Regional Distribution of Ionic Currents and Membrane Voltage Responses of Type II Hair Cells in the Vestibular Neuroepithelium

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INTRODUCTION

In chick auditory papilla, there is a correlation between types of ionic currents and membrane voltage responses of type II hair cells in the vestibular neuroepithelium. J. Neurophysiol. 82: 2451–2461, 1999. Basolateral ionic currents and membrane voltage responses were studied in pigeon vestibular type II hair cells using a thin slice through either the semicircular canal (SCC) crista or utricular macular epithelium. Whole cell tight-seal patch-clamp recording techniques were used. Current-clamp and voltage-clamp studies were carried out on the same cell. One hundred ten cells were studied in the peripheral (Zone I) and central (Zone III) zones of the SCC crista, and 162 cells were studied in the striolar (S Zone) and extrastriolar (ES Zone) zones of the utricular macula. One of the major findings of this paper is that hair cells with fast activation kinetics of their outward currents are found primarily in one region of the SCC crista and utricular macula, whereas hair cells with slow activation kinetics are found in a different region. In Zone I of the crista, 95% of the cells have fast activation kinetics (“fast” cells) and in Zone III of the crista, 86% of the cells have slow activation kinetics (“slow” cells). In the utriclar macula slice, 100% of the cells from the S Zone are slow cells, whereas 86% of the cells from the ES Zones are fast cells. Oscillation frequency (f) and quality factor (Q) of the damped oscillations of the membrane potential during extrinsic current injections were studied in hair cells in the different regions. The slow cells in Zone III and in the S Zone have a statistically significantly lower f as a function of the amplitude of injected current, when compared with the fast cells in Zone I and the ES Zone. Although Q varied over a small range and was <2.6 for all cells tested, there was a statistically significant difference between Q for the membrane oscillations of the slow cells and fast cells in response to a range of current injections.

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**METHODS**

White king pigeons (Columbia livia) of either sex, weighing from 200 to 350 g and with ages ranging from 3 to 12 mo, provided the tissue used in the present experiments. All experimental procedures were conducted after approval by the institutional animal care and use committee and followed the guidelines set forth by the National Institutes of Health and the American Physiological Society.

**Slice preparation**

SCCs and utricles were harvested and dissected free of each other. All tissue was then incubated in Dulbecco’s modified Eagle’s medium (DMEM) augmented with 24 mM NaHCO	extsubscript{3}, 15 mM PIPES, 50 mg/l ascorbate, and 1.5% fetal calf serum. The DMEM and tissue were maintained at 37°C, pH 7.4, and an osmolarity of 320 mosmol/kg, in a saturated 95% O\textsubscript{2}-5% CO\textsubscript{2} environment. At varying intervals, tissue was removed from the incubator, embedded in 4% agar, and quickly washed (Chromerge, Fischer Scientific) and sterilized were pulled recordings were made. Glass capillaries (Garner Glass No. 7052 or Sylgard), whereas others were coated with a 5% silanizing solution consisting of dimethyldichlorosilane (Sigma, No. D-3879) in chloroform (EM Scientific No. CX 1055-6). No difference was noted in the size of the final compensated electrode capacitance artifact using the two methods. In some experiments, perforated-patch (PP) recordings were made. Electrodes were pulled and polished to a tip diameter of ~1 μm. The tips and shanks of some of the microelectrodes were covered with silicone elastomer (Sylgard), whereas others were coated with a 5% silanizing solution consisting of dimethyldichlorosilane (Sigma, No. D-3879) in chloroform (EM Scientific No. CX 1055-6). No difference was noted in the size of the final compensated electrode capacitance artifact using the two methods. In some experiments, perforated-patch (PP) recordings were made. Electrodes were pulled and polished to a tip diameter of ~1.5–3.0 μm. The electrodes were back-filled with a solution containing a perforating agent. The perforating agent (either Amphotericin B, Sigma No. A-4888 or Nystatin, Sigma No. N3503) was dissolved completely in dimethyl sulfoxide (DMSO). Five milligrams of the perforating agent was dissolved in 50 μl of DMSO. This stock solution was diluted to a final concentration of 250 μg/ml in a back filling solution [back fill solution (PP), Table 1]. Care was taken to ensure that the final concentration of DMSO was <0.25%. The tip of the electrode was filled with a tip filling solution [tip solution (PP), Table 1]. For ruptured-patch recordings the access resistance was between 2 and 6 MΩ and for perforated-patch recordings the access resistance was indicated.

