. Because no type I hair cells have been reported in auditory receptors, the lagena appears to have a “vestibular-like” neuroepithelium. Data presented here are the first reports of the basolateral membrane properties of avian lagena type II hair cells. The electrical properties of these hair cells were most similar to vestibular hair cells (semicircular canal and utricle). This result supports the idea of a vestibular function for this end organ. This result also supports the hypothesis that basolateral membrane electrical properties of type II hair cells are alike across functionally distinct vestibular end organs. A similar result was noted for type I vestibular hair cells (31).

The results of this study suggest a possible role for the inactivating conductance in lagena hair cells and vestibular hair cells in general, which is to decrease the quality of the membrane resonance.

Finally, it is proposed that the membrane oscillations noted in vestibular hair cells may not necessarily represent a high-gain tuning mechanism as it does in resonating auditory hair cells but may represent the corner frequency of a tunable low-pass filter.

METHODS

All animal procedures were conducted in strict accordance with the guidelines of the American Physiological Society and the National Institutes of Health. The studies were conducted with the approval of the University of Texas Medical Branch Animal Care and Use committee.
Hair cell dissociation. Cells were dissociated as previously described (31). The auditory papilla and lagena were removed from anesthetized (50 mg/kg iv pentobarbital sodium) adult (8–40 wk old) white king pigeons (Columba livia) and placed into chilled DMEM. The DMEM was supplemented with 24 mM NaHCO3 and 10 mM HEPES. The lagena was identified as the area of the tissue covered with a layer of otoconia, and it was separated from the remaining papilla. The membrane over the otoconia was removed to enhance solution diffusion. At this point, the lagena was placed into room temperature DMEM containing 0.5 mg/ml protease (type XXIV, Sigma) for 10 min. After this incubation, the tissue was transferred to a DMEM solution containing 0.3% neonatal fetal calf serum, placed into an incubator, and maintained at 37°C in a 95% oxygen-5% carbon dioxide-saturated environment. The tissue was maintained in this environment for at least 3 h and up to 8 h. Cells were harvested by pinning the tissue to the floor of a Sylgard (Dow Corning)-coated petri dish and vacuuming the cells free using siliconized (Sigmacote, Sigma) pipettes that previously had been pulled and fire polished to have tips between 20 and 100 µm. The cells were then plated onto the bottom of conconavalin A (Sigma)-coated dishes into an external solution containing (in mM) 145 NaCl, 3 KCl, 2 CaCl2, 1 MgSO4, 15 HEPES, and 10 glucose. The pH was adjusted to 7.4, and osmolality was maintained between 310 and 320 mmol/kgH2O, as measured with a vapor pressure osmometer (Wescor 5500). Cells were viewed under bright-field or Nomarski differential interference contrast optics on an inverted Nikon microscope with a ×63, 1.4 numerical aperture oil immersion objective.

Cell identification. Dissociated type II hair cells were morphometrically discriminated from type I hair cells and studied. Measurements of neck width, cell body width, and cuticular plate width allowed for separation of hair cells into classes on the basis of the ratios of neck width to cuticular plate width (≥0.72) and neck width to cell body width (≥0.58). This method has been shown to have an accuracy of >90% when classifying cells as type I or type II (30).

Electrophysiological recording. The perforated patch variant of the whole cell recording technique [nystatin (Sigma) as the perforating agent] was used to measure both the voltage-clamp current and the current components of type II lagena hair cells (18). For each experiment, 2.4 mg of nystatin was dissolved in 10 µl DMSO and then diluted 1:1,000 with internal solution. Borosilicate glass (7052, Garner Glass) was pulled on a Brown-Fleming horizontal puller, fire polished on a Narashige (MF-83) microforge, and coated with Sylgard (Dow Corning). The electrode filling solution contained (in mM) 95 KMeSO3, 45 KCl, 15 HEPES, and 3 glucose. Seal resistances were ≥5 GΩ. Perforation began within 2 min of seal formation; access resistance typically plateaued within 15 min of perforation and remained stable for between 30 and 60 min at which time it began to increase. Series resistance, estimated from the peak capacitance transient (acquired from a 10-mV depolarizing pulse from −80 mV) ranged between 8 and 20 MΩ before compensation. Low series resistances were obtained, despite using the perforated patch recording technique by increasing the pipette tip diameter to between 3 and 6 µm (electrode resistances between 0.5 and 1.5 MΩ), often approaching the diameter of the cell. By reducing suction and working only with the most viable cells, excellent seals could be obtained with these large electrodes. Increasing the membrane surface area exposed to nystatin allowed for lower resistances to be obtained. Cells were only used if the final series resistance created a maximal voltage error of <20 mV and the clamp speed did not limit our analysis of the activation kinetics. Data were leak and capacity subtracted offline from current responses to hyperpolarizing voltage steps of 2–5 mV from a holding potential of −80 mV, a range where no active conductances were observed.

