Coding of Sound Intensity in the Chick Cochlear Nerve

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Received 21 May 2002; accepted in final form 19 August 2002


Tuning curves, spontaneous activity, and rate-intensity (RI) functions were obtained from units in the chick cochlear nerve. The characteristic frequency (CF) was determined from each tuning curve. The shape of each RI function was subjectively evaluated and assigned to one of four RI types. The breakpoint, discharge rate at the highest SPLs, and slopes of the primary and secondary segments were quantified for each function. The CF and RI type were then related to these variables. A new RI function was observed in which the discharge activity in the secondary segment diminished as stimulus level increased above the breakpoint. This function was called a “sloping-down” type. In 959 units, saturating, sloping-up, sloping-down, and straight RI types were identified in 39.2, 35.5, 12.6, and 12.7% of the sample, respectively. The slope of the primary segment was nearly the same in each of the four types and averaged 5.48 S · s⁻¹ · dB⁻¹ across all units. The slopes of the secondary segments formed four groupings when segregated by RI type based on the subjective assignments and averaged 0.03, 1.22, −0.90, and 3.95 S · s⁻¹ · dB⁻¹ in the saturating, sloping-up, sloping-down, and straight types, respectively. The data describing the secondary segments of all units were fit with a multi-compartment polynomial and showed a continuous distribution that segregated, with some overlap, into the different RI categories. The proportion of RI types, as well as the secondary and primary slopes were approximately constant across CFs. In addition, it would appear that the other parameters that define the four types were, for the most part, homogeneously distributed across the frequency axis of the chick inner ear. Specifically, a comparison of RI functions having a common CF suggested that the compressive nonlinearity that determines RI type may be a phenomenon localized to individual hair cells in the bird ear.

INTRODUCTION

The relation between neuron discharge rate in spikes per second (S/s) and stimulus intensity in dB sound pressure level (SPL) is described by the rate-intensity (RI) function. Rate-intensity functions have been reported in a number of avian species including the starling (Manley et al. 1985), pigeon (Richter et al. 1995), barn owl (Köppl and Yates 1999), chicken (Manley et al. 1991; Plontke et al. 1999; Salvi et al. 1992; Saunders et al. 1996), and emu (Yates et al. 2000). In these animals, three different types of RI functions have been identified: saturating, sloping upward, and straight.

Two of the three RI types exhibit multiple segments in their function. The “primary segment” represents sound driven activity from threshold stimulus levels to that level that elicits the so-called “breakpoint.” The growth of activity in this segment is very steep. Discharge activity between the breakpoint and the maximum stimulus level (e.g., 100 dB SPL) constitutes the “secondary segment” of the function, and this segment is used to define the RI type. As stimulus levels increase, the discharge activity over this segment may remain relatively constant (the saturating RI type) or may increase with a shallower slope than the primary segment (the sloping-up type). In some cases, the change in slope after the breakpoint approaches saturation only at the highest stimulus levels (e.g., 90–100 dB SPL). Sachs and Abbas (1974) referred to units with this RI shape as “sloping-saturating” types. Finally, a third type exhibits only one segment of sound-driven activity from threshold to the highest stimulus level, and these are referred to as “straight” RI types.

Elegant models that consider compressive nonlinear basilar membrane behavior and hair cell synaptic function have been used to describe RI types and characteristics (Eatock et al. 1991; Köppl and Yates 1999; Nizami and Schneider 1997; Sachs et al. 1989; Yates 1990, Yates et al. 1990, 2000). Other models have focused more on presynaptic (Neubauer and Heil 2002; Schoonhoven et al. 1997) or postsynaptic (Geisler 1997) processes. The RI functions in this paper are examined by measuring the slopes of the primary and secondary segments, and these are compared with the analytic model for describing RI functions developed by Yates (1990). In this presentation, we report the distribution of various RI parameters across the frequency axis of the basilar papilla. The frequency axis is defined by the “characteristic frequency” (CF) of a unit’s tuning curve, which is tonotopically distributed along the length of the papilla (see for example Chen et al. 1994, 1996).

METHODS

Animal preparation

White leghorn chicks (Gallus domesticus) between 6 and 12 days of age were anesthetized with an intra-muscular injection of a 25% ethyl carbamate (urethan) solution at a dose of 0.1 ml/10 g body wt. The airway was exposed to assure free breathing, and the soft tissue around the ear canal was excised to reveal fully the tympanic membrane. The surgical approach to the cochlear nerve via the recessus scala tympani has been detailed elsewhere (see for example Saunders et al. 1996). The treatment and maintenance of animals followed procedures approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

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Testing conditions

The skull was secured to a head holder with dental cement and then mounted on a frame located in a sound-attenuated acoustic chamber. A heating pad maintained body temperature at 41°C, and additional warming was achieved with a DC halogen lamp about 20.0 cm away from the head.