**Electrical recordings**

Ionic current and voltage recordings were made using conventional tight-seal whole cell patch-clamp techniques (Hamill et al. 1981). Access to the cell interior was achieved either by rupturing the cell membrane or by the use of a membrane-perforating agent (Horn and Marty 1988). Recordings were obtained using an Axoclamp 2A (Axon Instruments) bridge amplifier in both the voltage-clamp and the current-clamp mode. The Axoclamp-2A bridge amplifier was used because it has been shown (Masetto et al. 1999) that patch-clamp amplifiers can introduce significant distortions in the measurement of hair cell membrane voltage oscillations. In the majority of the experiments, ruptured patch (RP) recordings were made. Glass capillaries (Garner Glass No. 7052 or World Precision Instruments No. 1B150F-3) that had been acid washed (Chromerge, Fischer Scientific) and sterilized were pulled and fire-polished to a tip diameter of ~1 μm. The tips and shanks of some of the microelectrodes were covered with silicone elastomer (Sylgard), whereas others were coated with a 5% silanizing solution consisting of dimethyldichlorosilane (Sigma, No. D-3879) in chloroform (EM Scientific No. CX 1055-6). No difference was noted in the size of the final compensated electrode capacitance artifact using the two methods. In some experiments, perforated-patch (PP) recordings were made. Electrodes were pulled and polished to a tip diameter of ~1.5–3.0 μm. The electrodes were back-filled with a solution containing a perforating agent. The perforating agent (either Amphotericin B, Sigma No. A-4888 or Nystatin, Sigma No. N3503) was dissolved completely in dimethyl sulfoxide (DMSO). Five milligrams of the perforating agent was dissolved in 50 μl of DMSO. This stock solution was diluted to a final concentration of 250 μg/ml in a back filling solution [back fill solution (PP), Table 1]. Care was taken to ensure that the final concentration of DMSO was <0.25%. The tip of the electrode was filled with a tip filling solution [tip solution (PP), Table 1]. For ruptured-patch recordings the access resistance was between 2 and 6 MΩ and for perforated-patch recordings the access resistance was indicated.

**FIG. 1.** A: top view of a utricle that has been isolated and whose roof has been removed. White arrows point at the striolar (S) Zone. Lateral (L) and medial (M) to the striola are the extrastriolar regions. The long axis of the utricle is in an anterior (A)–posterior (P) direction. B: slice (180 μm thick) through the center of the utricle in a plane perpendicular to the long axis. The otocyst layer (black covering) is curved and thinned over the bulging otocystic membrane in the S Zone. Inset: section of the striolar epithelium in which it is apparent that the tallest stereocilia of 2 hair cells (pointed to by black arrows) oppose each other. C: sketch of the utricle indicating the S Zone and each of the extrastriolar (ES) subzones. In this and Fig. 2C the number of cells from which recordings were obtained in each subzone is indicated.

**TABLE 1. Composition of solutions**

<table>
<thead>
<tr>
<th>Solution</th>
<th>NaCl</th>
<th>KCl</th>
<th>CaCl\textsubscript{2}</th>
<th>MgSO\textsubscript{4}</th>
<th>MgCl\textsubscript{2}</th>
<th>HEPES</th>
<th>Glucose</th>
<th>Other</th>
<th>pH</th>
<th>Osmolality, mosmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular</td>
<td>145</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>15</td>
<td>10</td>
<td>Ascorbate (50 mg/L)</td>
<td>7.4</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Vibratome bath</td>
<td>145</td>
<td>3</td>
<td>0.1</td>
<td>7.5</td>
<td>15</td>
<td>10</td>
<td>As above</td>
<td>7.4</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Intracellular (ruptured patch)</td>
<td>140</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>11 EGTA</td>
<td>7.4</td>
<td>315</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tip Solution (perforated patch)</td>
<td>140</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>11 EGTA</td>
<td>7.4</td>
<td>315</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Backfill solution (perforated patch)</td>
<td>45</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>3</td>
<td>95 KmeSO\textsubscript{4}</td>
<td>315</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are in mM except for Osmolality. HEPES, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid.

