Regional Specification of Threshold Sensitivity and Response Time in CBA/CaJ Mouse Spiral Ganglion Neurons

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INTRODUCTION

Across all sensory systems, a fundamental organizing principle is the formation of submodality-specific primary neural maps that represent the output of sensory receptors that encode particular sensations. In the visual and somatosensory systems, for example, the visual world and dermatomes are organized into retinotopic and somatotopic maps where there is a straightforward translation of external space and surface into neural activity. These neural maps, however, serve analytical functions as well, and nowhere is this more evident than in the auditory system where sound frequency is mapped tonotopically onto the sensory end organ in the auditory system (von Bekesy system where sound frequency is mapped tonotopically onto the organ of Corti (Emadi et al. 2004; Raphael and Altschuler 1970). In the mammalian cochlea, mechanical, morphological, and electrical processes have been identified that decompose complex sounds into their component frequencies (Rubel and Fritzsch 2002) and represent them along the tonotopic axis of the organ of Corti (Emadi et al. 2004; Raphael and Altschuler 2003; Rubel and Fritzsch 2002). Systematic changes in the basilar membrane impedance, height of the stereocilia, hair cell length, angle of the reticular lamina, and afferent innervation density are all cellular features that demonstrate graded morphological features. Recent studies have also suggested that there is a neural contribution to this analytical gradient because electrophysiological properties such as accommodation, latency, and onset kinetics also vary with cochlear location (Adamson et al. 2002a,b; Davis 2003; Zhou et al. 2005).

In addition to the monotonically graded specializations, there is also a prominent nonmonotonic feature, the presence of a best-frequency range that is characteristic for different species (Fay and Popper 2000; Liberman 1982b; Rosowski 1991; Wever 1974). The long held tenet that middle ear mechanics are solely responsible for this classic auditory feature (Rosowski 2003) has recently come into question (Ruggero and Temchin 2002), thus suggesting that cochlear and/or neuronal response properties may also contribute to the augmented sensitivity at mid-range frequencies.

The highly organized nature of the spiral ganglion innervation patterns makes it possible to examine directly how the first neural element in the auditory system is mapped. The goal of the current study therefore was to determine how the spiral ganglion neuron electrophysiological features vary throughout the length of the cochlea. Are they arrayed in a systematic electrophysiological gradient consistent with frequency coding, or do they correspond to other patterns, suggesting an alternative level of neuronal organization? To accomplish this, we developed a novel “gangliotopic” culture system that preserves the relative location of neurons along the tonotopic gradient while enabling detailed electrophysiological analysis. By using this approach, we found that timing- and threshold-related features were distinctly heterogeneous, yet when averaged showed a different organization along the length of the spiral ganglion. Timing-related features, such as onset kinetics, latency, and accommodation, were graded from the high-frequency basal neurons to the low-frequency apical ones, corresponding to the tonotopic map. Threshold sensitivity, however, was highest in the middle region of the cochlea in a pattern that appears to be coincident with the best-frequency area. The differential distribution of these electrophysiological parameters suggests that the initial stages of auditory coding differentially process specific aspects of the sensory stimulus as conveyed by the hair cell receptors.

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Methods

Tissue culture

The neuronal cell bodies of the spiral ganglion are embedded in multiple layers of bone and tissue; therefore it was necessary to develop a culture preparation in which the tonotopic distribution of the neurons was retained coupled with intracellular recording methods (see following text) to establish the exact location of each recorded neuron. Cochleae were removed from postnatal day 5 to 8 (P5–P8) CBA/CaJ mice. The spiral ligament, stria vascularis, and organ of Corti were removed to isolate the spiral ganglion. Care was taken to recover the entire spiral ganglion; however, we noted that the basal region with its relatively open coil was more vulnerable to loss than the tightly coiled apical extreme. For gangliotopic cultures, the entire spiral ganglion was placed intact in a single culture dish with the apical region marked on the bottom of the plastic culture dish. For neuronal cultures, the ganglion was uncoiled and then divided into fifths using fine forceps; three of these five regions (base, middle, and apex) were plated into separate culture dishes. All spiral ganglion tissues, independent of the culture type, were plated in poly-L-lysine-coated dishes and maintained in growth medium [Dulbecco’s modified Eagle’s medium (DMEM), supplemented with 10% fetal bovine serum, 4 mM L-glutamine, and 0.1% penicillin-streptomycin] for 5–13 days at 37°C in a humidified incubator with 5% CO2. Procedures performed on CBA/CaJ mice were approved by the Rutgers University Institutional Review Board for the Use and Care of Animals (IRB-UCA), protocol 90-073.