The ear was stimulated with a closed-field sound system consisting of an earphone (Beyer Dynamic earphone, Model DT-48, Hicksville, NY) to which a sound tube (10-cm long, 5-mm diam) was attached. A 0.5-mm probe-tube microphone (Etymotic Research, Model ER-7, Elk Grove Village, IL) was fitted to the open end of the sound tube, and the latter was sealed against the exposed tympanic membrane. The second harmonic of the earphone was 60–74 dB below the fundamental at all frequencies. Output from the probe microphone was connected to the analyzer module of a frequency synthesizer (Audio Precision, Model “System One,” Beverton, OR), and converted to dB SPL (relative to 20 µPa). The generator module of the synthesizer produced tonal stimuli under computer control. An automatic calibration procedure achieved a constant SPL between 0.1 and 4.0 kHz, and by adjusting the synthesizer output voltage, different SPLs could be presented.

Recordings

Borosilicate glass microelectrodes (15–30 MΩ) filled with 3 M KCl were secured to a microdriver, inserted into scala tympani, and advanced in 1-µm steps. Electrical signals were amplified, connected to an oscilloscope, audio monitor, and level detector. The time between nerve discharges was measured with a 0.1-ms resolution, and these discharges were sampled over a 40-ms interval. Total discharge counts were stored on hard disk, and only well-isolated units were used for collecting data. When a unit was encountered, an initial estimate of spontaneous activity (SA) was determined from a 6-s sample of activity in the absence of sound stimulation.

Stimuli

Band-limited noise bursts (0.1–5.0 kHz, 40-ms duration, 5-ms rise/decay time) served as the search stimulus. This was switched to a tone burst when a unit was encountered. A tuning curve for each unit was constructed as described elsewhere (Saunders et al. 1996), and from this the CF, CF threshold, and frequency selectivity (Q10 dB) were determined. The relationship between unit discharge level, in spikes per second (S/s), and tone burst SPL was measured at the CF. The SPL was sequentially increased from 0 to 100 dB in 31 intensity steps of 3.33 dB each. Four equal-SPL tone bursts were presented at 100-ms intervals, and the total number of discharges was summed over the 40-ms stimulus interval in each of the presentations and converted to S/s. There was a 700-ms pause between intensity levels.

Determination of RI type and data analysis

The raw data for each RI function was subjected to a three-point running average using the routines provided in Sigma Plot (SPSS, Chicago, IL). This “smoothing” procedure made it easier to determine the particular RI type on visual inspection. Figure 1 shows raw (thin lines) and smoothed (open circles) data in examples of the four RI types.

The smoothed RI-function of each unit was visually examined, and, based on the appearance of the secondary segment, a subjective decision was made to assign it to one of four RI categories. This subjective evaluation provided a classification scheme against which other more objective methods for classifying RI type could be compared. The visual examination was unambiguous in over 86% of the units. In the remaining 14%, the type was resolved on the basis of the discharge behavior over the last four intensities (90.0, 93.3, 96.7, and 100 dB SPL). A unit might clearly exhibit sloping-upward behavior over much of the secondary segment, but if the four most intense levels exhibited asymptotic behavior, continued to slope upward, or tended downward, they were placed in the saturating, sloping-up, or sloping-down categories, respectively. The straight functions were obvious, although a slight change in slope might suggest a secondary segment. This subjective determination of RI-type was undertaken independently by two of the authors, and there was agreement in all but 9% of the jointly evaluated sample. In those units with a differing assignment, the authors reevaluated the unit together, and if no consensus could be reached it was discarded. The ambiguous functions arose, in all cases, from unusually noisy raw data in the secondary segment.
During a second pass through the data, a quantitative evaluation was undertaken independent of any consideration of RI type arrived at in the first pass. The quantitative analysis measured the slope of and the primary and secondary segments, as well as the maximum response, and SA. From these the threshold and breakpoint could be calculated. A computer program smoothed the RI data from each unit to an operational set of procedures. The first step averaged discharge activity over the five intensity steps from 0 to 13.33 dB SPL (the lowest CF threshold in our sample was 14 dB), and then a horizontal line was drawn on the RI plot indicating the level of SA. Next a regression line was fit to the primary segment of the curve. A minimum of six data points in the linear portion of the segment were identified and fit with a regression line (in more than 74% of the units, the primary segment was longer and the regression line could be calculated over more than 6 points). The line was extended to intersect the SA line, and the intercept point provided an operational definition of the threshold (see Fig. 1B). A second regression line was fit to the secondary segment of the RI function. A minimum of four data points (the 4 most intense levels: 90, 90.3, 96.7, and 100 dB SPL) contributed to this calculation. In most units, the secondary segment was sufficiently defined so that additional data points could be applied to the calculation. The intercept between the primary and secondary regressions (see Fig. 1B) defined operationally the stimulus SPL and discharge activity at the breakpoint. The number of dB between threshold and breakpoint defined the dynamic range of the primary segment. Finally, the average activity for stimuli at 96.6 and 100 dB SPL was assumed (using a ratio of 1.82:1) between 0.11 and 3.98 kHz (center frequencies of 0.15, 0.27, 0.49, 0.84, 1.63, and 2.96 kHz). The mean and SE for each parameter were determined for all units whose CFs fell within these bins.