A preliminary report of this work has appeared in abstract form (Weng and Correia 1998).
varied between 6 and 20 MΩ. The electrode junction potential and electrode capacitance were compensated using the amplifier’s analogue circuitry. No attempt was made to compensate for series resistance. However, the final series resistance produced a maximal voltage error of <10 mV, and the clamp speed did not limit the analysis of the activation kinetics.

**Data acquisition**

Stimuli were generated and signals were sampled using AD/DA converters (DigitData 1200, Axon Instruments) that were controlled by a PC running data acquisition software (Clampex 6.3, Axon Instruments). The bandwidth of the amplifier’s filter was set at 3 or 10 kHz depending on the experimental protocol. The digital sampling frequency was two to five times the analogue bandwidth of the recorded signal.

**Data analysis**

**REGIONS STUDIED.** The long axes of the vertical SCC cristae were partitioned into three zones, named as before (Masetto and Correia 1997a) and illustrated in Fig. 2, B and C. Zone I, the peripheral region, is indicated by PS, planum semilunatum. The crista is symmetrical about its apex. The zones of ½ of the crista are indicated by arrowheads. PS, planum semilunatum. C, sketch of the vertical SCC crista indicating widths of the zones and the subzones.

**TABLE 2. Summary of mean parameters from detailed analysis of 10 hair cells from each zone of the SCCs and utricle**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Zone I</th>
<th>Zone III</th>
<th>ES Zone</th>
<th>S Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>$V_e$, mV</td>
<td>$-63.58 \pm 8.44$</td>
<td>$-67.39 \pm 8.36$</td>
<td>$-58.90 \pm 8.32$</td>
<td>$-66.25 \pm 8.36$</td>
</tr>
</tbody>
</table>

**Voltage clamp (10 cells, voltage step from −60 to 0 mV)**

<table>
<thead>
<tr>
<th>Active properties</th>
<th>Voltage clamp (10 cells, voltage step from −60 to 0 mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>10</td>
</tr>
<tr>
<td>$f_e$, Hz</td>
<td>$95.33 \pm 9.58^\ast$</td>
</tr>
<tr>
<td>$\tau_o$, mS</td>
<td>$7.28 \pm 0.99$</td>
</tr>
<tr>
<td>$Q$</td>
<td>$2.18 \pm 0.23^\ast$</td>
</tr>
<tr>
<td>$V_{p}$, mV</td>
<td>$14.15 \pm 3.80$</td>
</tr>
<tr>
<td>$V_{e}$, mV</td>
<td>$-46.09 \pm 7.21$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Passive properties</th>
<th>Voltage clamp (10 cells, voltage step from −60 to 0 mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>10</td>
</tr>
<tr>
<td>$R_{in}$, GΩ</td>
<td>$1.49 \pm 0.45^\ast$</td>
</tr>
<tr>
<td>$C_{sw}$, pF</td>
<td>$17.32 \pm 4.93$</td>
</tr>
<tr>
<td>$\tau_{in}$, mS</td>
<td>$26.41 \pm 11.78^\ast$</td>
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**Current clamp (10 cells, 20 pA < $I$ < 120 pA and $V$ less than ±20 mV)**