An Axoclamp 2A (Axon Instruments) amplifier was used for all recordings. It was chosen because the bridge mode permitted accurate current-clamp measurements. The amplifier was coupled to a personal computer via an MII (Modular Instruments) interface and driven by the SPIKE software (Hilal Associates). Data were filtered at 3 kHz with an 8-pole Bessel filter and sampled at 10 kHz. Sinusoidal stimuli were produced by a Krohn-Hite (model 5920) function generator and delivered to the cell via the MII interface.

Drug applications. Gravity-driven perfusion pipettes were used for drug application. Pipettes (50 µm diameter) were placed above the cells so that normal external solution was being constantly perfused onto the cell. The drug-containing perfusion pipette was then manually moved over the cell, and perfusion was started. Ionic currents typically began to show effects of the drugs within 3 s. Both 4-aminopyridine (4-AP; 5 mM) and tetraethylammonium (TEA; 20 mM) were used. They were equimolar substituted for NaCl in the external solution. These concentrations were chosen because they are close to the concentrations previously used for avian semicircular canal hair cell studies (20). Although at these concentrations the drugs probably were not specific to particular classes of channel types, the results will demonstrate that they distinguished between the broad classes of rapidly activating, rapidly inactivating and slowly activating, slowly inactivating potassium conductances, just as they did in earlier studies of hair cells from semicircular canal (20).

Statistics and analysis. All statistical comparisons were performed using the SPSS software package (SPSS). Statistical comparisons using the Student’s t-tests were deemed significant if P < 0.01 and not significant if P > 0.05, unless otherwise stated. Tablecurve 2D (Jandel) was used for all curve fitting. The square of the correlation coefficient (coefficient of determination, r2), corrected for degrees of freedom, was calculated from least squares curve fits and indicates goodness of fit.

RESULTS

Electrical properties in voltage clamp. Examples of macroscopic currents from two cells representing the two extreme responses of type II lagena hair cells under voltage-clamp conditions are given in Fig. 1. The major component of the current in Fig. 1, A and C, is a rapidly activating, rapidly inactivating conductance, whereas the major component of the current from the cell on Fig. 1, B and D, is a slowly activating, slowly inactivating outward conductance. Most cells showed some combination of these two currents (Figs. 3 and 4). Although the macroscopic currents are probably composed of multiple underlying conductances [based on previous type I hair cell analysis of the currents in pigeon semicircular canal hair cells (20)], it is apparent from Fig. 1 that, at a macroscopic level, the major differences between these cells are in the rates of activation and inactivation. Although there appears to be a difference in the magnitude of the currents for the two representative cells in Fig. 1, generally no statistically significant difference existed between the two groups (see Table 1). This is probably due to the large intercell variability in current magnitudes within each sample.
Kinetics. The initial portion of the current responses following the capacitative artifact until the peak response at each voltage level (Fig. 1, C and D) was fitted by an exponential function

\[ Y = A + B(1 - e^{-\frac{t}{\tau}})^n \]  

where \( n \) was varied from 1 to 4 and the \( \tau \) was fitted. In the final analysis, \( n \) was chosen as 3, because it gave the best fitted values for \( A, B, \) and \( \tau \) (Fig. 1, C and D). This fit allowed us to measure the variations in activation rise time for the macroscopic currents. Cells were included in the analysis only if the series resistance was low enough that the clamp speed did not limit the measurement.

