Autocorrelation Analysis of Hair Bundle Structure in the Utricle

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Rowe, M. H. and E. H. Peterson. Autocorrelation analysis of hair bundle structure in the utricle. J Neurophysiol 96: 2653–2669, 2006. First published August 9, 2006; doi:10.1152/jn.00565.2006. The ability of hair bundles to signal head movements and sounds depends significantly on their structure, but a quantitative picture of bundle structure has proved elusive. The problem is acute for vestibular organs because their hair bundles exhibit complex morphologies that vary with endorgan, hair cell type, and epithelial locus. Here we use autocorrelation analysis to quantify stereociliary arrays (the number, spacing, and distribution of stereocilia) on hair cells of the turtle utricle. Our first goal was to characterize zonal variation across the macula, from medial extrastriola, through striola, to lateral extrastriola. This is important because it may help explain zonal variation in response dynamics of utricular hair cells and afferents. We also use known differences in type I and II bundles to estimate array characteristics of these two hair cell types. Our second goal was to quantify variation in array orientation at single macular loci and use this to estimate directional tuning in utricular afferents. Our major findings are that, of the features measured, array width is the most distinctive feature of striolar bundles, and within the striola there are significant, negatively correlated gradients in stereocilia number and spacing that parallel gradients in bundle heights. Together with previous results on stereocilia number and bundle heights, our results support the hypothesis that striolar hair cells are specialized to signal high-frequency/acceleration head movements. Finally, there is substantial variation in bundle orientation at single macular loci that may help explain why utricular afferents respond to stimuli orthogonal to their preferred directions.

INTRODUCTION

One of the major, unresolved questions in hair cell research is the significance of ciliary bundle structure. All hair bundles are built on the same basic plan—a staircase pattern of stereocilia, with or without a kinocilium—but the realization of this plan is highly variable. There are two reasons for believing this variability has information content, i.e., it is telling us something significant about how hair bundles work. First, it is orderly. Hair bundle structure differs systematically between species, between endorgans, and between different regions of a single epithelium (reviews: Eatoock and Lysakowski 2006; Hackney and Furness 1995; Lewis et al. 1985; Platt 1983; Saunders and Dear 1983; Smotherman and Narins 2000). Second, mechanical behavior depends on structure (Gordon 1978). So the strikingly diverse structures of hair bundles suggest they will differ in mechanical behavior and thus play different roles in signaling head movements.

The significance of bundle heterogeneity has proved difficult to analyze. Two major obstacles are the difficulty of making accurate measurements of such small, delicate structures and of acquiring large enough samples for quantitative comparisons. We are left with a rich, qualitative literature on differences in bundle structure and, with the possible exception of freestanding auditory hair bundles in alligator lizard (reviews: Aranysosi and Freeman 2004; Fettiplace and Fuchs 1999), little idea of what these differences mean to the behaving animal.

We are using computational and experimental approaches to study bundle structure and its functional significance in an otoconial organ, the utricle (Fontilla and Peterson 2000; Moravec and Peterson 2004; Nam et al. 2005, 2006; Rowe and Peterson 2004; Silber et al. 2004; Xue and Peterson 2006). As part of this effort, we developed new methods to quantify differences in bundle structure. Here we apply one of these methods, autocorrelation analysis of scanning micrographs (Rowe and Peterson 2004), to quantify several mechanically significant features of utricular bundles and characterize their spatial variation.

Spatial variation in bundle structure can arise from three sources. 1) Directional variation. Utricular bundles are organized into a series of radial transects that fan out from the medial margin of the macula (Fig. 1A, gray arrows) (Lindeman 1969). The activation axes (axes of maximum sensitivity) (Lowenstein and Wersall 1959; Shotwell et al. 1981) of bundles along a single transect have similar orientations; but average bundle orientation in neighboring transects differs, so bundles in each transect are maximally sensitive to a different direction of head movement. 2) Hair cell type. All vertebrates have type II hair cells; amniotes (reptiles, birds, mammals) have a second hair cell type (type I) (Wersell 1956). Spatial variation in bundle structure can arise if type I and II bundles differ in structure and also have different spatial distributions.

3) Zonal variation. Utricular bundles differ with position along a single radial transect. For example, there are differences between bundles in the striola (a crescent-shaped specialization in the macula and overlying otoconial membrane) and the extrastriola, even for bundles with parallel activation axes (e.g., Baird and Lowman 1978; Hillman 1976; Laperey et al. 1992; Lewis and Li 1975; Lim 1976; Platt and Popper 1981; Severinsen et al. 2003; Xue and Peterson 2006). This zonal variation is one major focus of the present study. It is a ubiquitous feature of vertebrate utricles; this suggests that it plays an important role in enabling these organs to detect and encode head movement. Furthermore, zonal variation in bundle structure covaries with utricular afferent properties such as discharge regularity and response dynamics (Baird and Lewis 1986; Goldberg et al. 1990; review in Lysakowski and Goldberg 2004); thus it may contribute to physiological diversity in afferents.
FIG. 1. Schematic diagram of the utricular macula in T. scripta illustrating 2 sources of systematic spatial variation in hair bundle structure (A) and random variation in bundle orientation at a single macular locus (B). A: gray arrows, average activation axes of utricular hair bundles. Zonal variation along a medial-to-lateral transect that spans all zones was quantified; the approximate location of this transect is indicated by the line of numbers. We also analyzed random variation in bundle orientation in small circular areas that approximate the collecting area of utricular afferents (A, a and b). At evenly spaced locations along the transect (A, gray numbers), we quantified variation in the orientation of activation axes (B, arrows), defined as the hexagon axis closest to the bundle’s axis of bilateral symmetry (ABS). In 1 analysis (a), we collected ≤25 samples at each location. These samples formed 2 concentric rings around a central sample. In a, the central sample is at location 6 and only 4 more widely spaced samples from the inner ring are illustrated for clarity. We eliminated samples that impinged on the borders of the transect, resulting in a total of 319 samples for the 2 utricles. In a 2nd analysis (b), we investigated whether varying sample radius from 10 to 40 μm (U50) or 50 μm (U5) would significantly increase the range of bundle orientations. In the figure, only four size variants of 1 sample at 1 location (3) are illustrated for clarity. B: stereociliary arrays. Arrows, hexagon axis closest to each bundle’s ABS (axis 1), i.e., the probable activation axis of the bundle. Numbers indicate the angle between axis 1 and a line parallel to the transect. The orientation of axis 1 in these 6 neighboring cells differs by 27° (33–6°). Because such local variations in axis 1 orientation show no systematic pattern (see also Fig. 7), we refer to this as random variation. Scale: 1 μm.