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<thead>
<tr>
<th>Active properties</th>
<th>Current clamp (10 cells, 20 pA &lt; $I$ &lt; 120 pA and $V$ less than ±20 mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>10</td>
</tr>
<tr>
<td>$f_e$, Hz</td>
<td>$64.54 \pm 16.59^\ast$</td>
</tr>
<tr>
<td>$\tau_o$, mS</td>
<td>$9.6 \pm 2.63$</td>
</tr>
<tr>
<td>$Q$</td>
<td>$2.02 \pm 0.69^\ast$</td>
</tr>
<tr>
<td>$V_{p}$, mV</td>
<td>$9.21 \pm 4.97$</td>
</tr>
<tr>
<td>$V_{e}$, mV</td>
<td>$-51.43 \pm 7.49$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Passive properties</th>
<th>Current clamp (10 cells, 20 pA current injection)</th>
</tr>
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<tbody>
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Values are means ± SD; $n$ is number of cells. SCCs, semicircular canals; ES, extrastriolar; S, striolar. Statistical comparisons (t-tests) were made between mean parameters from cells in Zone I vs. Zone III and in the S Zone vs. the ES Zone. $^\ast P < 0.001$. $^\dagger P < 0.01$. $^\ddagger P < 0.05$. 

FIG. 2. A: slice through the horizontal semicircular canal (SCC) ampulla in a plane parallel to the long axis of the crista. Zones I–III are demarcated by the arrowheads in this hemicrista. B: section through the ampulla of a vertical SCC. The plane of the section is through the long axis of the crista. The crista is symmetrical about its apex. The zones of ½ of the crista are indicated by arrowheads. PS, planum semilunatum. C, sketch of the vertical SCC crista indicating widths of the zones and the subzones.
These cells met three criteria: 1) their zero current potential ($V_z$) was more negative than $-40$ mV, 2) they produced recordings that lasted at least 10 min, and 3) they produced both voltage-clamp and current-clamp recordings. The distribution of the number of cells by zone was: 39 hair cells from Zone I; 71 hair cells from Zone III; 116 hair cells from the ES Zone and 46 from the S Zone. The number of cells studied in each subzone is indicated in Figs. 1C and 2C.

The total sample of cells was further sorted into fast and slow cells by comparing the time-to-peak ($T_p$) of the net current (Lang and Correia 1989). To study the properties of the ionic currents in hair cells residing in different zones of the SCCs (Zone I and Zone III) and the utricle (S Zone and ES Zone), 10 cells from each zone were randomly selected from the total sample as representative and analyzed as follows.

**PARAMETERS CHARACTERIZING MEMBRANE VOLTAGE AND CURRENT RESPONSE.** Input impedance ($R_{in}$), input capacitance ($C_{in}$), and membrane time constant ($r_{mem}$) were calculated from the measurement of the voltage ($V_{m}$) response to a $-20$-pA current pulse (250 ms long) from $V_z$ (see Table 2). $R_{in}$ was determined from the solution of the equation $R_{in} = V_{ss}/I (20 \text{ pA})$ where $V_{ss}$ is steady-state voltage measured just before the off time of the pulse. A single exponential function $[V_{ss} + V_{exp} \exp(-t/\tau_{e})]$ was fitted to the charging portion of the voltage response. The input capacitance, $C_{in}$, was calculated from the equation $C_{in} = r_{mem}/R_{in}$. A model cell with a 33-pF capacitor ($C_{in}$) in parallel with a 0.5-Ω resistor ($R_{in}$) and in series with a 10 Ω resistor (to simulate the resistance of the patch electrode) was tested using the above method. The calculated value of $C_{in}$ was in error by <6%.

The activation time constant ($\tau_a$) of the outward net current was estimated by fitting Eq. 1 to the rising phase of the current trace in response to a voltage pulse. The voltage pulse was 200 ms wide and varied from $-60$ to $0$ mV.

$$I(t) = I_0 + I_s (1 - e^{-t/\tau_a})$$

where $I_0$ is the initial current, $I_s$ is the steady-state current, and $\tau_a$ is the activation time constant. The parameter $n$ was varied from 1 to 4, but $n = 3$ gave the best fits and therefore was used in the final analysis.

Other parameters were calculated to quantify the outward current. These included peak current ($I_{p}$), peak conductance ($g_p$), steady-state conductance ($g_{ss}$), the ratio of $g_p$ to $g_{ss}$, and the time-to-peak current ($T_p$).