On the basis of activation time constants, cells were separated into two groups, “fast cells” and “slow cells.” Fast cells had activation time constants (\( \tau \) from Eq. 1) for the voltage step from \(-70 \) to \(-40 \) mV that were \(<10 \) ms (Fig. 2, A and B). However, because there was a broad distribution in the activation kinetics of the slow cells, this measurement alone was not precise enough to separate the cells (Fig. 2A). Therefore, the presence of a rapidly inactivating component, inactivation \( >20\% \) (ratio of peak current to steady-state current) for the voltage step from \(-70 \) to \(-10 \) mV at a point 100 ms into the step, was used as a second criterion. Fourteen of eighteen (78%) cells with activation time constants \(<10 \) ms also had rapid inactivation properties. Fourteen of fifteen (93%) cells with activation time constants \( >10 \) ms had slow or no inactivation over the time course measured.

It is clear from the above measurements that the activation kinetics vary with the inactivation properties. It is possible that the presence of inactivation artificially reduces the measured rise time of activation. To test this, a group of cells with a large inactivating component was fit with a double exponential equation, where the second exponential was used to account for inactivation. For five cells investigated, although there was a shift toward slowing the rise time, largely due to altering the peak of the activating component, the resulting measurements were still comparable to the measurements described above. The difference between activation \( \tau \) never exceeded \( \sim 15\% \), so for simplicity the original fitting paradigm was used for all cells.

Both fast and slow cells showed an exponential voltage dependence of the activation time constants (Fig. 2D). The mean time constants were statistically significantly different at each voltage point. The slow cells showed much greater variability in their activation time constants than did the fast cells. An exponential \( (Y = A + Be^{-\frac{t}{\tau}}) \) was fitted to the means for each group (Fig. 2D) and these were also statistically significantly different for the fast and slow cell samples (Table 1, parameter “voltage \( \tau \)”). The voltage \( \tau \) were \( 15 \pm 5 \) and \( 24 \pm 17 \) ms/mV for fast and slow cells, respectively. Thus, whereas the overall rise time was faster for the

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**Fig. 1.** Voltage-clamp responses from 2 cells that represent extreme examples of the 2 most commonly found macroscopic currents. Currents in A are examples from a cell whose largest component was a rapidly activating, rapidly inactivating outward conductance, whereas the largest component of currents recorded from the cell in B was a slowly activating, slowly inactivating outward conductance. Voltage stimuli were 10-mV incremental steps between \(-80 \) and \(+10 \) mV, from a holding potential of \(-70 \) mV. Examples of the fits to the activation time course for the voltage steps to \(-30 \) and \(0 \) mV for the fast response and \(-40 \) and \(0 \) mV for the slow cell response are given in C and D (solid lines). A simple exponential equation (Eq. 1, with \( n = 3 \)) was used to generate the curve fits. Time constants from the fits were \( 0.4 \) and \( 0.93 \) ms for the steps to \(-30 \) and \(0 \) mV and \( 38 \) and \( 18 \) ms for the steps to \(-40 \) and \(0 \) mV for the slow cell.
fast cells, the voltage dependence of the rise time was greater for the slow cells.

Figure 3A presents an example of a cell with a comparable proportion of both the fast and slow conductances. The two components could be separated by altering the holding potential ($V_h$). At a $V_h$ of $-50$ mV, the current was predominantly slowly activating (Fig. 3C), whereas at a $V_h$ of $-90$ mV, the rapidly activating component was unmasked (Fig. 3B). When the membrane potential was held at an intermediate value, a mixture of both conductances was revealed (Fig. 3A). Mixed cells were typically grouped with the fast cells on the basis of the above definitions.

Pharmacology. Fast and slow cells could also be distinguished pharmacologically. TEA blocked the major outward conductance in slow cells (Fig. 3E), whereas 4-AP blocked the major component of the fast cells (Fig. 4, A–C). Neither agent was capable of blocking all of the outward current in a given cell, suggesting that none of the cells had a single type of outward current. However, the different pharmacological profiles did correlate with the separation of cells on the basis of activation time constants, making these agents useful tools when characterizing the membrane voltage responses. This pharmacological profile was similar to that found in avian semicircular canal type II hair cells (20).