In addition to these three types of systematic spatial variation in hair bundle structure, utricular bundles also exhibit apparently random variation in structure at any one macular locus. One example is the spread of hexagon orientations (and thus activation axes) in neighboring bundles (Fig. 1B). Such random variation may help explain why afferents respond to movement directions perpendicular to their axes of maximal sensitivity (Dickman et al. 1991; Fernandez and Goldberg 1976; Si et al. 1997). Thus a second focus of this study was to quantify variation in hexagon orientation and estimate the effect this might have on directional tuning in afferents.

To quantify zonal and random variation in utricular hair bundles, we used autocorrelation analysis of stereociliary arrays on the hair cell’s apical surface (Fig. 1B). We also took advantage of the fact that in turtle utricle, type I hair cells have a sharply restricted distribution (Jorgensen 1974, 1988; Moravec and Peterson 2004; Xue and Peterson 2006) and significantly more stereocilia than neighboring type II hair cells (Moravec and Peterson 2004) to estimate differences between the arrays of different hair cell types. We presented some of these data in abstract form (Peterson and Rowe 2001).

M E T H O D S

Seven juvenile turtles, Trachemys (Pseudemys) scripta elegans, of both sexes (3.5- to 5-in carapace length; Kons Scientific, Germantown, WI) provided useful data. We used two turtles for a low-magnification view of the macula (Fig. 2) and images of intact bundles (Fig. 12) and five turtles for quantification (Table 1): autocorrelation analysis of a medial-to-lateral transect (U5, U50) and counts of total utricular bundles (U5 and 3 additional turtles). Animal care protocols are published (Brigha and Peterson 1994). We killed all turtles with Euthasol (390 mg pentobarbital sodium and 50 mg phenytoin sodium/ml; 0.5 ml im) and followed Ohio University Animal Care and Use Committee guidelines in all experiments.

Scanning electron microscopy

We perfused killed turtles transcardially with oxygenated turtle Ringer solution (Hounsgaard and Nicholson 1990) followed by fixative: 2% glutaraldehyde, 4% paraformaldehyde, 2 mM MgCl2 in 0.1 M sodium phosphate buffer, pH 6.2 to visualize intact bundles, or 5% glutaraldehyde, 4% paraformaldehyde, 2% sucrose, 0.2 M picric acid, 0.125 M phosphate buffer, pH 7.4 to visualize arrays. We dissected utricles in fresh fixative, postfixed them in 2% OsO4 for 1 h, followed by rinsing (10 times in distilled water), osmium-thiocarbohydrazide (OTOTO) treatment (Furness and Hackney 1986), dehydration (in graded ethanol series), critical point drying, sputter coating with gold palladium, and examination using a Jeol JSM-840 or a Zeiss DSM 962. Prior to post fixation we treated utricles in one of two ways. To visualize intact bundles, we removed the otoconial membranes using fine forceps. For autocorrelation analyses of stereociliary arrays, we removed otoconial membranes and hair bundles by sonication (30–60 s in 70% ethanol) or with a fine (0000) sable brush under high magnification, and we oriented the macula so that the viewing angle was normal to the transect. Arrays were considered measurable if they were intact and the hair cell surface was free of fissures, debris, and stereocilia.
Autocorrelation analysis: quantification of arrays

We quantified the number and arrangement of stereocilia as follows. We photographed arrays at ×5,000 and quantified features of interest using autocorrelation analysis. The term array refers to the number, spacing, and distribution of stereociliary remnants on the hair cell’s apical surface (Fig. 1B). We published details of this method previously (Rowe and Peterson 2004). Briefly, we used a custom program running under MATLAB (ver. 7; MathWorks) to record data from each hair cell (location of stereocilia and kinocilium, perimeter of apical surface and array) and to derive the following hair cell and bundle descriptors (see sections 2.2 and 2.3 and Figs. 1 and 2 in Rowe and Peterson 2004).

1) Area, orientation, and shape of the apical surface. For each hair cell, we fit an ellipse to the apical surface perimeter and used parameters of the fitted ellipse to specify the area, long and short axes, and orientation (relative to the axis of the transect) of the apical surface.

2) Area, long and short axes, and orientation of the stereociliary array were quantified using the same methods as for the apical surface.

3) Stereocilia number.

4) Stereocilia spacing. Average center-to-center spacing for each bundle is given by the initial peak in its autocorrelogram (see Fig. 2 in Rowe and Peterson 2004).

5) Axis of bilateral symmetry (ABS). Mean x and y coordinates of all stereocilia in an array estimate the array center. The orientation of a vector that originates at the array center and terminates at the kinocilium defines the ABS.

6) Estimated activation axis relative to ABS. Arrays are approximately hexagonal; thus they present three axes. Tip links generally run along the axis closest to the ABS (axis 1), so this is the best estimate of the bundle’s activation axis (Pickles and Corey 1992). It also specifies whether bundle geometry is “loose” or “tight” (Bagger-Sjöbäck and Takumida 1988; see Fig. 8 in Rowe and Peterson 2004).

7) Spacing slope is the change in average stereocilium spacing with distance from the kinocilium, measured parallel to the ABS. Slopes are negative if stereocilium spacing decreases toward the short end of the bundle.

Zonal variation

Our previous work indicates that the utricular macula in T. scripta can be divided into four zones (Figs. 2 and 3) based on differences in bundle structure, afferent terminal morphology, and calretinin-immunoreactivity of hair cells and afferents (Moravec and Peterson 2004; Xue and Peterson 2006; Xue et al. 2005). Zones 1 and 4 correspond to the lateral (LES) and medial (MES) extrastriolae, respectively. Zones 2 and 3 form the striola. In dehydrated utricles, zone 2 is ~20 μm wide. Zone 3 is a 50- to 60-μm-wide band of type I hair cells and any interspersed type II hair cells (Moravec and Peterson 2004; Xue and Peterson 2006).

To assess zonal variation in array structure, we compared arrays along a medial-to-lateral transect that spans all four zones (Fig. 2, ↔). Hair bundles along this transect have similar activation axes. Thus we were able to analyze zonal variation while holding directional variation nearly constant. Our previous work suggests that other aspects of bundle structure do not differ significantly with transect orientation (stereocilia number: Moravec and Peterson 2004; bundle heights: Xue and Peterson 2006); therefore zonal variation along the transect used in this study is likely to be representative of zonal variation along any transect.

We illustrate zonal variation in three ways. Scatter plots (Figs. 4, A, C, and E, and 5, A, B, D, and E) show how variable values change with distance from the line of polarity reversal (LPR). Zero on the abscissa represents a straight line fitted to the irregular trajectory of the LPR. Hair cell position is the perpendicular distance from this fitted reversal line to the hair cell’s kinocilium. Both turtles showed similar spatial trends, but we plot data for U5 and U50 separately because their values on some variables at some locations (usually the extrastriolae) are significantly different. Box plots (Figs. 4, B, D, and F, and 6) summarize broad differences between zones and hair cell types for the two utricles combined. Finally, bubble plots (Fig. 5, C and F) give semi-quantitative overviews of reciprocal patterns of stereocilia number and spacing within the striola.