**RESULTS**

**General aspects**

A total of 959 units were examined. This large sample overcame the difficulty of encountering units with very low or high CFs (Manley et al., 1991; Salvi et al., 1992; Saunders et al., 1996) and provided sufficient sample sizes in all frequency bins to conduct reliable statistical analysis. A summary of the results averaged across all CFs is found in Table 1.

**Sloping-down type**

An example of a sloping-down RI-function appears in Fig. 1D, and Salvi et al. (1992) noted the presence of this RI type but dismissed it as an artifact. In our data, multiple replications of the RI function produced the same sloping-down result. In addition, these types were just as likely to occur early in the testing session as late, thus ruling out a deteriorating preparation. Moreover, a review of these functions failed to reveal a sudden decline in spike rate at the 96.6- or 100-dB level due to some unrecognized recording artifact. Nevertheless, our procedure of presenting four successive tone bursts might cause them to interact with one another (because of incomplete recovery from adaptation) resulting in an overall suppression of discharge activity, especially at the higher SPLs. Such a phenomenon might cause the secondary segment to have a shallower slope or even a downward slope in what otherwise would be a saturating type. This possibility was explored by making a detailed comparison of RI functions in the same unit using the current mode of stimulus presentation (quadruple stimuli, sequential level changes) and a pseudo-random presentation mode. The latter procedure presented the array of intensity levels in a random sequence repeated four times. The comparison of RI functions in 31 units revealed no differences

![Table 1](http://jn.physiology.org/)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Saturating</th>
<th>Sloping Down</th>
<th>Sloping Up</th>
<th>Straight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>376</td>
<td>121</td>
<td>340</td>
<td>122</td>
</tr>
<tr>
<td>Characteristic frequency, Hz</td>
<td>1031.39 ± 35.57</td>
<td>1017.98 ± 61.46</td>
<td>997.77 ± 34.26</td>
<td>1278.22 ± 87.84</td>
</tr>
<tr>
<td>Threshold, dB SPL</td>
<td>38.72 ± 0.61</td>
<td>40.35 ± 1.14</td>
<td>38.30 ± 0.63</td>
<td>49.19 ± 1.23</td>
</tr>
<tr>
<td>Frequency selectivity, Q100 dB</td>
<td>5.21 ± 0.17</td>
<td>5.11 ± 0.30</td>
<td>5.15 ± 0.16</td>
<td>5.09 ± 0.29</td>
</tr>
<tr>
<td>Spontaneous activity, spikes/s</td>
<td>24.55 ± 1.00</td>
<td>27.41 ± 1.93</td>
<td>22.90 ± 0.96</td>
<td>18.96 ± 1.98</td>
</tr>
<tr>
<td>Maximum response, spikes/s</td>
<td>220.75 ± 3.22</td>
<td>180.08 ± 5.66</td>
<td>249.76 ± 3.44</td>
<td>232.03 ± 5.73</td>
</tr>
<tr>
<td>Dynamic range, dB</td>
<td>32.76 ± 0.46</td>
<td>34.12 ± 0.83</td>
<td>26.95 ± 0.38</td>
<td>55.03 ± 1.36</td>
</tr>
<tr>
<td>Break point, dB SPL</td>
<td>71.03 ± 0.63</td>
<td>68.85 ± 1.05</td>
<td>60.83 ± 0.67</td>
<td>—</td>
</tr>
<tr>
<td>Break point, spikes/s</td>
<td>198.03 ± 3.16</td>
<td>193.67 ± 6.18</td>
<td>189.77 ± 3.35</td>
<td>—</td>
</tr>
<tr>
<td>Primary angle</td>
<td>78.19 ± 0.25</td>
<td>77.12 ± 0.44</td>
<td>80.06 ± 0.17</td>
<td>74.66 ± 0.48</td>
</tr>
<tr>
<td>Secondary angle</td>
<td>1.83 ± 0.58</td>
<td>−40.55 ± 1.03</td>
<td>47.76 ± 0.63</td>
<td>73.66 ± 0.57</td>
</tr>
<tr>
<td>Primary slope, spikes/s/dB</td>
<td>5.40 ± 0.09</td>
<td>5.01 ± 0.17</td>
<td>6.24 ± 0.10</td>
<td>4.11 ± 0.14</td>
</tr>
<tr>
<td>Secondary slope, spikes/s/dB</td>
<td>0.03 ± 0.01</td>
<td>−0.90 ± 0.04</td>
<td>1.22 ± 0.03</td>
<td>3.95 ± 0.14</td>
</tr>
</tbody>
</table>

Entries represent the mean value, independent of characteristic frequency (CF); values are means ± SE.
in shape (including the sloping-down type) or in RI parameters from the two procedures (M. A. Crumling and J. C. Saunders, unpublished observations). It would appear that the 100-ms interval separating stimuli minimized interactions between tone bursts.