Boltzman functions were fitted to voltage-conductance ($V$-$G$) plots for the current blocked by 4-AP and the current blocked by TEA (Fig. 3F). The Boltzman functions were of the form

$$G/G_{max} = 1/(1 + e^{(V-V_0)/dV})$$  (2)

where $V_0$ is voltage of half-maximal current and $dV$ is the slope factor. The conductance ($G$) was estimated by using the potassium reversal potential as that of the channels. The best fitted values for the 4-AP-sensitive component were $-35$ mV ($V_0$) and $7.4$ (dV) and for the TEA-sensitive components, $11$ and $16$, respectively. From these parameters and the graphs in Fig. 3F, it can be seen that the fast conductance activates at more negative potentials.

An inactivation time constant for the macroscopic currents of the TEA-insensitive and 4-AP-sensitive currents was measured by fitting the time from the peak current to the steady-state current level with an exponential function. The time constant varied between 20 and 50 ms ($n = 9$). In contrast to the activation kinetics, no voltage dependence of the inactivating time constant was found (data not shown). These results are comparable to previously characterized inactivation properties (20, 25). No further characterizations of inactivation were performed due to the difficulty of isolating this conductance from the hair cell’s complement of conductances.

Steady-state properties. Fast and slow cells were compared in terms of their steady-state properties as
well as the pharmacology and kinetics of activation. Table 1 summarizes some of these comparisons. Values for the Boltzmann fits for the fast and slow cells are given in Table 1. Fast cells activate at a more negative potential and have a more negative voltage of half-maximal conductance than do the slow cells. The values are less different than those for the pharmacologically isolated conductances (Fig. 3) presumably due to the presence of both fast and slow components in each cell type tending to blur the differences.

Current clamp. Examples of membrane voltage responses of the fast and slow cells to a series of current pulse injections are given in Fig. 5. Both types of cells show passive responses to hyperpolarizing current injections. Both cells show low-frequency dampened oscillations at the onset of the stimulus. The frequency of oscillation increased with the magnitude of the current pulse injection. For comparable magnitudes of current injections, fast cells showed a higher frequency of oscillation (compare Fig. 5C with D and E with F). After the oscillations, the slow cells remain stable at a constant voltage (Fig. 5B), whereas the fast cells (Fig. 5A) continue to depolarize for the duration of the current pulse injection. A summary of some measures of the current-clamp response is given in Table 1. No statistically significant difference was found in zero-current potential, membrane time constant, or input resistance between the fast and slow cell types (Table 1). The initial portion of the voltage response was fitted by the electrical resonance equation, initially described by 10.220.33.2 on October 14, 2017.
as the solution to an inductance circuit in turtle auditory papilla (8) and adapted to pigeon semicircular canal hair cells (7). Figure 5, C-F, presents examples of the initial portion of a fast and slow cell response to 20- and 80-pA current injections. The solid lines in Fig. 5 represent fits using the equation

\[ V(t) = \left[ V_{pp} e^{-\frac{t}{\tau}} \right] \sin(2\pi ft + \phi) + V_s \]

where \( V_s \) is the steady-state plateau voltage, \( V_{pp} \) is the peak voltage above the plateau, \( \tau \) is the time constant of the envelope decay, \( f \) is the frequency of the oscillation, and \( \phi \) is the sinusoidal phase angle. The calculated values of \( f \) and \( \tau \) were then substituted into the equation

\[ Q_c = \sqrt{\frac{1}{\tau^2}} \]

(4)
to estimate the quality factor of the resonance (8). The \( Q_c \) and the \( r^2 \) are given with each trace. The slow cells were consistently fit better by Eq. 3 than were the fast cells.