### TABLE 1. Macular area, total hair cells, and number of measured hair cells

<table>
<thead>
<tr>
<th></th>
<th>Utricle</th>
<th>Transect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Area, sq. μm</strong></td>
<td><strong>Total Hair Cells</strong></td>
<td><strong>Lateral Hair Cells (%)</strong></td>
</tr>
<tr>
<td>Confocal Confocal</td>
<td>795,788</td>
<td>9,141</td>
</tr>
<tr>
<td>Confocal Confocal</td>
<td>833,830</td>
<td>9,049</td>
</tr>
<tr>
<td>Confocal Confocal</td>
<td>814,809</td>
<td>8,544</td>
</tr>
</tbody>
</table>

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and center-to-center spacing decreases with distance from the reversal line. Asterisks). Note that for probable type I hair cells, stereocilia number increases on stereocilia counts) were not considered measurable (examples marked with Fig. 1 C occasionally see identified type I hair cells very close to the reversal line (see be incorrectly categorized as type I hair cells. In confocal material, we stereocilia are closer to the reversal line than expected. These may or may not than 77 stereocilia, i.e., probable type I hair cells. Two bundles with 80 – 85 narrow toward the medial macula. Yellow highlighting: bundles with more zonal boundaries as follows. Hair cells lateral to the reversal line form zone 1 (LES); those between the reversal line and zone 3 form zone 2; those medial to zone 3 form zone 4 (MES). Resulting sample sizes for each zone and hair cell type are given in Table 2.

Statistical analysis of zonal variation

To facilitate comparisons with published studies using scanning electron microscopy (SEM), we made no correction for shrinkage. Exploratory and inferential statistics were implemented in Statistica (ver. 7.1; StatSoft) or S+ (ver. 7; Insightful). We used robust statistics for our analyses (Wilcox 2005) because many of our variable distributions were nonnormal (see discussion in Xue and Peterson 2006). Briefly, they are powerful but make few assumptions about the shape of underlying variable distributions. We implemented robust functions in S+. To compare bundles in different zones we used t1way (analog of 1-way ANOVA) and lincon (for multiple comparisons; Wilcox 2005, Chapter 7). To describe spatial gradients in and near the striola (Fig. 5, B and E), we used robust MM linear regression (available in S+). We summarized spatial trends across the macula using Loess fits (a form of smoothing using weighted, local regressions on data that cannot be fit with simple linear or quadratic equations) (Cleveland 1993). For the fits in Figs. 4, A, C, and E, and 5, A and D, we used a local quadratic fit (because there are local maxima and minima in our data), a Gaussian weighting function, and a span (which determines the degree of smoothing) of 0.1–0.2. We implemented Loess fits in S+.

Directional specificity

We also quantified the random variation in bundle orientation. We used one hexagon axis (axis 1, i.e., the hexagon axis closest to the ABS) to estimate each bundle’s activation axis, and we examined variability in axis 1 orientation for all bundles along the transect (see Directional specificity, Radial populations) and for bundles in small, circular areas with dimensions similar to the collecting areas of single utricular afferents (Directional specificity, Local populations). We used the small samples to examine two questions. What is the variation in axis 1 orientation for a small group of hair cells, such as might provide input to a single afferent (Fig. 1A, a)? How does variability in axis 1 orientation increase as sample area increases (Fig. 1A, b)? Answers to these questions may help explain some directional tuning characteristics of utricular afferents (Dickman et al. 1991; Fernandez and Goldberg 1976; Si et al. 1997). To estimate variation in hair cell activation axes that exists within the collecting area of a typical afferent, we sampled 8 (U5) or 9 (U50) locations evenly spaced along the medial-to-lateral transect. Figure 1A
shows approximate locations of the eight samples for U5. At each location, we simulated the collecting area of an afferent as a circle with a radius of 20 μm. This approximates the average size of utricular afferent terminals (Table 3). At each location, we also created 24 additional samples arranged in two concentric rings around the central sample (Fig. 1A, a). The inner ring comprised nine circular samples spaced at 45° angles around the central sample and displaced from it by one radius (20 μm). The outer ring comprised 15 circular samples, spaced at 22.5° angles around the central sample and displaced from it by two radii (40 μm). We eliminated samples that impinged on the borders of the transect, resulting in a total of 319 samples for the two utricles. These samples provided a robust estimate of the variation in bundle orientation to be expected within the collecting area of single utricular afferents.

For each of the samples (≈25) at each position along the transect, we recorded axis 1 orientation for all measured hair cells within the 20-μm-radius circle. We used these data to examine effects of variation in hair cell activation axes on afferent directional tuning. To do this, we simulated individual hair cell responses as the sum of two cosine functions,
\[ aH[\cos(\theta + \pi)], \] where \( \theta \) is the angle between stimulus direction and the hair cell excitatory axis, \( H[\cos(\theta + \pi)] \) is a half-wave rectified cosine phase shifted \( 180^\circ \), which partially cancels the negative half cycle of the first term (required because hair cell responses to excitatory and inhibitory stimuli are not symmetrical), and \( a \) is the asymmetry factor. Values for \( a \) were chosen to yield inhibitory/excitatory (I/E) ratios of 0.1, 0.2, or 0.5. The lower ratios correspond to values for hair cells reported in the literature (Holt et al. 1997; Hudspeth 1983; Shotwell et al. 1981; Vollrath and Eatoctk 2003). The upper bound of 0.5 takes into account the possibility that values in the literature are underestimates (Eatoctk 2000; Ricci et al. 1998). For each of the 319 samples, we simulated an afferent directional tuning curve by summing all hair cell profiles in that sample. Summation was linear and all hair cells were equally weighted. This is a reasonable approximation because local populations of hair cells in turtle utricle tend to be homoge-

[FIG. 5. Variation in stereocilia number (A–C) and spacing (D–F) with distance from the LPR. Stereocilia number and spacing are approximately constant in most of the MES and LES (A and D), but they exhibit marked spatial gradients within the striola (B and E). Stereocilia number increases from lateral to medial for most hair cells (B), and stereocilia spacing decreases (D). Regression lines in B and D summarize spatial trends in the variables for U5 (dark blue lines) and U50 (light blue lines). Equations are robust MM regression equations \( P < 0.001 \). Other plotting conventions as in Fig. 4. In B and E, 4 arrays with \( \geq 77 \) stereocilia appear to be in zone 2 (i.e., within 15 \( \mu \)m of the reversal line). They may or may not be misclassified; type I hair cells occasionally occur very close to the LPR. C and F: bubble plots showing zonal differences in stereocilia number and spacing for U5 hair cells in the striola and adjacent extrastriolae. Coordinates represent distance from the upper left corner of the transect. Each symbol represents 1 measured array. Symbol size is scaled to the number (C) and center-to-center spacing (F) of stereocilia. Note the reciprocal relation between stereocilia number and spacing in striolar zones 2 and 3.]
Hair bundles were assigned to macular zone and hair cell type as described in METHODS. * Carapace lengths: 5" (U5) and 3.75" (U50).