The membrane voltage response to a current pulse (250 ms wide) was fitted with a sinusoidal function that decays to a plateau (Ricci and Correia 1999). The magnitude of the pulse varied from 20 to 120 pA.
RESULTS

Figure 3 presents histograms of log $T_p$ measured for 272 cells residing in either Zone I or Zone III of the SCC or the ES Zone or the S Zone of the utricle. Average values for $T_p$ are also presented. A statistically significant difference ($P < 0.001$, repeated measures ANOVA) existed between the values of $T_p$ for cells in the different zones. The values of $T_p$ were statistically significantly smaller for cells from the ES Zone ($t$-test, $P < 0.001$) and Zone I ($t$-test, $P < 0.001$) when compared with cells from Zone III and the S Zone. The median value of $T_p$ for all cells was 3.83 ms. This $T_p$ value separated cells into two groups, which we have designated as “fast” and “slow” cells. Thus the ES Zone of the utricle and Zone I of the SCC contained predominately fast cells, whereas the S Zone of the utricle and Zone III of the SCC contained mostly slow cells. There was no statistically significant difference between
values of \( T_p \) for the subzones of Zone III of the SCCs and the subzones of the ES Zone so the data were pooled.

A random sample of 10 cells from each zone was chosen for further analysis. Normalized outward current traces from these cells are presented in Fig. 4A (cells from the SCC) and in Fig. 4B (cells from the utricle). The current traces from cells of Zone III and the S Zone show slower activation, slower inactivation and delayed peaks. These characteristics of the current traces did not change with repeated testing. Ninety percent of the cells from Zone III and 100% of the cells from the S Zone were classified as slow (dotted lines) using the \( T_p \) criterion while 100% of the cells from Zone I and 90% of the cells from the ES Zone were classified as fast (solid lines).

Traces of outward currents, inward currents and membrane voltages from a fast cell from Zone I of the SCC and a slow cell from Zone III are shown in Figs. 5, A–F. Stimulus protocols used throughout the study for all cells are presented in the top panel of each column. The activation and inactivation of the net outward current is faster for the fast cell (Fig. 5A) as compared with the slow cell (Fig. 5B). Mean parameters quantifying these kinetic differences for the random sample of 10

<table>
<thead>
<tr>
<th>Type of Conductance</th>
<th>Zone I</th>
<th>Zone III</th>
<th>ES Zone</th>
<th>S Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outward</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast</td>
<td>37/39 (95)</td>
<td>10/71 (14)</td>
<td>94/116 (81)</td>
<td>0/46 (0)</td>
</tr>
<tr>
<td>Slow</td>
<td>2/39 (5)</td>
<td>61/71 (86)</td>
<td>22/116 (19)</td>
<td>46/46 (100)</td>
</tr>
<tr>
<td><strong>Inward</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRIK1</td>
<td>16/43 (37)</td>
<td>14/76 (18)</td>
<td>44/106 (41)</td>
<td>11/43 (26)</td>
</tr>
<tr>
<td>I</td>
<td>9/43 (21)</td>
<td>34/76 (45)</td>
<td>24/106 (23)</td>
<td>13/43 (30)</td>
</tr>
<tr>
<td>IRIK1 + I</td>
<td>4/43 (9)</td>
<td>13/76 (17)</td>
<td>20/106 (19)</td>
<td>7/43 (16)</td>
</tr>
<tr>
<td>None</td>
<td>14/43 (33)</td>
<td>15/76 (20)</td>
<td>18/106 (17)</td>
<td>12/43 (28)</td>
</tr>
</tbody>
</table>

Number in parentheses is percentage of cells. For abbreviations, see Table 2.