For slow cells \( Q_c \) increased with frequency as predicted by Eq. 4 (Fig. 6A). Surprisingly, the \( Q_c \) remained relatively constant for the fast cells as \( f \) increased. A plot of \( f \) against \( Q_c \) (Fig. 6A) shows this linear relationship for slow cells and lack of relationship for the fast cells. Additionally, the \( Q_c \) was lower for the high-frequency responses than would be predicted, suggesting that some factor was suppressing \( Q_c \) in fast cells, possibly inactivation. In an attempt to address this

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Table 1. A summary of the voltage-clamp and current-clamp measurements made on lagena type II hair cells

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Fast Cells</th>
<th>Slow Cells</th>
<th>Student's t-Test, P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells</td>
<td>22</td>
<td>17</td>
<td>0.37</td>
</tr>
<tr>
<td>Zero-current potential, mV</td>
<td>(-57 \pm 10)</td>
<td>(-54 \pm 7)</td>
<td>0.37</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>1,301 \pm 948</td>
<td>758 \pm 778</td>
<td>0.114</td>
</tr>
<tr>
<td>Membrane time constant, ms</td>
<td>21 \pm 13</td>
<td>16 \pm 8</td>
<td>0.201</td>
</tr>
<tr>
<td>Peak conductance, nS*</td>
<td>26 \pm 26</td>
<td>45 \pm 48</td>
<td>0.2</td>
</tr>
<tr>
<td>Steady-state conductance, nS*</td>
<td>14 \pm 15</td>
<td>44 \pm 49</td>
<td>0.025</td>
</tr>
<tr>
<td>Activation threshold*</td>
<td>(-51.5 \pm 11)</td>
<td>(-34 \pm 12)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Voltage of half-maximal conductance, mV*</td>
<td>(-35 \pm 10)</td>
<td>(-5 \pm 10)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Sensitivity, ( dv^* )</td>
<td>7.4</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>Voltage ( \tau ), ms/mV*</td>
<td>15 \pm 5</td>
<td>24 \pm 17</td>
<td>0.006</td>
</tr>
<tr>
<td>Frequency, Hz</td>
<td>56 \pm 22</td>
<td>23 \pm 17</td>
<td>0.002</td>
</tr>
<tr>
<td>Quality factor</td>
<td>0.98 \pm 0.45</td>
<td>0.76 \pm 0.48</td>
<td>0.497</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Measurements made in voltage-clamp. P values are taken from Student’s t-tests between 2 groups of cells. Voltage \( \tau \) is a measure of the voltage dependence of the activation time constant and is obtained from the exponential fit to the time constants vs. voltage step plots, as shown in Fig. 2D. \( dv \), slope factor.

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Fig. 4. A: currents elicited from a fast cell in response to 10-mV incremental voltage steps between \(-100\) and \(10\) mV; \( V_h = -90\) mV; 4-AP (5 mM) blocks fast component, leaving slow component (B) unaffected. C: difference \( (A - B) \) reveals fast component blocked by 4-AP. D: voltage response to a 40-pA current injection given in the presence and absence of 5 mM 4-AP. Hair cell zero current potential \( (V_z) \) was depolarized by 20 mV, and the frequency of the oscillation was decreased in the presence of 4-AP. Fits are based on Eq. 3 and gave frequency values of 55 and 17 Hz for control and 4-AP-treated cells, respectively. Quality of resonance remained near 1 under both conditions.
possibility, current-clamp experiments were performed in the presence and absence of 4-AP. In two cells where the inactivating component was large, application of 4-AP resulted in a depolarization of the hair cells and a loss of oscillations. In two other cells that were of the mixed variety, 4-AP also depolarized the hair cells and significantly reduced the frequency of the oscillations. An example of this response is given in Fig. 4D. The calculated $Q_c$ values were virtually unchanged, but it is clear from the traces that the 4-AP responses become more dampened. Because $Q_c$ is also dependent on the magnitude of the current, it is possible that the 4-AP response reflects a decrease in current amplitude and is not a manifestation of a reduction in the inactivating component. Plotting $f$ vs. $Q_c$ in Fig. 6A for control data and 4-AP treatment implies that the relationship shifted from that of the fast cells to that of the slow cells. With the present data it is not possible to conclude that the inactivating conductance is directly responsible for the reduced quality of the oscillations. However, it is clear