Immunofluorescence

Prior to antibody application, tissue was fixed in 100% methanol (−20°C for 6 min), rinsed (3× for 5 min) with 0.01 M phosphate-buffered saline (PBS; pH 7.4) and blocked for 1 h with 5% normal goat serum. The primary antibody (monoclonal anti-β-tubulin, 1:350, Covance, β-TUJ1) was applied and incubated for 1 h at room temperature, then rinsed three times with PBS for 5 min. Fluorescein-conjugated secondary antibody (anti-mouse Alexa-Flour 594, Molecular Probes, 1:100) was subsequently applied for 1 h at room temperature; rinsed three times with PBS for 5 min; DABCO (1,4-diazabicyclo[2.2.2]octane) was applied for the preparation to viewing and storage.

Electrophysiology

Electrodes were coated with silicone-estamolar (Sylgard) and fire polished (Narishige MF-83). Resistances ranged from 4 to 6 MΩ in standard pipette and bath solutions. Pipette offset current was zeroed immediately before contacting the cell membrane. Current-clamp measurements were made with the Axopatch Axon Instrument 200A amplifier. The internal current solution was as follows (in mM): 112 KCl, 2 MgCl₂, 0.1 CaCl₂, 11 EGTA, and 10 HEPES-NaOH, pH 7.45 with 0.05% Lucifer yellow (Sigma) or 1 mM pyramine (8-hydroxy-2-(di-n-propylamino) tetraline, Sigma). The bath solution (in mM) was 137 NaCl, 5 KCl, 1.7 CaCl₂, 1 MgCl₂, 17 glucose, 50 sucrose, and 10 HEPES-NaOH, pH 7.45; 350 mM KCl. Recordings from the neuronal cell somata were made at room temperature (19–22°C), although differential temperature-dependent alterations have been noted in the current magnitude and kinetics (Cao and Oertel 2005); the properties of voltage dependence, threshold, and adaptation appear to be temperature insensitive (Cao and Oertel 2005; Crumling and Saunders 2005). Data were digitized at 10 kHz with a CED Power 1401 interface in an IBM-compatible personal computer and filtered at 2 kHz; the programs for data acquisition and analysis were generously contributed by Dr. Mark R. Plummer, Rutgers University.

Successful labeling of multiple neurons in each culture necessitated very rapid assessment of the neuronal firing features so that each cell could be retained for further immunocytochemical analysis and subsequent mapping. For this reason, we focused on whole cell current-clamp recordings because they enabled a clear profile of the firing features from the cells in which recordings were made. The current-clamp recordings were considered acceptable when they met the following criteria: stable membrane potentials, low noise levels, discernable membrane time constant on step current injection, and overshooting action potentials (magnitudes of ≥70 mV). If any of these parameters changed during an experiment, indicating compromised cell health or metabolic failure, the remaining data were not included in the analysis.

Determination and calculation of cochlear position

The position of each neuron was documented both before and after fixation for immunocytochemistry with a series of photographs taken with Hoffman and fluorescence optics. Hoffman optics captured the position of each neuron and the surrounding physical markers, such as scratch marks on the dish and distinctive tissue growth patterns, while fluorescence photos indicated the intracellular dye fill (green). This allowed documentation of the exact position of the recorded neuron at the conclusion of each recording even in cases in which the cell or dye label was lost during subsequent processing. All photographs were aligned, scaled, rotated, and adjusted for opacity in Adobe Photoshop. The documented neuronal position before immunostaining was used to calculate its relative distance within the gangliotopic culture; fluorescence images of anti β-tubulin antibody labeling indicated the overall neuronal distribution. Images were acquired with a Hamamatsu C4742-95 charge-coupled digital (CCD) camera using Zeiss Axiovision or IPLAB software.