**Determination of RI type**

The quantitative model developed by Yates (1990) for evaluating RI functions has been successfully applied to avian data (Köppl and Yates 1999; Richter et al. 1995; Yates et al. 2000). The RI functions are fitted to a pair of nonlinear equations (Eqs. 2 and 3), where $A_0$ is the spontaneous discharge rate, $A_1$ is the maximum increase in discharge rate above the spontaneous level, $A_2$ is the sound pressure (in Pascals) half-way between the spontaneous and maximum levels of activity, $A_3$ is the sound pressure level (in Pascals) at the breakpoint, $A_4$ is the exponent of the power-law slope in the secondary segment of the RI function, $P$ is the sound pressure level (in Pascals) at the tympanic membrane, and $R$ is the predicted discharge rate. The raw data and running average were compared with model fits to the RI data in 120 units randomly selected from the data set. The model was fit to the raw data using the curve fitting routines of the Sigma Plot program (SPSS). Results for six units appear in Fig. 2. The raw data show the expected variability while the solid black line is the smoothed curve. The open circles show the results of the model fit, and these closely approximate the smoothed curve. The coefficient of determination ($r^2$) between the raw data and the model ranged between 0.94 and 0.99 in

$$d = \left[ \frac{A_1^{(A_4-1)} + P^{(A_4)}}{A_0^{(A_4-1)} + P^{(A_4)}} \right]^{1/A_4}$$

$$R = A_0 + \frac{A_1}{A_2 + d^2}$$

107 units (13 units were the sloping-down type and poorly fit by the model).

**Primary and secondary angles**

Figure 3 illustrates angle histograms (at 3.5° intervals) in primary and secondary segments, organized by the subjectively categorized RI type. Figure 3A shows the distribution of all primary angles, while B–E, depict the primary angles in each of the four RI types. The average primary angle for the saturating, sloping-down, sloping-up, and straight RI types was 78.2, 77.1, 80.1, and 74.7°, respectively (see Table 1). A two-way ANOVA showed that the primary angle differed significantly by type ($F = 29.62; P < 0.01$). The meaningful difference was the shallower primary angle of the straight type.

Figure 3F shows the secondary angles in 837 units (there was no statistical difference between the primary and secondary segment of the straight types and therefore they are omitted from this figure). Figure 3, G–I, plots the secondary angles on the basis of the subjective categorization. While there is

![Image](http://jn.physiology.org/)

**FIG. 2.** RI functions are illustrated for 6 representative units. The raw data, running average, and Yates (1990) model fit is shown for each. The running average and the fitted function are nearly identical in each example.
some overlap, the subjective designation of RI type, and the value of secondary angles, show considerable correspondence (see Table 2).

The results in Fig. 3F show a distribution of secondary angles that appear to separate into three groupings. These data were fit to a three-compartment polynomial (Eq. 4), where $Y$ is the number of events, $X$ is the angle, $A_i$ is the peak value, $B_i$ is the mean angle, and $C_i$ is the width factor of the respective

$$ Y = \sum_{i=1}^{3} A_i \cdot e^{-0.5(X-B_i)^2/C_i} \quad (4) $$

binomial function. The results appear in Fig. 4A, and the coefficient of determination ($r^2$) of the fit to the histogram data was 0.95 ($P < 0.01$). The Gaussian functions associated with each of the peaks are teased out of the overall polynomial, and a histogram (and Gaussian fit) to the straight RI angles is added (●). The intercept of the Gaussians provides an objective means of determining the range of secondary angles that segregate the four distributions (Table 2). For example, the saturating RI functions are those units with a secondary angle between $-28.2$ and $+26.5^\circ$. The wide range of angles for the saturating functions might surprise the reader; however, the slope-to-angle transformation is deceptive, and angles of $-28.2$ and $26.5^\circ$ have slopes of only $-0.54$ and $0.50 \text{ S} \cdot \text{s}^{-1} \cdot \text{dB}^{-1}$.

Figure 4B now plots the slopes of the secondary segments, and the data are again fit by a polynomial. The exponential growth of slope concentrates the “saturating” functions around the zero value, while the slope of the “straight” functions (●) are distributed over a wider range. Figure 4, A and B, reveals that the angle transformation simply normalizes the slope to a ratio scale.

The first section of Table 2 reports the percentage of RI types determined by visual inspection. The middle section of Table 2 indicates the angle intervals from Fig. 4A and the percent of units falling into these intervals. For example, the range of angles associated with the saturating type ($-28.2$ to $26.5^\circ$) comprises 41.9% of the units in the sample. The bottom
section of Table 2 indicates the distribution of RI categories determined from the model fit to the RI functions. The ratio between the A3/A2 components categorizes RI functions, and ratios larger than 3.0 are considered saturating. Those between 0.5 and 3.0 are sloping up, and less than 0.5 straight. In the sample of 107 units, 13, 72, and 15% were saturating, sloping-up, or straight RI types by these criteria. The dominance of the sloping-up type using the model fit was similar to that reported in the pigeon, owl and emu (Köppl and Yates 1999; Richter et al. 1995; Yates et al. 2000). Table 2 also reports our subjective categorization of RI types in these 120 units, and it is similar to that in the total sample. The difference in the incidence of RI types using the model and either the subjective or angle assignment is striking.

