Stiffness of the Gerbil Basilar Membrane: Radial and Longitudinal Variations

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Submitted 8 May 2003; accepted in final form 28 September 2003

The basilar membrane within the mammalian cochlea plays an integral role in the mechanical processing that takes place before sound information is encoded into the afferent firing of the auditory nerve. Variations of basilar membrane stiffness are likely to be critical in the manifestation of several phenomena, including the separation of sound frequencies along the length of the cochlea. Furthermore, our results indicate qualitative changes of stiffness–deflection curves as a function of radial position; in particular, there are differences in the rate of stiffness growth with increasing tissue deflection. Longitudinal coupling within the basilar membrane/organ of Corti complex is determined to have a space constant of 21 μm in the middle turn of the cochlea. The bulk of our data was obtained in the hemicochlea preparation, and we include a comparison of this set of data to data obtained in vivo.

INTRODUCTION

The basilar membrane within the mammalian cochlea plays an integral role in the mechanical processing that takes place before sound information is encoded into the afferent firing of the auditory nerve. Variations of basilar membrane stiffness are likely to be critical in the manifestation of several phenomena, including the separation of sound frequencies along the length of the cochlea, traveling wave behavior, and possibly the proper functioning of the cochlear amplifier. In this communication, we present basal membrane stiffness data obtained in the gerbil hemicochlea preparation (Edge et al. 1998; Hu et al. 1995, 1999; Richter et al. 1998b) using piezoelectric-based sensors. A hemicochlea, prepared by cutting a freshly isolated cochlea in half along its modiolar plane, provides a clear cross-sectional view of the tissue structures and allows measurement probes to be positioned with great accuracy. Accordingly, we were able to measure stiffness at multiple positions across the width of the basilar membrane for several longitudinal locations. We use these data to discuss the following mechanical aspects of the basilar membrane: 1) coupling along its length, 2) variation of elastic properties across its width, 3) the gradient of stiffness along its length, and 4) the role of this stiffness gradient in accounting for the frequency-place map of the cochlea. In addition to the data obtained in the hemicochlea preparation, control measurements were obtained from an in vivo preparation that allows access to the tissue at a location about 2.5 mm from the basal end of the basilar membrane. At this location along the gerbil cochlea, the stapedial artery overlies the bone of the cochlear wall and makes it difficult to open a hole in the wall without excessive bleeding. We provide methods on ligating and removing a portion of the stapedial artery to allow access to the basilar membrane at this location.

Previous basal membrane stiffness measurements

The mechanical properties of cochlear tissues were first measured by von Békésy (1960), who reported that the elasticity of the cochlear partition changed by a factor of 100 from the base to the apex of the 35-mm-long human cadaver cochlea. Despite the basic concerns regarding the freshness of his cadaver preparations (e.g., see Völdrich 1978), his magnitude for the base-to-apex stiffness gradient is remarkably close to more recently reported values for the basilar membrane (Emadi and Dallos 2000; Naidu and Mountain 1998). Measurements of basilar membrane stiffness have also been obtained in guinea pig (Gummer et al. 1981; Miller 1985) and in gerbil (Naidu and Mountain 1998; Olson and Mountain 1991, 1994). All of these investigators have provided data on point stiffness as a function of tissue deflection for each recording site.