A curve was drawn by eye in Photoshop to fit the gangliotopic axis; each neuronal position was taken as the closest point to the fitted curve. Based on this analysis, the average length of the 10 longest gangliotopic cultures was 4.5 ± 0.57 (SD) mm, only slightly shorter than that measured from the inner hair cell region from adult CBA mice (5.13 ± 0.27 mm) that represents frequencies between 7.2 and 61.8 kHz (Müller et al. 2005). During removal of the spiral ganglion, some tissue was lost from both ends; because the basal end of the ganglion is relatively uncoiled when removed from the animal, we determined that this end of the tissue was more foreshortened than the apical end. To normalize one preparation to the next, measurements of cochlear position were made from the apical end, and the following formula was used to convert these measurements to the more standard convention of having the basal end be position = 0. Neuronal position from base = [(max length – actual length) * alignment constant] + (actual length – distance from apex).

In the formula, max length is the length of a completely intact spiral ganglion (rounded to 5.0 mm from the value given by Müller et al., 2005). Actual length is the measured length of the individual spiral ganglion in gangliotopic culture. Distance from apex is the measured position of an identified neuron from the apical end of the cultured tissue. The alignment constant (fraction of missing tissue that is from the base) is a factor used to compensate for missing portions of the spiral ganglion. Although examination of the ganglion enabled us to judge to some extent the amount and location of missing tissue, precise quantification was not possible. We therefore developed an objective and globally applied rule for compensation that avoided any culture-dependent bias. In gangliotopic cultures with actual lengths greater than the overall mean (3.7 mm), we applied a constant of 0.7 when calculating the location of a given neuron, thus weighting its position slightly toward an apical location. In gangliotopic cultures with total lengths that were lower than the mean, a significant portion of the apical regions was also missing, and we used 0.3 as our alignment constant; thus shifting its location slightly in the opposite direction.
RESULTS

Previous studies from our laboratory have shown that the peripheral auditory neurons, which compose the spiral ganglion, display specific electrophysiological features that differ between neurons that innervate the high- and low-frequency regions (Adamson et al. 2002b). However, to determine more precisely the ganglionic distribution of these features, we took advantage of the highly organized nature of the peripheral auditory system to evaluate the entire spiral ganglion, including those neurons that innervated the middle most-sensitive frequency region.

The primary preparation that we utilized was the gangliotopic culture because it allowed a direct comparison of a neuron’s specific electrophysiological features to its relative location within the ganglion. Whole cell patch-clamp recordings were made from 127 neurons in which the positional location was determined with intracellular dye injection and mapping techniques. To confirm these findings, we also took a more conventional approach and made dissociated cell cultures from portions of the spiral ganglion. Unlike our previous studies in which we divided the ganglion into thirds and separately cultured the apical and basal portions (Adamson et al. 2002b), here we divided the ganglion into fifths and separately cultured the extreme basal, middle, and apical regions. Although these dissociated neuronal cultures did not provide the precise positional information of the gangliotopic preparation, they allowed us to be more confident that we isolated the end regions of the spiral ganglion for comparison with the middle and made identification with intracellular dye injection unnecessary. Whole cell patch-clamp recordings were made from 138 neurons in the neuronal cultures; 38, 52, and 48 from the base, middle, and apex, respectively.

An example experiment in which multiple recordings were made from a gangliotopic preparation is shown in Fig. 1. Two whole cell current-clamp recordings were made from cells localized to the basal region of the preparation (Fig. 1A; 1 and 2), and two recordings were made from neurons positioned at the extreme apical end (Fig. 1A; 3 and 4). Similar to our previously reported experiments from neuronal cultures in which cells were isolated separately from the apex and base of the cochlea (Adamson et al. 2002a,b), we noted that the two basal neurons differed predictably from the apical ones (Fig. 1B). Responses of the basal neurons to prolonged (240 ms) depolarizing current injection showed uniformly rapid accommodation (maximum number of action potentials fired, AP$_{\text{max}}$ = 1; Fig. 1B, 1 and 2), whereas the two recordings from the apical neurons showed heterogeneous accommodation. The AP$_{\text{max}}$ levels determined for the recordings from the neurons labeled 3 and 4 were 2 and 20, respectively (Fig. 1B, 3 and 4). Latency from depolarization onset to the peak of the action potential, measured at the threshold voltage for action potential firing (red and black traces, just supra- and subthreshold, respectively) was abbreviated in the basal region (8 and 8.9 ms for neurons 1 and 2, respectively) compared with the apical region (11.7 and 15.3 ms for neurons 3 and 4, respectively).