Distribution of RI variables and type across frequency

Figure 5 considers the distribution of primary and secondary angles across CF. Figure 5, A and C, shows primary angle data, whereas B and D illustrate the secondary angles. The top panels show all the units plotted against their CFs, while the bottom panels summarize results across the six frequency bins. The parameter in each panel is the subjectively determined RI type. The primary angles exhibit considerable consistency within type and across frequency. A two-way ANOVA on the secondary angle data (Fig. 5D), in which RI type and frequency were the main factors, revealed significant differences only among types (F = 1635, P < 0.01).

Figure 6A shows the CF thresholds for all units differentiated by type, whereas Fig. 6B is organized by binned frequency intervals. The saturating, sloping-down, and sloping-up types have similar thresholds, but the straight types are consistently 8–12 dB poorer in threshold across CFs. Figure 6C shows the percentage of RI types in each frequency bin, and these proportional data preclude any indication of variability. Nevertheless, the occurrence of RI types appears to be independent of CF.

The relationship between RI type and frequency selectivity (data not shown) revealed a significant frequency effect (as CF increased the value of Q increased). However, the ANOVA revealed that the values of Q could not be distinguished by RI type (P > 0.05). In addition, the maximum response, primary segment dynamic range, and breakpoint were analyzed across the binned frequency intervals for each of the RI types (data not shown), and the smallest maximum response occurred in the sloping-down type, while the sloping-up type had the largest maximum. For all RI types, the

Table 2. Rate-intensity type determinations

<table>
<thead>
<tr>
<th></th>
<th>Sloping Down</th>
<th>Saturating</th>
<th>Sloping Up</th>
<th>Straight</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjective determination from visual examination</td>
<td>n</td>
<td>121</td>
<td>376</td>
<td>340</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>12.6</td>
<td>39.2</td>
<td>35.5</td>
<td>12.7</td>
</tr>
<tr>
<td>Objective determination by secondary angle alone</td>
<td>Angle, degree</td>
<td>-63 to -28.3</td>
<td>-28.2 to 26.5</td>
<td>26.4–67.9</td>
<td>68.0–83</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>99</td>
<td>402</td>
<td>351</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>10.3</td>
<td>41.9</td>
<td>36.6</td>
<td>11.2</td>
</tr>
<tr>
<td>Determination from model fit</td>
<td>Model fit to RI functions</td>
<td>n</td>
<td>13</td>
<td>50</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>10.8</td>
<td>41.6</td>
<td>31.6</td>
<td>15.8</td>
</tr>
</tbody>
</table>
| RI, rate intensity. * 107 of 120 unit sample are reported because 13 units were of the sloping-down type poorly fit by the model.
maximum response was smallest at the highest and lowest frequencies and larger in the mid-range.

Spontaneous activity and RI type

The relation between threshold, SA and RI type is highly correlated in mammalian 8th nerve activity. High-SA, low (sensitive)-threshold units are associated with saturating RI types, while low-SA, high (insensitive)-thresholds occur in straight types. Intermediate levels of SA and threshold are correlated with sloping-saturating units (Winter et al. 1990; Yates 1990; Yates et al. 1990). These relations are weakly correlated in the chick ($r^2 = 0.39$). Table 1 indicates that straight RI types have the highest thresholds and lowest levels of SA, whereas saturating and sloping-up types have lower (but identical) thresholds, and SA levels only 4 and 6 S/s faster than the straight types. The ANOVA on SA revealed that the difference between types (Table 1), was nevertheless significant ($F = 2.86, P < 0.05$).

Narrowband analysis

Köpl and Yates (1999) noted that compressive nonlinear cochlear mechanics should give rise to a shared micromechanical input to sensory hair bundles on hair cells with a similar CF. If this was true, then certain RI parameters should be predictable on the basis of this shared input (Cooper and Yates 1996; Müller and Robertson 1991; Winter et al. 1990; Yates 1990). However, the analysis of units with similar CFs in the owl and emu cochlear nerve failed to yield RI parameters that supported these predictions (Köpl and Yates 1999; Yates et al. 2000). The idea of a shared input was explored in our data by identifying clusters of units (in the same animal) with a similar CF. A total of 27 such clusters was identified, each containing between three and seven units. The difference between the highest and lowest CFs within a cluster averaged only 39 Hz across all clusters.