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**Fig. 5.** Current-clamp responses from a fast cell (A) and a slow cell (B). Stimuli used were 20-pA incremental steps between −30 and 100 pA. $V_z = −56$ for the slow cell and $V_z = −63$ mV for the fast cell. Both cells show a passive response to hyperpolarizing stimuli and a dampened oscillatory response to depolarizing stimuli. For both responses, frequency of the oscillations increased with stimulus current magnitude. Responses of the slow cell (B) depolarize to a constant level, whereas responses of the fast cell (A) continue to depolarize for duration of the pulse. Scale bars represent 20 mV and 100 ms. Expanded views of the initial oscillatory voltage response from a fast cell (C) and a slow cell (D) for 20 (C, D) and 80 pA (E, F) current injections are shown. Solid lines through data points are from fits using the resonance equation (Eq. 3) described in RESULTS. Coefficient of determination ($r^2$) as well as the resonant frequency ($f$) and quality factor ($Q_c$) are given with each trace. Slow cells consistently showed a better fit to the resonance equation than fast cells.

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**Fig. 6.** Plots correlating data from voltage and current-clamp measurements. A: plot of $f$ (Eq. 3) against $Q_c$ (Eq. 4) shows a linear relationship for slow cells (●); however, fast cells show no clear relationship (○). ▲, Control data for cell in Fig. 4; ●, measurements from the same cell in the presence of 4-AP. Application of 4-AP appears to switch cell between fast and slow groups. B: exponential plot of activation time constant measured in voltage clamp against $f$ measured in current clamp for a 30-pA current stimulus. Time constant was calculated from the exponential fit described by Eq. 1. ■, Slow cells; ○, fast cells.
from the plot of Fig. 6 that the hair cells that show a significant inactivating component have a reduced Qc.

The activation time constant, measured in voltage clamp (Figs. 1, C and D, and 2D), plotted against f, and measured in current clamp, shows an exponential relationship (Fig. 6B). In this plot, the activation time constant was taken from the exponential fit to the voltage vs. activation time constant plot (Fig. 2D) at the voltage of the steady-state membrane oscillation measured in current clamp. These data suggest that the whole cell macroscopic activation kinetics determine the frequency of the membrane oscillation. Data from the two cell types fall at different extremes along the plot, suggesting that the two major conductances serve similar functions but over different frequency ranges and that the majority of mixed cells creates a continuum of frequency responses.

The correlation between activation kinetics and oscillation frequency was further supported pharmacologically. Application of 4-AP to a fast cell caused membrane depolarization and a decrease in the oscillation frequency of the hair cell from 55 to 17 Hz (Fig. 4D). These results suggest that the 4-AP-sensitive conductance was important in establishing the cells’ resting potential as well as in determining the f of the hair cell. The mean values of f for lagena hair cells are similar to those found in type II hair cells from the pigeon’s semicircular canal (7). One difficulty in the past with the interpretation of resonance being physiologically significant for the semicircular canals was that the mean frequencies reported were higher than those usually attributed to head motion. This is especially true because data were acquired at room temperature, suggesting that the frequencies would be even higher at physiological body temperatures (40°C for pigeon). However, the membrane potential oscillation frequencies observed must represent frequencies passed by the filtering properties of the hair cells, otherwise they would not be observed. These filtering properties are determined by the active conductances and passive membrane properties of the hair cells, which differ among hair cells. Therefore, the range of frequencies seen in different cells would represent frequencies passed by the hair cell filter, the higher frequency values representing an extended bandwidth. This would be particularly true in cells with a low Qc (a low-pass filter).

To test the above hypothesis, cells were injected with sinusoidal as well as pulse currents and the membrane voltages were recorded. Figure 7 summarizes the results. One example from a fast cell shows the response to a 30-pA current pulse (Fig. 7A) as well as the response to a series of 30-pA sinusoidal current injections (Fig. 7, B–E). Figure 7F presents the normalized peak-to-peak voltage response from six different hair cells plotted against the stimulus frequency. Cells were chosen that reflect different oscillation frequencies and different activation kinetics. In each case, the only difference between the cell responses is the cutoff frequency of the frequency response function. The slope of each cell’s roll off on a log-log plot was near −1, suggesting a first-order transfer function. Furthermore, a plot of the break frequency, the frequency at which the two linear portions of the frequency response plot (Fig. 7G) cross (23), against the oscillation frequency measured from pulse injections, shows a linear relationship with a slope of 1. This suggests that the oscillation frequency seen with pulse injections represents the upper limit of the flat frequency response function of a low-pass filter. Cells with the faster activation kinetics have an extended bandwidth.