Measurements of AP$_{\text{max}}$ plotted as a function of position from the basal end of the ganglion for the example experiment (red diamonds) and the complete data set (black diamonds) showed heterogeneous values that had an overall increase in
the number of neurons that slowly accommodated to a prolonged stimulus in the apex compared with the base (Fig. 1C). To calculate the ratios of rapidly and slowly accommodating neurons, we utilized an AP_max value reported in a previous study as the dividing point (Mo and Davis 1997a). Neurons that fired eight or less action potentials were categorized as rapidly accommodating; neurons that fired nine or more action potentials were categorized as slowly accommodating. Using these criteria, the relative proportion of slowly to rapidly accommodating cells increases from the base to the apex of the ganglion used to prepare neuronal cultures. The averaged AP_max increased systematically from the base to the apex of the ganglion (Fig. 1C, gray bars; 0.11, 0.25, 0.43 for the base, middle, and apex, respectively).

Although heterogeneous, latency measurements showed a trend when the total data set was evaluated according to gangliotopic position (Fig. 1D). The basal region showed fewer neurons with prolonged latencies, whereas the apical neurons showed greater heterogeneity. The middle region, which had heretofore been largely unexplored, did not show any particular specialized AP_max or latency values.

To compare these results to neuronal cultures, the data from the gangliotopic cultures were averaged separately for the apical, middle, and basal fifths, corresponding to the division of the ganglion used to prepare neuronal cultures. The averaged AP_max increased systematically from the base to the apex in both neuronal (5.3 ± 1.5, n = 37; 8.6 ± 1.4, n = 48; 15.1 ± 1.9, n = 44) and gangliotopic cultures (2.3 ± 0.8, n = 20; 5.5 ± 1.6, n = 25; 6.5 ± 1.8, n = 20; Fig. 1E). Similarly, the average latency was systematically prolonged from the initial rapid responses in the base, to the intermediate levels in the middle, with the slowest responses of the apical neurons in both neuronal (10.1 ± 0.5 ms, n = 38; 11.9 ± 0.4 ms, n = 50; 13.4 ± 0.8 ms, n = 48) and gangliotopic (10.1 ± 0.7 ms, n = 21; 10.6 ± 0.9 ms, n = 25; 13.5 ± 1.5 ms, n = 20) cultures (Fig. 1F). Comparisons of data obtained from the two prepreparations, therefore yielded very similar results.

Furthermore, when the time constant of the underlying voltage change in response to a depolarizing step current injection was measured at threshold level (Fig. 2A), we also noted a small but progressive increase in the slow onset time constant as a function of spiral ganglion position (Fig. 2B). When each basal, middle, and apical fifth of the gangliotopic data was averaged and compared with onset tau measurements from neuronal cultures (Fig. 2C), the same pattern emerged. Basal neurons displayed overall faster onset time courses (5.6 ± 0.3 and 5.8 ± 0.7 ms) than apical neurons (7.9 ± 0.6 and 8.8 ± 1.0 ms) with middle measurements falling in between (6.7 ± 0.4 and 6.4 ± 0.4 ms) in the gangliotopic and neuronal cultures, respectively. Time-dependent electrophysiological features, although heterogeneous, appear to be organized in a monotonic gradient along the tonotopic axis, increasing from the rapid basal end to the slower apical end.