Four of these clusters, representing low-, mid-, and high-frequency CFs, appear in Fig. 7. Figure 7C, for example, had one straight, two saturating, and two sloping-up RI types. The levels of spontaneous activity, threshold, breakpoint, dynamic

![Image](http://jn.physiology.org/)

**FIG. 5.** The primary (A and C) and secondary (B and D) angles are presented for each RI type as a function of characteristic frequency (CF). C and D: the data are summarized over sequential frequency bins. Inset: definitions of the angles. The vertical bars show ± SE.

**FIG. 6.** A: CF thresholds are plotted for each unit and distinguished by RI type. B: the average threshold, binned across successive frequency intervals and delineated by RI type, is plotted. C: the proportion of RI types in each of the binned intervals is presented. The 4 types within each interval sum to 100%.
The decibel level of the breakpoint in sloping-up units with a common CF should be that SPL where the shared nonlinearity begins (Yates et al. 2000) and should be independent of threshold. Independent because threshold is determined by synaptic processes rather than the mechanical input to the hair cell. The results in Fig. 8A fail to support this prediction and show that the breakpoint significantly increases as CF threshold increases. This occurs in units with narrowly defined CFs. The same reasoning applies to the primary segment dynamic range. A shared input in sloping-up units should show a decreasing primary segment dynamic range as threshold increases. This is because the breakpoint would occur at a common SPL (the beginning of the compressive nonlinearity). Figure 8B, however, shows that there is little correlation between threshold and primary segment dynamic range (slope = -0.09, \( P > 0.05 \)). Additionally, if the compressive portion of the mechanical input was shared in sloping-up units with a common CF, then the secondary segments of these units should have similar angles (or slopes). Figure 8C, however, reveals that the secondary angles vary widely in units with relatively common CFs. Figure 8D describes the angle of the secondary segment in sloping-up units, and the primary angle of straight units, for narrow CF bands. At each sample point, there were between 7 and 12 units and the CFs on average were within ±1.29% of the mean CF. Theoretically, the compressive portion of the mechanical input should determine these angles, and if it did, we would predict that they should be both the same. Figure 8D shows that within a type the angles are very similar across frequency, but straight units exhibit almost a 33° steeper angle than the secondary segment of the sloping-up types. Finally Fig. 8E shows the dynamic range in the primary segment of saturating units (the dB difference between threshold and the breakpoint) at different CFs. This range is determined, in part, by the synaptic processes of the hair cell which determines the threshold and theoretically should be relatively constant around 19 dB (Köppl and Yates 1999; Yates 1990; Yates et al. 2000). While none of the saturating units examined showed a primary segment dynamic range less than 19.5 dB, they were, nevertheless, very variable, with dynamic ranges as high as 45–60 dB. Collectively, the panels in Figs. 7 and 8 argue against a shared mechanical input.

**Discussion**

The stimulus presentation used in this paper is different from the pseudorandom presentation used by other authors. As reported in the preceding text, a comparison of RI functions in the same unit obtained with the methods described here and a pseudorandom mode of stimulus presentation, showed no difference in RI type or RI parameters (angle, slope, maximum response, or breakpoint) (M. A. Crumling and J. C. Saunders, ...
unpublished observations). Thus we believe that the data presented here are a valid indication of RI activity in the chick cochlear nerve.

**New observations**

The occurrence of a sloping-down RI type was previously dismissed as an artifact of poor electrode contact (Salvi et al. 1992). This was not a problem in our data, and there is every reason to believe our observations were valid. The downward angle in the secondary segment may be due to neural inhibition. However, there is little support for this because individual cochlear nerve fibers do not interact with one another and efferent fibers in the cochlear nerve only synapse directly on the hair cell, not the primary neuron as in mammals (Fischer 1992, 1994; Warr 1992). Moreover, the anesthetized preparation should minimize any effect of efferent feedback on the hair cell afferent output. The sloping-down type might originate from a particular population of hair cells, or a unique papilla location, but evidence to support this possibility is lacking. Finally, this type did not arise from the sequence of stimulus presentation used. A complete accounting of this RI type awaits further research.

Another new observation was the continuous distribution of secondary angles (or slopes) extending from negative values to angles greater than $+80^\circ$. This distribution segregated into multiple components corresponding to RI types (see Fig. 4), but the transition from one to the next had overlap, suggesting that the subjective categorization of RI functions could lead to erroneous decisions. Additionally, the homogeneity across frequency in the proportion of RI types, and the values of primary and secondary angles, suggested that the mechanisms determining RI type were independent of the basilar papilla tonotopic axis.

**Determining RI type**

Differences were seen in the proportion of RI types reported by our subjective or angle evaluations and that determined by the Yates model (Table 2). The model used the ratio between “breakpoint” and “sensitivity” ($A_3/A_2$) to categorize RI functions (Köpl and Yates 1999; Richter et al. 1995; Yates et al. 2000).

2000). Ratios more than 3.0 constituted a saturating type. Köappl and Yates (1999) noted that the defining characteristic of their saturating units was not the ultimate saturation but rather the steep primary slope and relatively abrupt transition to saturation. Ratios between 0.5 and 3.0 defined a sloping-up type, but this may yield an ambiguous result.