**RESULTS**

Figure 2 is a low-magnification scanning micrograph of the utricle of *T. scripita* that was sonicated to remove otoconial membrane and hair bundles. The fan-shaped macula forms a shallow bowl except posteriorly, where the neuroepithelium is more steeply curved. The striola is visible as a crescent-shaped band that parallels the lateral margin of the macula (arrowheads). There are ∼8,500 total hair cells (Table 1); roughly 30% of these are lateral to the line of polarity reversal. Macular areas (Table 1) were measured using undehydrated whole mounts only (n = 2); they are slightly underestimated due to foreshortening of the curved macula.

We analyzed arrays from medial-to-lateral transects in two animals (U5, U50); the transects were 107 and 79 µm wide, respectively. Median hair cell number for the two transects was 765.5; of these, ∼66% bore measurable arrays (Table 1). Table 2 summarizes the resulting data base. In this and other tables, we assigned arrays to macular zone and hair cell type as described in METHODS. Figure 3 shows examples of arrays analyzed in striolar zones 2 and 3 and adjacent LES (zone 1) and MES (zone 4). Putative type I hair cells (those having more than 77 stereocilia) are highlighted.

**Zonal variation in stereociliary arrays**

Array structure varies with medial-to-lateral position across the macula. Both utricles showed similar patterns, but length and area measurements from the extrastriola of U50 were sometimes larger than those in U5. This is probably due to differential shrinkage rather than developmental stage because U5 was slightly larger than U50 (Table 2). Array features that exhibit the most striking regional variation are illustrated in Figs. 4–6. Two other array features are not illustrated because they show little zonal variation: orientation of the presumptive activation axis (Axis 1 orientation) and changes in stereocilia spacing with distance from the kinocilium (Spacing slope).

**TABLE 2. Samples for height measurements: number of measured bundles**

<table>
<thead>
<tr>
<th></th>
<th>Type II</th>
<th>Type I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone 1</td>
<td>Zone 2</td>
</tr>
<tr>
<td>U5*</td>
<td>557</td>
<td>70</td>
</tr>
<tr>
<td>U50*</td>
<td>418</td>
<td>77</td>
</tr>
</tbody>
</table>

Hair bundles were assigned to macular zone and hair cell type as described in METHODS. * Carapace lengths: 5" (U5) and 3.75" (U50).

**Statistical analysis of directional specificity**

To compare overall differences in the distribution of apical surface, ABS, and axis 1 orientations we used a Kolmogorov-Smirnov test. We used *t*-test and *lincon* to investigate the effect of increasing sample radius on variability of axis 1 angles (putative activation axes) and on orthogonal afferent response magnitude, assuming I/E asymmetry of 0.1, 0.2, and 0.5. To assess the effect of I/E asymmetry on orthogonal afferent response magnitude, assuming I/E asymmetry while holding sample radius on variability of axis 1 angles (putative activation axes) we used *ancova* (robust analysis of covariance, radius as covariate) (Wilcox 2005; section 11.8). Axis 1 ranges and orthogonal response magnitudes for U5 and U50 were not significantly different, so we collapsed them for analysis.

**Hair bundle counts**

We counted total bundles from one SEM montage and three utricular whole mounts stained with the F-actin probe phalloidin to visualize hair bundles (Table 1). Two utricles scanned at ×10 magnification provided total bundle counts. A third, scanned at ×40, provided total bundle counts and enabled us to distinguish hair cells medial and lateral to the LPR.

**TABLE 3. Collecting areas of utricular afferents**

<table>
<thead>
<tr>
<th>Location</th>
<th>Diameter, µm</th>
<th>Area, µm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baird and Schuff (1994) (frog)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bouton: striola</td>
<td>41.96</td>
<td>1383</td>
</tr>
<tr>
<td>juxtastriola</td>
<td>43.78</td>
<td>1505</td>
</tr>
<tr>
<td>extrastriola</td>
<td>27.85</td>
<td>609</td>
</tr>
<tr>
<td>Si et al. (2003) (pigeon)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calyx</td>
<td>19.36</td>
<td>294.5</td>
</tr>
<tr>
<td>Dimorph</td>
<td>29.70</td>
<td>692.6</td>
</tr>
<tr>
<td>Bouton</td>
<td>34.70</td>
<td>945.4</td>
</tr>
<tr>
<td>Fernandez et al. (1990) (chinchilla)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calyx</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Dimorph</td>
<td>57.30</td>
<td>2578.6</td>
</tr>
<tr>
<td>Bouton</td>
<td>54.30</td>
<td>2315.7</td>
</tr>
<tr>
<td>Average ± SD</td>
<td>38.62 ± 13.17</td>
<td>1290.47 ± 819.22</td>
</tr>
</tbody>
</table>
II hair cells in zone 2 are not significantly different from those of probable type I hair cells (Fig. 4D).

Array length is less strongly correlated with array area (U5: \( r = 0.47, P < 10^{-6}; U50: r = 0.32, P < 10^{-6} \)). Instead of peaking in the striola as do array area and width, striolar array lengths are roughly equal to (U5) or slightly less than (U50) those in the MES (Fig. 4, E and F). Thus arrays in the striola are large because they are distinctively wide; they are not distinctively long. We consider the significance of wide striolar arrays in the Discussion. Bundles in the LES (zone 1) have significantly shorter arrays than other macular bundles (Fig. 4F).

**Stereocilia number and spacing**

Stereocilia numbers, like array area and width, are highest in a band that begins at the LPR and is 50–60 \( \mu \text{m} \) wide, i.e., in the region we operationally define as the striola (Fig. 5A). Stereocilia numbers in the striola are significantly greater than those in the extrastriolae (zones 1 and 4), whether or not one includes putative type I hair cells (Moravec and Peterson 2004). Stereocilia spacing is approximately uniform across the macula except for a narrow band just medial to the LPR (zone 2; Fig. 5D), where spacing is significantly greater than elsewhere in the macula (Fig. 6B).