Because the responsiveness of each primary neuron can have a significant impact on signal detection and encoding in any sensory system, we also evaluated electrophysiological thresholds. This parameter was obtained from current-clamp recordings by adjusting the amount of current injection to produce just supra- and subthreshold responses (Fig. 3A, thin and thick lines, respectively). The threshold voltage was taken as the peak voltage level at threshold in which the neuron did not fire an action potential (Fig. 3B, arrowheads). Four recordings obtained from a single gangliotopic culture experiment (Fig. 3, A and B) aligned according to their original position from the base (left) to the apex (right), showed a nonmonotonic relationship between the threshold level and relative position. The two recordings from the middle region showed the reduced threshold levels making it easier to fire an action potential (−52.2, left and −48.0 mV, right) compared with the apical and basal thresholds, with the basal threshold displaying the most elevated value (−43.3 mV), making it more difficult to fire an action potential.

When these values (black diamonds) were plotted with the full data set (gray diamonds), it is clear that the four recordings from this single experiment exemplify the overall pattern of threshold data (Fig. 3C). The most reduced threshold levels were not localized on either end of the ganglion, rather they were found toward the mid-range, whereas the thresholds levels were elevated in neurons localized to the basal region. To determine whether similar patterns of threshold distribution were also found within the neuronal cultures, we plotted a frequency histogram of the number of observations at each threshold level for the apical, middle, and basal cultures (Fig. 3D). This analysis revealed that recordings made from the neuronal cultures isolated from the middle fifth of the spiral ganglion (dark gray bars) also possessed the most reduced threshold levels (arrow). Consistent with both of these types of analysis, an average of the base (−42.9 ± 1.2 mV, n = 21), middle

FIG. 2. The subthreshold membrane kinetics became progressively prolonged from the basal to apical neurons. A: double-exponential functions (dotted line) were well fitted to 2 recordings at threshold onset (thick black). The long-latency apical neuron (right) showed slower onset time constant than the basal neuron (left). Inset: superimposed recordings at threshold with (gray) and without (thick black) an action potential (shown above). B: time constant of the slow exponential component (slow onset tau) plotted as a function of neuronal position. Unusually large onset time constants found for 1 base, 1 middle, and 2 apical neurons (14.8, 31.1, 18.1, and 38.7, respectively) were not included in the data set to reflect the main population. C: regional comparison of slow onset tau for gangliotopic (gray bars) and neuronal (white bars) cultures, respectively, showed significant differences F (2,198) = 6.79; P = 0.0014. Dashed line fitted to combined means.
Regional specification of SGNs

**FIG. 3.** Mid-ganglion neurons had elevated sensitivity. A: current-clamp recordings from basal (left) to apical (right) neurons in a single gangliotopic preparation. Traces show responses at electrophysiological threshold with (thin) or without (thick) an action potential. B: measurements of threshold voltage were taken at the peak of the voltage trace (angle brackets). C: threshold plotted as a function of location in gangliotopic cultures. Black diamonds highlight the relative position of the recordings shown above. D: histogram of the number of observations for each threshold voltage from separately isolated neurons. The most sensitive neurons (arrow) were isolated from middle cochlea regions. E: mid-range threshold sensitivity was significantly different from flanking regions when averages were calculated for each culture preparation [one-way ANOVA, \(F(2,201) = 30.98; P < 0.0001\)]. Dashed curve: second-order polynomial fit to combined means.

\((-51.3 \pm 0.7 \text{ mV}, n = 25\), and apical \((-49.9 \pm 0.8 \text{ mV}, n = 20\) fifth of the gangliotopic data compared extremely well with that obtained from neuronal cultures (left to right: \(-44.3 \pm 0.8 \text{ mV}, n = 38\); \(-48.8 \pm 0.5 \text{ mV}, n = 52\); \(-47.2 \pm 0.6 \text{ mV}, n = 48\); Fig. 3E).