Figure 2A reveals a function whose model fit has an A3/A2 ratio of 4.19. The smoothed curve in this function showed asymptotic behavior and had the angle of a saturating unit (1.12°, slope of 0.02 S · s⁻¹ · dB⁻¹). Thus the subjective and regression categorizations were consistent with the classification from the model fit. Figure 2, B and C, has A3/A2 ratios of 1.21 and 0.71, categorizing them as sloping-up types. The subjective evaluation and angle results (0.34 and −1.04°) defined these as saturating types, which is at odds with the model classification. Finally, the subjective, angle, and model classifications all agreed in Fig. 2, D–F.

The use of an A3/A2 ratio offers a rational way of determining RI type. However, it seems something of a stretch to subsume RI shapes like those in Fig. 2, B and C, into the sloping-up category. Hundreds of RI functions with the shapes illustrated in Fig. 2, A–C, were identified by our criteria as saturating, and this accounts for the percent difference in the incidence of saturating types seen in Table 2. We are concerned that the A3/A2 ratios from the model may be in need of additional refinement. This is not meant as a criticism. The Yates model is perhaps the most elegant application of mammalian nonlinear basilar membrane mechanics to peripheral neural signal processing found in the literature. The extension of the model and its ratio criteria to avian RI data, however, may be more compelling when the mechanistic basis of the cochlear amplifier in birds is better understood (Manley 2001).

Contributions of a global or localized cochlear amplifier to RI type

The different RI types in mammalian auditory nerve recordings result from the interaction between a compressive nonlinear input to the inner hair cell (IHC) hair bundle and the synaptic properties found at the base of the cell. The nonlinearity arises from a “cochlear amplifier” attributed to the motile properties of the outer hair cell (Cooper and Yates 1994; Müller and Robertson 1991; Robles and Ruggero 2001; Winter et al. 1990; Yates 1990). At low SPLs, the presumptive IHC stereocilia displacement increases linearly with a dB/dB relation between increasing SPL and displacement. The cochlear amplifier, at some mid-range level introduces a compressive nonlinearity into the growth of hair bundle motion, resulting in a fractional dB/dB relation between SPL and displacement (see Robles and Ruggero 2001; Yates 1990; Yates et al. 1990). The mechanical input over adjacent IHCs is similar and thus “shared” in the output activity of the many afferent nerve terminals innervating each IHC. This “global” input is thus shared in the RI behavior of the hundreds of 8th nerve fibers with nearly identical CFs.

Mechanisms at the hair cell synapse determine the levels of SA and threshold sensitivity (Winter et al. 1990). In cat, and to a lesser extent the guinea pig, synaptic behavior is well correlated with synaptic morphology (Liberman 1982; Merchante-Perez and Liberman 1996; Tsuji and Liberman 1997). Synaptic boutons on the modiolar side of the IHC are large, associated with large diameter dendritic fibers, and a single presynaptic ribbon that is relatively short. Boutons located at the lateral extreme of the IHC are small, associated with finer bore dendrites and a larger presynaptic ribbon. The numbers of tethered neurotransmitter vesicles on these ribbons of different length should vary accordingly. Between these extremes, the pre- and postsynaptic morphology is graded.

Labeled neurons in cat have confirmed that large synaptic endings are associated with high-SA, high-sensitivity (e.g., low-threshold) units, which should give rise to the saturating RI type. Conversely, small boutons are associated with low-SA, poor-sensitivity (e.g., high-threshold) units, likely to exhibit straight RI functions (Merchan-Perez and Liberman 1996). Intermediate synaptic specializations, SA, and threshold levels, should be associated with sloping-saturating RI types.

In mammals, low-threshold, high-SA, saturating units would be expected to exhibit sound-driven activity that begins in the linear portion of the mechanical input. Sound-driven activity in these units increase to the level of synaptic saturation over a relatively small dynamic range but always occurring below the SPL of the compressional input (Winter et al. 1990; Yates 1990). Units with lower levels of SA and poorer thresholds have primary RI segments that initially respond within the linear portion of the mechanical input. As intensity increases, the discharge rate circumscribes that SPL where compressional input behavior begins. At sound levels above the compressive “breakpoint” neural activity exhibits a shallower growth, revealing the sloping RI type. Low-SA, high-threshold units, exhibit sound-driven activity at SPLs associated with the compressional portion of the input and exhibit the behavior of straight RI types.

Figures 7 and 8 presented data from units (within the same chick or across animals) that represented narrow CF bandwidths on the papilla surface. The presumption, based on the mammalian model, was that a shared mechanical input to hair cells with similar CFs should exhibit similar RI properties. These properties were a similar rate of growth for the linear input segment (primary RI segment), the SPL level of the breakpoint, and the angle (slope) of the compressive input (secondary RI segment). Table 1 and Fig. 3 show that the primary angle (slope) in saturating, sloping-up and -down RI types were very similar. It would appear that the linear portion of the hair cell input has a common origin. The results in Figs. 7 and 8, however, provided little support for the concept of a shared input.