Within the striola, stereocilia number and spacing change systematically from lateral to medial (Fig. 5, B and E). Stereocilia numbers increase (Fig. 5B) and spacing decreases (Fig. 5E). The dependence of stereocilia number on spacing is significant (MM regression: Wald test \( P = 6.7 \times 10^{-7} \)). Thus hair bundles in zone 2 have wider center-to-center spacing than other utricular bundles, and bundles in zone 3 have the highest stereocilia counts (Fig. 5, C and F). In addition, the number and spacing of stereocilia on presumptive type I hair cells (zone 3 only) show a significant dependence on position from the reversal line (MM regression: Wald test \( P = 1.8 \times 10^{-5} \) and \( P = 1.6 \times 10^{-9} \), respectively). Thus there are systematic spatial gradients in stereocilia number and spacing from lateral to medial margins of the striola.

**Directional specificity**

We asked two questions about the directional specificity of hair cells in the utricle. First, how precise is the directional specificity of a radial strip of hair cells that appear, grossly, to be aligned parallel to each other? Such radial populations are presumed to be important functional units of the utricle because they are “tuned” to the same direction of force by the parallel orientation of their activation axes. Second, how tight is the directional specificity of a small, local hair cell population, which might provide the input to a single afferent?

**Radial populations**

To assess directional specificity (tightness of bundle alignment) in a radial strip of receptors we examined hair cells of the transect. We compared three measures of hair cell orientation. 1) Apical surface orientation. This is the measure used to construct classical maps of hair cell orientation in otoconial organs, but it has only a loose relation to the activation axis.
(Rowe and Peterson 2004). 2) Axis of bilateral symmetry (ABS). The ABS is more functionally relevant than apical surface orientation because it runs from the kinocilium (the presumed site of force application) through the centroid of the bundle; but it is not equivalent to the activation axis unless it runs along the axis that carries the gating springs (elastic elements that help tense mechanotransduction channels when the bundle deflects toward the kinocilium). 3) Axis 1, the hexagon axis closest to the ABS. This is the best estimate of the hair cell’s activation axis because it is most likely to carry the bundle’s gating springs.

Vector plots confirm the subjective impression gleaned from scanning micrographs that the apical surfaces of hair cells in the transect are aligned approximately in parallel (except for a small group of cells at lower right in this utricle; Fig. 7, left). Alignment of the ABS is slightly less regular (not shown), and alignment of axis 1 is markedly irregular (Fig. 7, right). Distributions for apical surface area, ABS, and axis 1 relative to the transect line are shown in Fig. 8, A—C. The distribution for axis 1 (Fig. 8C) is significantly broader than for the other two variables (Fig. 8, A and B; Kolmogorov-Smirnov test for goodness of fit, \( P < 0.02 \)). A nearly identical pattern was observed in a second utricle. This broad distribution of axis 1 relative to the transect line suggests that hair cells in a radial strip such as the transect are not as tightly tuned to a single direction of head movement as their apical surfaces suggest.

Dispersion of axis 1 around the transect line estimates variability in bundle activation axes relative to a single direction of force (i.e., a force parallel to the transect line). Three factors sum to produce this dispersion: orientation of apical surfaces relative to the transect line, orientation of the ABS relative to the apical surface, and orientation of the hexagonal array relative to the ABS. The latter variable measures whether the bundle is “loose” or “tight” (Bagger- Sjöback and Taku- mida 1988; Rowe and Peterson 2004). Multiple regression analysis suggests it is most important in producing the spread

<table>
<thead>
<tr>
<th>Array area, ( \mu m^2 )</th>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Zone 3</th>
<th>Zone 4</th>
<th>All zones</th>
<th>Zone 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0, 2.79–3.25</td>
<td>6.7, 6.20–7.11</td>
<td>4.2, 3.83–4.57</td>
<td>2.6, 2.54–2.73</td>
<td>2.8, 2.67–2.85</td>
<td>6.9, 6.57–7.31</td>
<td></td>
</tr>
<tr>
<td>Array width, ( \mu m )</td>
<td>1.53, 1.45–1.62</td>
<td>2.61, 2.51–2.70</td>
<td>1.75, 1.59–1.92</td>
<td>1.09, 1.07–1.10</td>
<td>1.15, 1.13–1.17</td>
<td>1.25, 1.24–1.26</td>
</tr>
<tr>
<td>Array length, ( \mu m )</td>
<td>2.49, 2.35–2.62</td>
<td>3.28, 3.12–3.43</td>
<td>3.03, 2.64–3.42</td>
<td>3.34, 3.23–3.46</td>
<td>3.13, 3.05–3.21</td>
<td>3.51, 3.39–3.62</td>
</tr>
<tr>
<td>Stereocilia number</td>
<td>40, 37.7–42.3</td>
<td>71, 67.9–74.1</td>
<td>56, 49.6–62.4</td>
<td>45, 44.0–46.0</td>
<td>45, 44.1–45.9</td>
<td>96, 92.5–99.5</td>
</tr>
<tr>
<td>Stereocilia spacing, ( \mu m )</td>
<td>0.30, 0.30–0.31</td>
<td>0.34, 0.33–0.35</td>
<td>0.29, 0.28–0.31</td>
<td>0.28, 0.28–0.29</td>
<td>0.29, 0.285–0.29</td>
<td>0.29, 0.28–0.30</td>
</tr>
<tr>
<td>Axis 1 angle, radians</td>
<td>0.02, −0.03–0.06</td>
<td>0.01, −0.10–0.11</td>
<td>0.03, −0.06–0.13</td>
<td>−0.02, −0.04–0.00</td>
<td>−0.01, −0.03–0.00</td>
<td>−0.02, −0.06–0.03</td>
</tr>
<tr>
<td>Spacing slope</td>
<td>0.001, −0.001–0.003</td>
<td>−0.004, −0.006–0.001</td>
<td>−0.010, −0.013–0.007</td>
<td>−0.009, −0.009–0.008</td>
<td>−0.007, −0.008–0.007</td>
<td>−0.007, −0.008–0.006</td>
</tr>
</tbody>
</table>

Median, 95% confidence intervals of the median.
of axis 1; the squared semi-partial correlation, which gives the proportion of axis 1 variance uniquely accounted for by the looseness or tightness of the array was 0.66 (U5) and 0.67 (U50). Thus the dispersion of axis 1 around the transect line (Fig. 8C) arises primarily because the hexagonal array of stereocilia is rotated relative to the ABS.