To determine whether the distribution of relevant ion channels correlated with the nonmonotonic pattern of threshold voltages, we evaluated the contribution from the low-voltage-activated \(I_h\) current. This current type is active at voltages close to the action potential threshold, and its magnitude can be assessed in current-clamp recordings as a hyperpolarizing "sag" in response to hyperpolarizing step current injections. A separate gangliotopic experiment is shown in Fig. 4 in which four identified neurons were

**FIG. 4.** Threshold levels were related to the magnitude of hyperpolarizing inward rectification. A: neuronal location was shown for 4 recordings made from a single experiment. The specific culture was stained with anti-\(\beta\)-tubulin antibody (red); the dotted line was drawn by eye to approximate the apex to base tonotopic axis. Neurons 1 and 4 were identified with pyranine intracellular dye. Neurons 2 and 3, although not visible with anti-\(\beta\)-tubulin antibody staining, could be precisely located by overlaying physical culture markers documented in images acquired immediately before and after recordings were completed. Inset: high-magnification pictures for neurons 1 and 4. Scale bar: 20 \(\mu\)m. B: current-clamp traces from the same 4 neurons illustrate the relationship between threshold voltage and hyperpolarizing sag magnitude: the higher the threshold (left to right), the lower the sag magnitude. Dashed lines indicate their highest (\(-45.8 \text{ mV}\)) and lowest (\(-57.6 \text{ mV}\)) threshold levels. The holding potential and hyperpolarization level achieved were shown as the lower 2 dotted lines. C: hyperpolarizing sag magnitude plotted as a function of neuronal position. The 4 neurons were shown in color. Dashed curve is a second-order polynomial group data fit (\(R^2 = 0.23\)). D: hyperpolarizing sag magnitude plotted as a function of threshold voltage for 97 recordings. Red and black lines are linear fits to the individual experiment and to the grouped data (\(R^2 = 0.22\), respectively.
evaluated for threshold as well as their response to hyperpolarizing current injections. Recordings 1 and 2 were taken from cells closest to the apex, recording 3 was from the mid-region, and recording 4 was toward the base (Fig. 4A). When these recordings were aligned according to threshold (Fig. 4B), an inverse relationship to the magnitude of the hyperpolarizing sag (from a nadir of -185 mV) was observed. This measurement is indicative of the magnitude of the inward rectification contributed by the cationic hyperpolarization-activated current ($I_h$). One would expect from these observations that the hyperpolarizing sag magnitude would also be distributed nonmonotonically throughout the ganglion, and this was the case. As seen in Fig. 4C, each of the four recordings showed the expected inverse relationship with threshold, with the greatest magnitudes in the middle region (colored diamonds), a pattern also observed in the full data set (black diamonds). Furthermore, this general relationship is evident when hyperpolarizing sag magnitude is plotted directly against threshold voltage (Fig. 4D).

To determine if the relationship between hyperpolarizing sag magnitude and threshold held for all of the neurons in the spiral ganglion or was limited to a subset, we evaluated these parameters separately for neurons that displayed different firing patterns in both neuronal and gangliotopic culture recordings. Interestingly, we found that neurons with an APmax of only 1 showed the strongest relationship between the magnitude of the hyperpolarizing sag and the threshold (Fig. 5A; $R^2 = 0.36$). The gangliotopic distributions were also more refined such that the magnitude of the hyperpolarizing sag showed a more prominent enhancement in the mid-regions (Fig. 5B; black diamonds) and thresholds showed the mirror image (Fig. 5C). The same distribution patterns were also observed in neuronal cultures for both hyperpolarizing sag magnitude and threshold (Fig. 5, B and C, respectively; gray bars). For neurons having an APmax $>1$, the hyperpolarizing sag magnitude was not correlated with threshold (Fig. 5D; $R^2 = 0.08$); furthermore, the positional distributions of the hyperpolarizing sag magnitude (Fig. 5E) and threshold levels (Fig. 5F) were not as precisely aligned to the mid-regions for both gangliotopic and neuronal cultures (black diamonds and gray bars, respectively).

In summary, our analysis of spiral ganglion neurons response properties revealed local heterogeneity superimposed on regional gradations. Somewhat surprisingly, the average threshold voltage showed a different distribution pattern along the tonotopic gradient than that observed for accommodation, latency, and onset tau. Rather than showing a progressive change from base to apex, there was an increase in sensitivity in the mid-region of the gangliotopic cultures, which was most pronounced for rapidly accommodating neurons. The magnitude of the hyperpolarizing sag also showed this distinctive distribution pattern yet with an inverse relationship, suggesting that the $I_h$ current may play a related role in regulating threshold levels in spiral ganglion neurons.