FIG. 6. Curve fits of current traces and oscillatory voltage responses for a fast cell from Zone I (A and B) and for a slow cell from Zone III (C and D). The equations fitted and best fit lines (dark solid lines) are shown in each graph. A \( \chi^2 \) estimate of goodness-of-fit is also shown. Outward currents (A and C) were produced by a 60 mV (−60 to 0 mV to −60 mV) voltage pulse. The starting points of the curve fits were \( t = 20 \) ms, the start of the voltage pulses. The voltage responses (B and D) were produced by an 80-pA current pulse injection. The starting points of the curve fits were \( t = 25 \) ms, the start of the current pulses.
cells, whose outward currents are presented in Fig. 4, are summarized in Table 2. The mean peak current \( I_p \) and mean peak chord conductance \( g_{p} \), are statistically significantly larger, whereas the mean steady-state conductance \( g_{ss} \), \( T_p \), and the mean activation time constant \( t_a \) are statistically significantly smaller for fast cells from Zone I and the ES Zone when compared with equivalent parameters for cells from Zone III and the S Zone.

Inwardly rectifying currents found in both fast and slow cells were of two types (Masetto and Correia 1997a): a fast inwardly rectifying current, IRIK1, (Fig. 5C) that relaxed at very hyperpolarized potentials (>120 mV) and a slowly activating inwardly rectifying current, \( I_h \), (Fig. 5D). In a given hair cell, IRIK1 or \( I_h \) could be present either singly or together. In some hair cells, no inwardly rectifying currents could be detected. Table 3 summarizes the percentages and distributions of cells with fast and slow outward currents, IRIK1 and \( I_h \) inwardly rectifying currents in Zones I and III of the SCCs and the S and ES Zones of the utricle. IRIK1 occurs singly 40% of the time in zones (Zone I and ES Zone) containing fast cells but only 21% of the time in zones (Zone III and the S Zone) containing primarily slow cells. The current \( I_h \) occurs singly in 22% of the cells in Zone I and the ES Zone and singly in 39% of the cells in the S Zone or Zone III. Thus IRIK1 occurs almost twice as often in fast cells and \( I_h \) occurs almost twice as often in slow cells (see Fig. 5, C and D, for examples).

Figure 5, E and F, illustrates membrane voltage responses for fast and slow cells, respectively. The voltage responses for fast and slow cells depended on the composition of the ensemble of outward and inward conductances. In Fig. 5E, it can be seen that the inactivation of the fast outward conductance produced a continuous voltage depolarization during the step at higher levels of current injection. The slow conductance produced a plateau of voltages during the duration of the pulse (Fig. 5F). The hyperpolarized response for the fast cell, which contained IRIK1, had a more rapid activation, and the voltage plateaued during the pulse duration. The hyperpolarized response of the slow cell containing \( I_h \) (Fig. 5F) activated more
slowly and repolarized during the duration of the pulse. The onset of repolarization was keyed to the activation of $I_h$. Membrane depolarization and repolarization following pulse onset occur faster in the fast cell (Fig. 5E) than in the slow cell (Fig. 5F). This difference in membrane oscillation frequency was quantified by curve-fitting Eq. 2 to membrane voltage responses to extrinsic current injections (Figs. 6, B and D) and then by comparing the mean best fitted parameters (presented in Table 2). For current injections ranging from 20 to 120 pA, mean frequency of oscillation, $f$, and the quality of resonance, $Q$ were statistically significantly greater for fast cells from Zone I and the ES Zone when compared with slow cells from Zone III and the S Zone.

Current-voltage ($I-V$) plots for mean $I_h$ and mean $I_o$ from 10 hair cells in each of the two zones of the SCCs and each of the two zones of the utricle are shown in Fig. 7, A and C, respectively. Comparable voltage-current ($V-I$) plots are shown in Fig. 7, B and D. The data points in the $I-V$ plots were not corrected for cell size because it can be seen from Table 2 that there was no statistically significant difference between values of $C_m$ for cells in different zones.

The $V-I$ plots in Fig. 7, B and D, indicate that the steepest change in membrane potential occurs around rest over the range of current injection from $-40$ to $+20$ pA. Rectification begins at $+20$ pA for cells from all zones but less so for the peak voltage from cells of the S Zone of the utricle. The slopes of linear curve-fits over the range $-40$ to $+20$ pA were about the same order of magnitude ($\sim 1$ mV/pA or $1 \text{ G} \Omega$). However, the slow cells from Zone III and the S Zone were the least sensitive, i.e., had the smallest slopes (see values in the legend of Fig. 7). Although the peak membrane voltage response was not statistically significantly different for cells from Zone I and Zone III, it was statistically significantly different for cells from the S Zone and the ES Zone ($P < 0.01$, repeated measures ANOVA). The opposite was true for the steady-state response. Lack of rectification of the peak voltage response contributed to the difference between cells from the S Zone and the ES Zone. Rectification of the steady-state response for the cells from Zone III contributed to the difference between cells from Zone I and Zone III.