The preceding analysis assumes that axis 1 corresponds to the hair cell’s activation axis (the hexagon axis that carries the gating springs). This assumption is least likely to be true for tight bundles; in a perfectly tight bundle, the ciliary array is rotated such that the ABS is equidistant (30°) from two hexagon axes, either of which may carry the gating springs. Thus the greater the rotation of axis 1 from the ABS (∼30°), the greater the possibility that axis 1 is not the activation axis. To assess the effect of mis-identifying the activation axis we removed the “tightest” bundles (defined arbitrarily as bundles in which the ciliary array was rotated more than 20° from the ABS). This restricted distribution (Fig. 8D) is not significantly different from the total distribution of axis 1 (Fig. 8C; Kolmogorov-Smirnov test, \( P > 0.2 \) for both utricles), and it displays significantly greater dispersion than the distribution of apical surfaces or of the ABS (Fig. 8A and B; Kolmogorov-Smirnov test, \( P < 0.01 \) for both utricles). Thus using a more conservative criterion for identifying activation axes does not change the conclusion that hair cells along the transect are less directionally specific than their apical surfaces suggest.

Local hair cell populations

It would also be useful to know the directional properties of hair cells that provide input to single afferents because this helps us understand the origin of afferent directional tuning. Two factors will introduce variability in axis 1 orientation. First, axis 1 (putative activation axis) exhibits local, apparently random differences in orientation (Fig. 1B). Figure 9 shows the orientation of axis 1 for all measured hair bundles in eight (U5) or nine (U50) samples. Samples are equally spaced along the transect (center-to-center spacing ∼60 μm); each sample is 40 μm diameter (average collecting area of utricular afferents; Table 3). Thus each sample approximates a local population of hair cells that could provide the input to a single afferent. These local populations have a preferred direction but are broadly tuned. Average orientation of all 40 μm-diameter samples combined is indicated by vertical dashed lines. Over 90% (U5: 90.2%, U50: 94.7%) of all bundles are oriented within ±25° of this population average (vertical dotted lines). For all 176 samples from U5 the mean range of orientations was 47.6 ± 10.14° (mean ± SD); for the 143 samples from U50, the mean range was 45.8 ± 13.33°.

To examine the effects of this variation in activation axes on afferent directional tuning, we represented individual hair cell responses as asymmetrical cosine functions. Figure 10 shows one example. For an I/E ratio of 0.2, mean orthogonal response amplitude for all samples combined was 8 ± 2.2% (mean ± SD) of the maximum response in one transect and 7.9 ± 1.9% in the second. Reducing the I/E ratio to 0.1 increased the mean values to 9 and 8.9%, respectively. Increasing the I/E ratio to 0.5 reduced the mean values to 5 and 4.9%.

A second factor affecting the directional specificity of hair bundle populations is the systematic directional variation in bundle orientation across different radial transects (Fig. 1A, b).
One consequence is that the larger the collecting area of an afferent, the more likely it is to sample hair cells with different bundle orientations. To learn how the spread of bundle orientations changes with increased sample area, we varied the radius of all samples at each of the eight (U5) or nine (U50) epithelial locations from 10 to 40 μm (U50) or 50 μm (U5). Figure 11A illustrates results for the two utricles; they were not significantly different. A robust ANOVA on the two utricles combined indicated that there is a significant effect of sample radius on the range of axis 1 angles (P ≤ 0.000001). Each 10 μm increment in sample radius through 40 μm produced a significant increase in the range of axis 1 angles; results for samples of 40–50 μm did not differ.

To explore the influence of collecting area radius on directional tuning of afferents, we repeated the analysis shown in Fig. 10 but with additional samples of radii 10, 30, 40, and 50 μm (U5 only). Results for the two utricles did not differ significantly (Fig. 11B). The magnitude of the simulated orthogonal response depended on whether we assumed a hair cell I/E response asymmetry of 0.1, 0.2, or 0.5 (ancova, radius as covariate; P < 0.01 for all radii). It also showed a significant overall dependence on sample radius (P = 0.0000014) for I/E asymmetries of 0.1 or 0.2, but the only significant difference was between samples of radius 10 μm and the other, larger samples. Sample radius had no effect for I/E ratios of 0.5. Figure 11C shows normalized data from A and B to facilitate their comparison. The simulated magnitude of orthogonal responses (circles) showed a weaker dependence on sample radius than did axis 1 range (squares).

**DISCUSSION**

This study describes spatial patterns in the ciliary arrays of utricular hair bundles, i.e., the number, spacing, and distribution of stereocilia on the hair cell surface. These data are important for the
important results are that 1) the features measured, array width is the single most distinctive feature of striolar hair bundles, 2) within the striola there are negatively correlated gradients in stereocilia number and spacing, and 3) neighboring arrays vary 45–50° in orientation. We consider the functional implications of these results in the following text.

Methodological issues

Two factors affect the accuracy of our results. Stereocilia are generally easy to distinguish from microvilli (Peterson et al. 1996). But in a narrow band just medial to zone 3, the distinction was often difficult at the short end of the bundle. In this juxtastriolar region, heights of the tallest stereocilia increase toward striolar values (Xue and Peterson 2006). Thus stereocilia at the short end of the bundle may be difficult to identify because they are being resorbed as stereocilia at the tall end of the bundle elongate; such redistribution of actin occurs in developing chick auditory bundles (Tilney et al. 1992). One possibility is that we saw no dip in array length in the juxtastriolar region of U50, as we did in U5 (Fig. 4E) and utricular slices (Xue and Peterson 2006), because we incorrectly identified microvilli (as stereocilia) at the short end of the bundle. Alternatively U50 could represent a slightly different developmental stage. Our result that array lengths in the MES are as long or longer than those in the striola is consistent with conclusions from Xue and Peterson (2006).

For analysis of directional specificity, we assumed that the hexagon axis closest to the ABS (axis 1) is the hair cell’s activation axis. In intact bundles, the activation axis bears tip links (Gillespie et al. 2005; Nicolson 2005). Considerable evidence suggests that tip links typically (Pickles and Corey 1992; Pickles et al. 1991) follow a single axis of the bundle’s hexagonal array, although anomalously oriented tip links have been described (Bagger-Sjöbäck and Takumida 1988; Hackney et al. 1988; Pickles et al. 1989). In auditory hair bundles (e.g., Pickles et al. 1989; Tilney et al. 1992) and frog sacculae (e.g., Jacobs and Hudspeth 1990), this axis tends to parallel the ABS, i.e., the arrays are “loose.” But in some “tight” vestibular bundles (Bagger-Sjöbäck and Takumida 1988; Flock 1964), arrays are rotated ≤30° relative to the ABS. The greater the rotation, the greater the uncertainty in identifying the activation axis. To gauge the effect of this uncertainty, we compared variability in estimated activation axes for all arrays versus arrays rotated only 0–20° from the ABS, i.e., we removed the “tightest” bundles. The two distributions were not significantly different (Fig. 8, C and D). Thus it is unlikely that errors in identifying activation axes of the tightest bundles, if any, would change our results.