Equally interesting was the synaptic contribution to RI functions in the chick. The correlation among SA, threshold level, and RI type showed only a weak relationship. There are relatively few afferent synapses per intermediate and tall hair cell (average of 2.1), and the size of the postsynaptic boutons on the tall hair cell is fairly uniform (Fischer 1992, 1994). The organization of the presynaptic dense bodies shows gradients across the length and width of the papilla, and there also appears to be a gradient in the voltage-gated calcium channels (Martinez-Dunst et al. 1997).

We need to ask what evidence exists in the bird papilla for: nonlinear and compressive papilla mechanics, shared mechanical input to hair cells with a common CF, and synaptic specialization? We assume that most of the units in this report originate from intermediate and tall hair cells that communicate with the majority of the afferent fibers in the cochlear
nerve. How movements of the cochlear partition stimulate tall and intermediate hair cell stereocilia is not well understood, although the tectorial membrane may play an important role in this process (see for example Saunders et al. 1996; Smolders et al. 1995). Moreover, there is evidence that the mechanical input to these hair cells may not be uniform across an iso-frequency strip. Gradients of tuning curve sharpness and input to these hair cells may not be uniform across an iso-papilla location (Martinez-Dunst et al. 1997), they remain to be described fully. A presynaptic mechanism is thus another factor that may contribute locally to the shape of the RI function (Neubauer and Heil 2002; Schoonhoven et al. 1997).

Hair bundle shape in chick tall hair cells is another possible contributor. Tall hair cells have stereocilia that exhibit a profile of increasing hair height that is either straight or curved across the successively taller rows of the bundle (Duncan et al. 2001). The curvature can be mild or pronounced, and these bundle shapes may be randomly distributed across tall hair cells. Models (Duncan 1999) of tip-link tension in bundles with different shapes suggested a complex distribution of gating spring forces through the bundle. This raises the possibility that the sequence of gating transduction channels with changing stimulus intensity may vary with the degree of bundle curvature, resulting in unique patterns of membrane depolarization and cochlear nerve activation. The important point is that the contributions to discharge activity from hair bundle motility, synaptic body behavior, and hair bundle shape are potential mechanisms whose contributions should be explored in future research.

The peripheral ear of mammals achieves diversity in RI functions by the interaction of synaptic mechanisms and a global compressive nonlinear mechanical input to the hair cell. The same RI types emerge in the avian ear through a compressive nonlinear mechanical input that may be localized to individual hair cells and a far more generalized synaptic mechanism. If these observations prove to be true, then we see a marvelous example in the organ of Corti and the basilar papilla of different evolutionary mechanisms achieving the same end.

The authors greatly appreciate the comments of Drs. Thomas Parsons, Michael Anne Gratton, Mark Crumling, and Stephen Wolfe. The authors also thank Dr. R. Keith Duncan for suggesting the angle analysis. An anonymous reviewer expended considerable time and effort to help us get this manuscript “right” and for that we are grateful. The technical assistance of R. Kurian, A. Lieberman, and M. Graham was also appreciated.

This research was supported by awards from the National Institute on Deafness and Other Communication Disorders and the Pennsylvania Lions Hearing Research Foundation.

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**Sources of the compressive nonlinearity in the chick ear**

Considerable interest has been directed toward a cochlear amplifier in anurans, reptiles, and birds associated with a calcium-dependent sensory hair bundle motility at the site of the transduction channel (Hudsphet and Martin 2002, Manley et al. 2001). This motility, yet to be characterized in bird hair cells, may be related to the mechanisms of hair bundle adaptation and the control of tip-link tension. It could produce compressive nonlinear behavior in the mechanical movements of the sensory hair bundle in individual hair cells (Eguiluz et al. 2000; Manley 2000, 2001; Manley et al. 2001; Martin et al. 2000).

Another possibility arises from the fact that approximately two afferent nerve fibers terminate on each tall hair cell (Fischer 1992, 1994), and within each cell there are about 15 dense bodies. Thus there are multiple dense bodies and release sites associated with each synapse. Additionally, the size and number of dense bodies per hair cell appear to vary in an orderly way across the length and width of the basilar papilla (Martinez-Dunst et al. 1997). Tethered to the dense bodies are synaptic vesicles, and the number of vesicles released with membrane depolarization is partially known (Spassova et al. 2001). However, the summed contribution to exocytosis at multiple release sites has yet to be determined. Furthermore, vesicle release is dependent on the proximity of voltage-gated calcium channels to each site, and while these also vary with papilla location (Martinez-Dunst et al. 1997), they remain to be described fully. A presynaptic mechanism is thus another factor that may contribute locally to the shape of the RI function (Neubauer and Heil 2002; Schoonhoven et al. 1997).