Figure 8 is a plot of the activation time constants, $\tau_a$, for cells in each zone over the membrane potential range from $-30$ to $40$ mV. Generally, the values of $\tau_a$ declined to an asymptote as the membrane potential increased. The time constant of the decay, $\tau'_a$, was determined by fitting the equation for a single exponential shown in the figure. The best-fitted parameters of the equation for each zone are presented in the figure legend. The activation time constants for cells in the S Zone (100% slow) are statistically significantly slower than those for other zones and are more than 3 times those of the other zones at $-30$ mV and more than 1.75 times at $40$ mV. Moreover, the time constant of decay value, $\tau'_a$, for cells in the S Zone is nearly 1.5 times that of the cells in the ES Zone and 2–3 times that of cells in the 2 zones of the SCCs. Thus the hair cells in the S Zone have long activation time constants that remain long over a large voltage range.

Figure 9, A–D, graphically summarizes, for cells in each zone, the membrane oscillation responses to extrinsic current injections. Figure 9A shows that the oscillation frequency, $f$, increases toward an asymptote as the magnitude of current injection increases. The time constants are faster for fast cells in Zone I and in the ES Zone. Furthermore, the ranges of oscillation frequencies are higher for the cells of the ES Zone and Zone I. The mean frequencies at each current level are statistically significantly different for cells from Zone I compared with Zone III ($P < 0.01$, repeated measures ANOVA) and for cells from the S Zone compared with cells from the ES Zone ($P < 0.001$, repeated measures ANOVA). Except for the lowest current injection, there is an ordering of the values of oscillation frequencies for a given level of current injection. The S Zone shows the lowest $f$, Zone III the next lowest, Zone I the next lowest, and the ES Zone has the highest $f$ value. For the lowest current injection (20 pA), the oscillation frequencies range from $-30$ for the cells from the S Zone to $-65$ for cells from Zone I and the ES Zone. As pointed out previously (Correia et al. 1989), however, these values could be underestimates by as much as 2 octaves because the recordings were made at $20^\circ\text{C}$ below the pigeon’s usual body temperature ($40^\circ\text{C}$). Figure 9B is a plot of the time constant of damping, $\tau_Q$, of the membrane potential oscillations as a function of the current injection magnitude. The damping time constant, $\tau_Q$, declines to an asymptote as the magnitude of current injection increases. At each level of current injection the $\tau_Q$s are not clearly separated. The parameters of the best-fitted exponential functions are given in the figure legend.

Equation 3 implies that the quality of resonance, $Q$, is a function of the frequency of oscillation, $f$, and the oscillation damping time constant, $\tau_Q$. As in auditory hair cells, the slow cells found in Zone III of the SCCs and the S Zone of the utricle demonstrated a statistically significant negative correlation between $Q$ and $f$ (Fig. 9, C and D). But, like fast cells in the pigeon lagena (Ricci and Correia 1999), fast cells in Zone I of the SCC and the ES Zone of the utricle express currents with $Q$ values that are constant about a level of $Q \approx 2.2$, particularly when $f > 100$ Hz. A statistically significant differ-
ence exists between the mean values of $Q$ for cells from Zone I compared with cells from Zone III and between the mean values of $Q$ for cells from the ES Zone compared with cells from the S Zone except for the two highest frequencies. These results suggest that the quality of resonance is “clamped” to a constant value at higher frequencies for fast vestibular hair cells in the SCC and utricle as in the lagena (Ricci and Correia 1999).