**FIG. 5.** The strongest correlation between threshold and hyperpolarizing sag magnitude was found in rapidly accommodating neurons. A–C: analysis of rapidly accommodating neurons (APmax = 1). A: hyperpolarizing sag magnitude plotted as a function of neuronal threshold for gangliotopic (black diamonds) and neuronal cultures (gray diamonds). Dashed lined shows a linear fit to the total data ($R^2 = 0.36$). B and C: hyperpolarizing sag magnitude and threshold, respectively, plotted as a function of gangliotopic position (diamonds). Bar charts were from neuronal cultures for comparison. Dashed curves show second-order polynomial group data fits ($R^2 = 0.21$ and 0.25 for B and C, respectively). D–F: analysis of neurons with an APmax $>1$. D: hyperpolarizing sag magnitude plotted as a function of neuronal threshold for gangliotopic (black diamonds) and neuronal (gray diamonds) cultures. Dashed line shows linear group data fit ($R^2 = 0.085$). E and F: hyperpolarizing sag magnitude and threshold, respectively, plotted as a function of gangliotopic position (diamonds). Bar charts were from neuronal cultures for comparison. Dashed curves show second-order polynomial group data fits ($R^2 = 0.12$ and 0.11 for E and F, respectively).
The primary neural map in the peripheral auditory system that is established to decode sound into its component parts is a frequency map that aligns high and low tones in precise locations along the length of the cochlea. From elegant studies of the cochlea (von Békésy 1970), it was recognized that like the strings of a harp, the vibrations of the basilar membrane are systematically graded. However, the length and width of the fibers that compose the basilar membrane are not the only elements of the cochlea that vary with position. Developmental biologists have provided numerous examples of cochlear features that are graded during different stages of development (Rubel 1978; Rubel and Fritzsche 2002; Ruben 1967). Moreover, recent investigations have shown that many of these gradients are retained and ultimately define the organization of the fully functioning inner ear. Orderly variations in the receptor somata size and stereocilia length, even the electrical properties in nonmammalian vertebrate hair cells, are graded along the tonotopic map (Raphael and Altschuler 2003). Therefore to relate the diverse electrophysiological properties that have been observed in spiral ganglion to frequency specificity, it is crucial to determine whether these features are distributed in a similarly graded manner. Interestingly, we found that the time-dependent features of the spiral ganglion neuronal membrane properties were locally heterogeneous, yet they did indeed change along the tonotopic gradient.

In contrast to this, we found that electrophysiological thresholds were distributed nonmonotonically. Neurons with the most reduced thresholds were found within the middle region of the ganglion associated with the frequency range of best hearing. Additional experiments are currently underway to determine whether threshold levels are altered specifically by limiting the high-frequency sensitivity to lower basal neuron excitability or by enhancing the mid-frequency sensitivity to increased mid-ganglionic neuron excitability or both. Nevertheless, it is interesting to note that threshold sensitivity appears to parallel the best frequency range, which is typically located toward the mid-cochlear region (Müller et al. 2005; Taberner and Liberman 2005). Heterogeneity in both threshold and timing-related features was noted in previous work from this laboratory in which dissociated cultures of apical and basal thirds of the ganglion were used (Adamson et al. 2002b). This variation in response properties implicated at least two possibilities: that the heterogeneity was present locally or that it varied systematically within each ganglion region. With our newly developed preparation, it is now clear that the heterogeneous properties are distributed locally. Furthermore, our plot of threshold level versus gangliotopic position compares well with in vivo measurements of threshold plotted as a function of characteristic frequency (Furman et al. 2006; Kiang et al. 1965; Liberman 1978; Müller et al. 2005; Taberner and Liberman 2005). In each of these studies, despite the local variation in response properties that precludes strong statistical differences between cochlear locations, the patterns are clear. And although the exact parameters of the in vitro and in vivo recordings are necessarily different, each type of evaluation suggests the possibility that analysis of frequency at any one cochlear location is accomplished by a small population of neurons that further dissect the signal according to other features such as intensity.