**DISCUSSION**

The presence of otoconia, type I hair cells (30, 38), and projections to the vestibular nuclei (4) suggest that the lagena is primarily a vestibular end organ in pigeon. The electrophysiological properties of the type I hair cells in the lagena are similar to those found in other vestibular end organs, again supporting a vestibular function (31). Data presented here demonstrate that the electrical properties of lagena type II hair cells are also very similar to type II hair cells reported in other vestibular end organs such as pigeon semicircular canal (7, 20) and pigeon utricle (T. X. Weng and M. J. Correia, unpublished observations) and guinea pig semicircular canal (15, 27). Together these data suggest a vestibular function for the avian lagena. The similarity of the electrical response properties of type II hair cells across functionally distinct vestibular end organs suggests that the electrical properties serve similar functions in each of the receptors.

What then determines the different transfer functions noted for primary afferent discharge from the otolith organs and semicircular canals? Natural candidates include the mechanical properties of the otolithic membrane and the cupula as well as hair bundle mechanical properties and the coupling of the hair bundles to the overlying membranes (2). It has recently been suggested that the adaptation process of the mechanoelectric transducer contributes a high-pass filter to hair cell tuning properties and that the filtering characteristics are calcium dependent (29). Differential distributions of calcium binding proteins in hair bundles of vestibular cells may serve to vary the properties of this high-pass filter and offer an additional mechanism for specializing hair cell response properties (3). Differences in hair cell synaptic properties and the transfer characteristics of the primary afferents may also play a role (16).

Our voltage-clamp data suggest that in lagena (and possibly all vestibular) type II hair cells, the activation kinetics of the macroscopic currents vary to produce a continuum in the frequency response of these cells. At one end of the continuum are cells whose macroscopic outward current activates rapidly and typically inactivates rapidly. At the other end of the continuum are cells whose macroscopic outward current activates slowly and shows little inactivation. Most of the recorded cells fell somewhere in between these two extremes. This separation was also supported by the pharmacological data.
For cells with a limited inactivating conductance, the quality of resonance varied with frequency (Fig. 6A). As the relative proportion of the inactivating component increased, the quality of the voltage oscillation became virtually independent of frequency and remained constant, near one (Fig. 6A). It is possible that the role of inactivation is to limit the quality of resonance at higher frequencies. This property would create a system of cells that could pass a broad range of frequencies with comparable gains. The bandwidth would depend on the resting membrane potential. It would also suggest that both the frequency range and the quality would be highly dependent on membrane potential. Because the resting potentials of vestibular hair cells are at the steep portion of the inactivation Boltzman function (20, 24, 25), slight changes in membrane potential could significantly increase or decrease the proportion of inactivating conductance available. In turn, the amount of inactivation would extend (during hyperpolarization) or limit (during depolarization) the frequency response range of the cell. By this scheme, vestibular hair cells could act as tunable variable filters. This concept has been proposed for semicircular canal primary afferent responses (26). Additionally, the idea of hair cells having different “operating modes” has been suggested for toadfish semicircular canal hair cells (33).

Similarly, an argument has been made for vestibular type I hair cells as tunable filters (28). The difference here is that the dominant current, $I_{K_1}$, in type I hair cells is ~50% activated at the resting membrane potential. Therefore the bandwidth of the frequency response of the type I hair cells is in general greater than for type II hair cells (28), and the gain is significantly reduced.