**DISCUSSION**

One of the major findings of this paper is that hair cells with outward currents that have fast activation kinetics ($T_p$, $t_a$, Table 2) are found primarily in one region of the SCCs and the utricle, whereas hair cells with slow activation kinetics are found in a different region. Cells with fast activation kinetics (fast cells) are found in the peripheral zone (Zone I) of the SCC and the extra-striolar zones of the utricle. Cells with slow
activation kinetics (slow cells) are found in the central zone (Zone III) of the SCC and the striolar zone (S Zone) of the utricle. This result extends previous observations (Ricci and Correia 1999) that fast and slow cells are present in the pigeon’s vestibular neuroepithelium and that they are regionally distributed in the pigeon SCC cristae (Masetto and Correia 1997a). A regional distribution of cells, with different mixtures of ionic currents, has also been noted previously in frog crista (Masetto et al. 1994). Also, inwardly rectifying currents in vestibular type II hair cells are regionally distributed and paired with fast and slow cells (Table 3). IRIK1 occurs 2 times more frequently in Zone II (predominantly populated by fast hair cells) than in the hair cells in Zone III (predominantly populated by slow hair cells). I$_h$ was found in the cells of Zone III 2 times more frequently in the slow cells of Zone III than in the fast cells of Zone I. Similar, but less pronounced, differential distributions were found in the utricle. IRIK1 was found 1.5 times more often in the hair cells of the ES Zone than in the hair cells of the S Zone. I$_h$ was found 1.3 times more often in cells in the S Zone than the ES Zone (Table 3). Thus fast cells are more likely to have the inward rectifier current I$_h$, and the slow cells are more likely to have the inward rectifier current I$_h$.

Striolar cells were strikingly different from cells in the other regions, particularly those from the ES Zone. First, the activation time constant, $\tau_a$, for striolar cells was statistically significantly longer and decayed slower as a function of membrane potential than for cells in the other regions (Fig. 8). For example, the value of $\tau_a$ for striolar cells in response to a step from $-60$ to $0$ mV was on average 5 times that of cells from the ES region (Table 2). Second, the time constants of inactivation were longer for cells from the striolar region. This fact is reflected by the statistic $g_{in}/g_{on}$, which for striolar cells was approximately one-third that of cells from the ES Zone (Table 2). These comparisons suggest that the fast activating–fast inactivating current found in pigeon vestibular hair cells [presumably an A-type K$^+$ current (Lang and Correia 1989)] is less prominent in striolar cells. Finally, striolar cells and cells from Zone III of the SCC (slow cells) showed oscillations to small current injections that were lower in frequency than those from fast cells from Zone I and the ES Zone. Ricci and Correia (1999) have recently suggested that the low-quality oscillations noted in some dissociated lagenar hair cells during extrinsic sinusoidal and pulse current injections are not resonant frequencies but are the cutoff frequencies of the low-pass membrane filter. When the sinusoidal frequency response cutoff frequency was regressed against the oscillation frequency resulting from pulse stimulated, a straight line with a slope of one was obtained. The data in Figs. 9 and 10 of the present study lend further support to this notion. In Fig. 10, the mean admittance magnitude for 10 striolar cells begins to decrease near 50 Hz. This value corresponds to an interpolated value of 50 Hz resulting from analysis of membrane voltage oscillations in response to pulse current injections into 10 striolar cells (Fig. 9A). It is possible that hair cells in different regions of the SCC and utricle neuroepithelia may act as low-pass filters with different corner frequencies, which may be tuned at a given membrane potential by the mixture of activated ionic currents. These different filtering properties may result from differential topographical gene expression, because following complete loss of hair cells due to streptomycin ototoxicity, new hair cells with the same mixtures of ionic currents repopulate the same regions of the neuroepithelium as their predecessors (Masetto and Correia 1997a,b).

There is evidence for systematic regional variation in the response properties of both SCC and otolith organ afferents (Boyle et al. 1991; Goldberg 1991; O’Leary and Dunn 1976). Also, discrete groups of vestibular efferents project to different regions of the epithelia of the otolith organs and SCCs (Purcell and Perachio 1997). The results of the present study suggest that the different filtering properties of hair cells in different regions of the utricle and SCC must also be considered also in the interpretation of responses from the vestibular periphery.