Relation to previous work

Numerous studies have visualized ciliary arrays on inner ear and lateral line hair cells using light microscopy (e.g., Engström et al. 1962; Lindeman 1969), freeze fracture (e.g., Favre et al. 1986; Jacobs and Hudspeth 1990), transmission (e.g., Flock 1964; Flock and Wersäll 1962; Hackney et al. 1993; Morita et al. 1997), or scanning electron microscopy (e.g., Lim 1971; Platt and D’Andrea 1982; Severinsen et al. 2003; Tilney and Saunders 1983). Few studies quantified the images (Platt and D’Andrea 1982; studies reviewed in Jacobs and Hudspeth
Zonal variation

WHICH FEATURES DISTINGUISH STRIOLAR BUNDLES? Striolae are commonly described as a band of “big” bundles (e.g., Platt 1983; Rosenhall 1970). In what sense are they big? Probably not in heights. Kinocilia typically, and the tallest stereocilia frequently, are shorter in the striola than the extrastriola (e.g., Fontilla and Peterson 2000; Jorgensen 1988, 1989; Jorgensen and Christensen 1989; Lapeyre et al. 1992; Lewis and Li 1975; Lim 1977; Platt 1993; Rosenhall 1970; Xue and Peterson 2006). Apical surface areas of striolar bundles tend to be “large” (e.g., Lewis and Li 1975; Lindeman 1969; Lindeman et al. 1973; Severinsen et al. 2003). In turtle utricle, this is because they are wider than extrastriolar bundles (Jorgensen 1974; present results). Wide striolar bundles (i.e., wide parallel to the LPR) have also been reported in bats (Kirkegaard and Jorgensen 2001) and rodents (Lindeman et al. 1973). Indeed, Lindeman et al. (1973) commented that striolar bundles “bear a striking resemblance to the hair bundles of the inner hair cells in the cochlea.” Thus bundle width is a major distinguishing feature of striolar hair bundles.

The functional significance of wide striolar bundles is uncertain. One hypothesis is that they allow numerous stereocilia to be arranged in a shallow staircase (i.e., with few rows from short to tall ends of the bundle). This allows a significant height step between successive rows (Fig. 12). Finite element models suggest that a large height step may 1) maximize the ability of endolymphatic shear flow to tense gating springs, thereby 2) producing very rapid peak transduction currents that 3) enable wide striolar bundles to signal high-frequency head transients (Fig. 12D) (Nam et al. 2005). In mammalian cochlear, high-frequency auditory stimuli are detected by the wide, shallow bundles of inner hair cells, which are probably stimulated exclusively by endolymph flow (Fridberger et al. 2006; Nowotny and Guummer 2006; reviewed in Robles and Ruggero 2001).

INTERNAL ORGANIZATION OF THE STRIOLA. As a group, striolar bundles differ from extrastriolar bundles, but they are not homogeneous. Structural subtypes of striolar bundles have been described in anamniotes, which have only type II hair cells (Lewis and Li 1975; Platt 1983), and in birds (Jorgensen 1989) and mammals (Lim 1977), where they were attributed to differences in hair cell type. In turtle utricular striola, the striking inverse relation between stereocilia number and spacing (Fig. 5, B and E) parallels gradients in bundle heights (Xue and Peterson 2006) (Figs. 6C and 7). These trans-striolar gradients clearly reflect differences in striolar bundles. For example, ciliary spacing in zone 2 is greater than in zone 3; confocal images suggest this is because stereocilia shaft diameters are larger (E. H. Peterson and W. J. Moravec, unpublished). Trans-striolar gradients also reflect differences between type I hair cells within zone 3 (Fig. 5, B and E). Interestingly, type I cells contacted by calretinin-immunoreactive calyces (probable calyx afferents) (Desai et al. 2005) and calretinin-negative calyces (dimorphic afferents) occur laterally and medially, respectively, in zone 3, and these subgroups of type I hair cells differ significantly in bundle heights (Xue et al. 2005). Thus trans-striolar gradients in stereocilia and spacing may also reflect differences in the type I hair cells contacting these two afferent types.

IMPLICATIONS FOR RESPONSE DYNAMICS. Our present and previous results together suggest that there are significant differences between striolar and extrastriolar bundles and, within the striola, correlated gradients in stereocilia number, spacing and heights. Figure 12 illustrates these features for representative bundles from the MES (A), medial (B), and lateral (C) margins of the striola. Our results support earlier suggestions (Baird 1994a,b) that these zonal differences enable hair cells to signal progressively higher frequencies and, perhaps, to have more phase advanced responses with increasing proximity to the LPR (left to right in Fig. 12). For example, preliminary steady-state stiffness measurements from turtle utricular bundles show striking zonal gradients that are parallel to, and well predicted by, bundle structure (Moravec et al. 2005; Spoon et al. 2005). Higher stiffness suggests an ability to follow higher frequency stimuli. In agreement with this, preliminary recordings of transduction currents in response to sinusoidal fluid jet stimuli suggest that best frequencies of striolar hair cells are higher than those in the MES (25 vs. 1 Hz) (W. J. Moravec and R. A. Eatock, unpublished results). Similarly, variations in bundle shape suggest that the effectiveness of endolymph flow in tensing gating springs will increase with proximity to the LPR (Fig. 12D). Simulations suggest that with such fluid forcing, transduction channels are activated more rapidly than under point loading via the kinocilium (Nam et al. 2005). This may lead to an advance in the response phase of utricular hair cells and, perhaps, of utricular afferents.