The resonance equation best fit cells with little or no inactivating conductance. Cells with inactivation really only fit the initial cycle and then the fit became poor. It is possible that resonance as an enhancement to the
frequency response is important only in a subset of hair cells and that the oscillations observed in cells with inactivation really indicate some other form of tuning. The extension of the frequency response relationship beyond what is passively possible might be one of these functions. Another possibility is that a portion of vestibular hair cells phase lock. That is, subsets of vestibular hair cells may encode different properties of a stimulus and it is only in the summation of hair cell output through complex afferent innervation patterns that afferent firing patterns are achieved. The reason for suggesting this comes from the similarity in the types of conductances and in the subthreshold membrane voltage oscillations found in cells of the central auditory pathways believed to be responsible for the preservation of acoustic timing information (see Ref. 37 for review). For example, cells of the medial nucleus of the trapezoid body have a low-threshold potassium conductance that is responsible for maintaining the temporal fidelity of synaptic transmission and a high-threshold delayed rectifier that facilitates high-frequency responses (6). Cells of the nucleus magnocellularis carry precise temporal information encoded as phase-locked activity (36). Subthreshold responses from these cells look remarkably similar to those reported here for vestibular hair cells and show similar pharmacological sensitivities (36, 40). Some vestibular hair cells have similar electrical properties as the cells described above, and it is possible that the conductances are serving similar purposes, i.e., creating a phase-locked response. Perhaps the complexities in the primary afferent responses are derived from the integration of hair cells encoding different aspects of a mechanical stimulus. Vestibular hair cells may be an excellent model for the study of these types of responses because they are presynaptic cells that are easily voltage clamped.

Whether the membrane oscillations seen in vestibular type II hair cells reflect the characteristic frequency for these cells or are the corner frequency of a low-pass filter remains to be determined. Until the responses to mechanical stimulation are investigated with reference to the electrical properties of the hair cell, the physiological relevance of the membrane oscillations will be questionable (11). It is important to note that, although electrical resonance has been described in a variety of hair cell end organs, only in the turtle auditory papilla has the characteristic frequency, measured by mechanical stimulation of the hair cell, been correlated with the electrical resonant frequency (8). In fact, some evidence exists that electrical resonance can be demonstrated, but the resonant frequency does not correlate with the characteristic frequency (11). Additionally, nonresonant hair cell responses have been reported (27). Other possible roles for the active conductances suggested herein would give a physiological significance to these nonresonant responses.

Our voltage-clamp data do not parcel out the specific number and types of conductances present in these hair cells. Presumably, there is a calcium conductance, some cells show inward rectification, and the nature and components of the slowly activating conductance have not been characterized. The large variability in the activation properties of the slowly activating conductance might suggest that there are multiple types of conductances present. Also, whether the slowly activating conductance is a delayed rectifier or a calcium-dependent conductance or a combination of both remains to be elucidated. The nature of the slowly activating conductance appears to be quite variable across species and between investigators (15, 19, 20, 27, 33–35). Perhaps all are correct and the differences lie in which cells are being dissociated. That is, like in the turtle papilla, cells contain multiple slowly activating conductances, some of which are calcium dependent and some of which are voltage dependent (14).

Our interpretation and conclusions in this paper do not hinge on the quantitative assessment of specific channel types but have focused on the general characteristics of the macroscopic conductances to determine the properties that vary the membrane voltage responses of lagena type II hair cells. The analysis has allowed us to draw substantive conclusions regarding the filtering properties of vestibular hair cells, the role of channel inactivation in altering the filtering properties of these hair cells, the functional significance of voltage oscillations, and the function of the avian lagena.

**Perspectives**

Over the past 15 years a great deal of effort has been made by a variety of investigators to understand the electrical filtering properties of vestibular hair cells, yet no unifying theory exists to explain the variety of response properties that have been described. The work presented here has attempted to use the naturally occurring variability in hair cell responses to identify the elements involved in shaping the hair cell’s response. Several ideas have been presented here that may help our understanding of vestibular hair cells. We have demonstrated that, as with type I hair cells, type II hair cells show remarkably similar electrical properties between functionally different end organs, suggesting that the mechanisms for specialization lie elsewhere. We have shown that a population of type II hair cells responds to current injections with dampened low-frequency resonance, where the quality of the resonance varies, as predicted, with the frequency. A second population of cells shows more dampened membrane oscillations where the quality and frequency do not covary. These cells were remarkably similar to phase-locking cells of the auditory brain stem nuclei and might represent a functionally distinct population. Fast lagena cells were characterized by having an inactivating conductance whose function might be to limit the quality of the membrane oscillations at higher frequencies. Understanding how these different populations of hair cells are distributed in the epithelium and also understanding how the primary afferents are coupled to these populations of cells will be an important direction for future investigations.

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