The heterogeneity within the ganglion, however, makes the simple demarcation between rapidly and slowly accommodating neurons somewhat difficult. Although a convenient classification was previously made based on a distinct gap in the data (Mo and Davis 1997b), this undoubtedly belies the true complexity of this feature as evidenced by correlations between threshold and the hyperpolarizing sag magnitude. Rather than observing a compelling connection between these two parameters in rapidly versus slowly accommodating neurons, the clearest relationship was seen in the population of neurons that fired only a single action potential in response to prolonged depolarization. This may indicate that a subclass of rapidly accommodating neurons is responsible for systematically representing threshold variations in the cochlea.

The electrophysiological heterogeneity, which is a hallmark of the spiral ganglion neurons, is also observed in certain whole cell currents. For example, the $I_h$ current displayed marked differences in half-maximal voltage ($V_{1/2}$) that varied from cell to cell, despite stable Na$^+$ current properties (Mo and Davis 1997b). Therefore a possible explanation for the heterogeneity in the hyperpolarizing sag could be, in part, accounted for by the extraordinarily wide range of steady-state levels of the voltage dependence of activation. The $V_{1/2}$ obtained from Boltzmann fits to activation curves ranged by 44 mV and were affected by cAMP application (Mo and Davis 1997b). This large range of $V_{1/2}$ values appears to be exceptional to auditory neurons (Caicedo and Ebyalin 1999; Mo and Davis 1997b; Shaikh and Finlayson 2003, 2005), some of which show a differential distribution (Yamada et al. 2005), most likely contributing to an aspect of sensory processing unique to this system. Because the $I_h$ current contributes to regulating levels of excitation in other sensory ganglion cells (Doan et al. 2004) as one of its many functions (Robinson and Siegelbaum 2003), it may also play a similar role in the spiral ganglion. Thus we speculate that the well-characterized categories of spontaneous rate (Kawase and Liberman 1992; Liberman 1982a; Schmidetz 1989), which are related to threshold levels in the spiral ganglion, may be orchestrated by more than the size and shape of the presynaptic terminal of the hair cell receptors (Merchan-Perez and Liberman 1996); the neurons themselves may also contribute. Future experiments will be directed toward determining the specific association among threshold, $I_h$ current density, and $V_{1/2}$ in these spiral ganglion neurons.

Because timing and sensitivity are differentially localized along the length of the ganglion, and therefore relative to the tonotopic map, it will be important to decipher how the underlying ion channel composition is regulated. Previous studies (Adamson et al. 2002a,b) have shown that voltage-gated ion channel $\alpha$-subunit distributions in postnatal and adult animals, and hence spiral ganglion electrophysiology, is potentially regulated by brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3). Neurons supplemented with low concentrations of BDNF uniformly showed rapid accommodation, abbreviated latency, and rapid onset tau, along with enhanced ion channel $\alpha$-subunits associated with the rapid basal neurons (Kv3.1, Kv1.1, Kv1.2, BK). In contrast, neurons supplemented with low concentrations of NT-3 showed a more
heterogeneous profile that included a greater proportion of cells with slow accommodation, prolonged latencies, and slow onset tau, along with enhanced Kv4.2 α-subunits (Adamson et al. 2002a). The broad outline of these experiments easily explains how the gradations in the timing features could be systematically shaped from the base of the cochlea to the apex. The two opposing neurotrophin gradients noted in postnatal and adult animals (Fritzsch et al. 1997; Schimmang et al. 2003; Sugawara et al. 2007) could account for this pattern (Davis 2003) and are consistent with the present findings that show a systematic gradient in these features.

In contrast, regulatory mechanisms that could account for the U-shaped distribution of threshold levels are presently unknown. An intriguing hypothesis is that the absolute concentration levels of BDNF and NT-3 may alter threshold differently from other electrophysiological parameters. We had found previously a number of electrophysiological features actually changed nonmonotonically in basal neurons exposed to slight NT-3 concentration differences (Zhou et al. 2005). It is possible that the promiscuous binding of NT-3 to trkB (Segal 2003) is utilized to construct a complex, possibly nonmonotonic, map from ligands distributed in a simple gradient. What needs to be studied further is whether these electrophysiological properties are differentially regulated by each of the neurotrophins and, if so, exactly how this is achieved.

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