Directional specificity

Previous investigators reported that utricular afferents, although cosine tuned, give excitatory responses for stimulus directions orthogonal to their optimal axis. This was surprising because classic maps of apical surface orientation suggested that neighboring hair cells had nearly parallel orientations (e.g., Lindeman 1969) (see also Fig. 7A). In squirrel monkey, orthogonal response magnitudes are 15% of the average of peak excitatory and inhibitory response magnitudes (Fernandez and
FIG. 12. Differences in the structure of hair bundles in the medial extrastriola (A) and striola (B and C). The upper image in each panel shows representative hair bundles; below the micrographs are simplified drawings of each bundle type in lateral view along with the associated arrays. In the lateral views, only a few rows of stereocilia are depicted for clarity; heights of kinocilium and stereocilia are from Xue and Peterson (2006). The arrays are tracings from micrographs of sonicated bundles. A: bundles in the medial extrastriola (MES) have long narrow arrays. Stereocilia are short (relative to the kinocilium) and the height step between adjacent stereocilia is very small. B: type I bundles (striolar zone 3) have wide arrays with numerous, closely packed stereocilia. The kinocilium is shorter than on MES bundles and stereocilia are taller, with larger steps between adjacent stereocilia. In the micrograph, the type I hair cell (I) can be identified because the epithelium was fractured to reveal its globular soma. The calyx is visible as debris on the soma surface (Lapeyre et al. 1992). A cylindrical type II hair cell (II) appears to wrap around the type I soma. Confocal micrographs of identified hair cells in utricular slices indicate that this is a very common configuration in striolar zone 3 (Xue and Peterson 2006). C: bundles in striolar zone 2 are homogeneous, with wide arrays and few, widely spaced stereocilia. The tallest stereocilia equal the kinocilium in height, and the height step between adjacent stereocilia is very steep. Center-to-center spacing of stereocilia is wider than elsewhere in the macula (Fig. 6B); this probably reflects the fact that stereocilia shaft diameters are also significantly greater in this zone (E. H. Peterson and W. J. Moravec, unpublished). D: schematic diagram (redrawn from Nam et al. 2005) illustrating some mechanical consequences of different bundle configurations. Simulations suggest that shear flow of endolymph (arrows) produces fluid drag on stereocilia, thereby tensing gating springs and opening transduction channels (Nam et al. 2005). This fluid drag is proportional to the height step between adjacent stereocilia (∆yss; because large height steps increase the area for fluid drag that tensions gating springs) and the height at which fluid force is applied to each stereocilium (yi; because shearing force increases with distance from the apical surface). The black arrow shows one example. Both ∆yss and yi increase with proximity to the LPR (from A to C), suggesting that fluid drag will become increasingly effective. Because time to peak channel activation is much faster in fluid-forced bundles than when force is applied to the kinocilium via its attachment to the otoconial membrane (Nam et al. 2005), we anticipate that striolar bundles (B and C) will respond more rapidly than MES bundles, enabling them to signal higher frequency head transients and leading to a relative phase lead when stimulated by sinusoidal stimuli.

Goldberg 1976). In pigeons (Si et al. 1997), they are 3–18% of the maximum excitatory responses, and in gerbils (Dickman et al. 1991), they average 4.4 and 7.9% for regular and irregular fibers, respectively. Fernandez and Goldberg (1976) discussed three possible mechanisms for orthogonal responses in afferents: 1) variation in excitatory axis orientation of presynaptic hair cells (citing Flock 1965), 2) distortion in elastic components of the otoconial membrane during orthogonal force application, and 3) excitatory responses of individual hair cells to orthogonal bundle deflections. Subsequent results indicate that, although isolated hair cells can respond to orthogonal bundle deflections for some stimulus configurations (Shotwell et al. 1981), generally they do not. Thus alternative 3 is unlikely but cannot be entirely dismissed. Alternative 2 remains plausible, but awaits experimental test. Our simulations indicate that alternative 1, variation in array orientations, can result in orthogonal response magnitudes comparable to those reported experimentally. Simulated orthogonal responses in afferents were substantial, even at the smallest collecting areas examined, and only increased by 15–25% as collecting area diameter varied from 20 to 100 µm. This suggests that the dominant factor producing orthogonal responses is random variation in hair cell excitatory axis orientation and that increases in directional variation, in collecting areas =100 µm in diameter, play a minor role.

Our result differs from that of Fernandez and Goldberg (1976), who concluded that variation in the preferred axes of presynaptic hair cells was insufficient to produce significant orthogonal responses. Their simulations differed from ours in three ways. First, our measured range of hair cell excitatory axis orientations was ~45–50° on average and reached values of 60° in some samples, whereas Fernandez and Goldberg assumed an upper bound of 30° (values reported by Flock 1965). Second, magnitudes of asymmetry between excitatory and inhibitory hair cell responses in most of our simulations were greater than values used by Fernandez and Goldberg, and our results indicate that orthogonal response magnitude is strongly dependent on this asymmetry (Fig. 11). The more
pronounced asymmetries in our simulations (I/E ratios of 0.1–0.2) were based on published data (Holt et al. 1997; Hudspeth 1983; Shotwell et al. 1981; Vollrath and Eatock 2003). However, these values may be too low due to the use of [Ca2+] significantly higher than those of normal endolymph (Eatock 2000). Experiments (Hacohen et al. 1989; Ricci et al. 1998) indicate that, at in vivo endolymphatic [Ca2+] resting MET currents (and hence I/E ratios) could be 30–50% of maximum values. Finally, we used half-wave rectification to represent the I/E asymmetry of individual hair cells, whereas Fernandez and Goldberg used a quadratic term. Although there is no a priori basis for choosing between these formulations, we avoided the quadratic term because at the higher values of I/E asymmetry we employed (0.1, 0.2), it results in an anomalous excitatory peak at stimulus orientations that produce maximum afferent inhibition in experiments. It is important to note that the orientation of the macula in vivo, and hence the rest position of the bundle is not necessarily perpendicular to gravity as it typically is in physiological experiments, not the same for all hair cells because the epithelial surface is curved in many animals, and not static. Thus the magnitude and significance of these response asymmetries during normal behavior remains to be established.

**Defining the striola**

Since 1933, when Werner first introduced the term, numerous authors have suggested definitions of the striola (see discussion in Severinsen et al. 2003). Typically, these definitions are based on one or a small number of descriptors, e.g., a groove in the otoconial membrane, a band of “big” hair bundles, or the site at which bundles reverse orientation. We (Rowe and Stone 1977) and others (Migliore and Shepherd 2005) have argued that an alternative to relying on such descriptors is to develop a functional definition, i.e., one based on a group of features that together define a “functional niche” (Rowe and Stone 1977) or “functional phenotype” (Migliore and Shepherd 2005). In the striola, for example, this constellation of features would encompass properties of the otoconial membrane, hair cells, and afferents. Do our data on turtle hair bundles move us closer to a functional definition of the striola?

Our results on stereociliary arrays (this paper) and on bundle heights (Xue and Peterson 2006) and stereociliary numbers (Moravec and Peterson 2004) of identified hair cells together present a detailed picture of how striolar hair bundles in turtle utricle differ from those in the extrastriola. As a group, striolar bundles are distinctive because of their wide arrays (present results), high stereociliary counts (Moravec and Peterson 2004; present results), and bundle heights (Fontilla and Peterson 2000; Xue and Peterson 2006). As noted in the preceding text, these structural features lead to stiffer bundles in the striola than in the extrastriola (Spooner et al. 2005), which suggests that they signal higher head accelerations and stimulus frequencies. The same structural features may also lead to faster transduction currents as predicted by our models (Nam et al. 2005) and supported by preliminary evidence that best frequencies are higher in striolar than extrastriolar bundles (W. J. Moravec and R. A. Eatock, unpublished results). These results contribute to a growing body of evidence that the functional role of the striola is to transduce the upper end of the stimulus range experienced by a behaving organism, i.e., the highest frequencies (Baird 1994a; Eatock and Lysakowski 2006) and the highest accelerations. As a corollary, we expect that the exact descriptive features of the striola will differ across species because each species places different dynamic demands on its vestibular organs.

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