Gummer et al. (1981), who made measurements in excised guinea pig cochleae (<1 h postmortem) on the scala tympani side of the basilar membrane near the base of the cochlea (0.8- to 2.3-mm region), observed an initial constant “plateau” of stiffness (corresponding to linearly elastic behavior) extending over a deflection range of 1 to 3 μm after initial contact with the tissue, followed by a quadratic growth of stiffness with further increases in deflection. Miller (1985) made basilar membrane compliance measurements at radial positions spanning the width of the basilar membrane in excised guinea pig cochleae at locations 1.0–2.5 mm from the base. She observed 3 distinct plateaus of stiffness with divisions between the segments occurring at average tissue deflections of 2.4 and 4.7 μm. Near the center of the gerbil basilar membrane, Olson and Mountain (1991) observed the following features as a function of increasing tissue deflection: 1) an initial stiffness plateau whose length depended on the noise level, 2) a rise in stiffness over a short distance, 3) a second plateau of stiffness, and 4) a quadratic increase in stiffness beyond the second plateau. Extending their point stiffness measurements to positions spanning the entire width of the basilar membrane, Olson and
Mountain (1994) found that, near the spiral lamina and near the spiral ligament, the stiffness increased relatively steeply with increasing tissue deflection. At the arcuate zone (below the fluid-filled tunnel of Corti), they observed that the basilar membrane was initially relatively compliant and exhibited a monotonic increase in stiffness with increasing tissue deflection. Below the foot of the outer pillar cell, the basilar membrane exhibited a relatively stiff plateau followed by a quadratic increase in stiffness with increasing deflection. The magnitude of the plateau stiffness measured at the pectinate zone was in between those measured at the arcuate zone and below the outer pillar foot; for deflections beyond the plateau, the stiffness increased quadratically. Naidu and Mountain (1998) made stiffness measurements in an in vitro gerbil cochlear turn preparation and, at a given position along the length of the cochlea, observed stiffness–deflection curves similar in shape to those seen by Olson and Mountain (1991, 1994). Naidu and Mountain also examined the longitudinal gradient of basilar membrane stiffness by measuring at locations near 1–4, 8, and 12 mm from the base of the gerbil cochlea. According to their data, the basilar membrane stiffness decreased by a factor of 56 from the base to the apex at radial locations below the outer pillar foot, below the outer hair cells (OHCs), and below the Hensen cells. Below the arcuate zone, the stiffness decreased by a factor of 20 from base to apex.

The basilar membrane has been approximated by a parallel beam structure (Allaire et al. 1974; Gummer et al. 1981), and the plateau stiffness has been suggested to represent the stiffness of the beams and therefore the physiologically relevant stiffness of the basilar membrane (Gummer et al. 1981; Miller 1985; Naidu and Mountain 1998; Olson and Mountain 1991). This beam structure presumably has as its substrate the fibers embedded in the cottonty ground substance of the basilar membrane (Iurato 1962). Miller (1985), for example, suggested that the cells and ground substance of the basilar membrane could each be approximated as an incompressible fluid layer with low shear strength. From the perspective of a point force, these layers would appear as floppy springs, relative to the embedded fibers, until fully compressed. From the perspective of a distributed pressure, the 2 layers would be incompressible at all tissue deflections. For the latter situation (i.e., the physiological situation), the embedded fibers would dominate the effective compliance of the basilar membrane. In this case, physiologically relevant information can be derived from measurements of the structural properties of these fibers.

**METHODS**

The procedures for care and use of all animals reported on in this study followed guidelines from the National Institutes of Health and were approved by the Northwestern University Animal Care and Use Committee.

**Preparation of hemicochlea**

Methods on preparing a hemicochlea are available in the literature (Edge et al. 1998; Hu et al. 1999; Richter et al. 1998b). Briefly, adult gerbils (Meriones unguiculatus) were sedated with chloroform, anesthetized with intraperitoneal sodium pentobarbital (200 mg/kg body weight), killed by rapid cervical dislocation, and decapitated. One bulla was extracted and trimmed to expose the cochlea. The bulla was bathed in modified artificial perilymph (described below), and a sectioning system was used to make a planar cut from apex to base along the modiolar plane of the cochlea. This cut effectively removed one half of the cochlea and left behind a hemicochlea. The hemicochlea was oriented to yield a cross-sectional view of the tissues in a selected cochlear turn and placed on the stage of an upright microscope (Leitz Ergolux AMC), which was located on a vibration isolation table (Newport model VW-3646-OPT) and fitted with a 100 Nikon water immersion objective. The tissue was illuminated off the optical axis using a fiber-optic light guide. All experiments were conducted at room temperature (18°C).

The artificial perilymph solution contained (in mM): 5 KCl, 45 NaCl, 105 NaOH, 10 HEPES, and 100 lactobionic acid (Sigma L2398). The solution was adjusted to pH 7.3–7.4 and 315 mOsm and was oxygenated immediately before use in an experiment by bubbling with a mixture of 5% carbon dioxide and 95% oxygen for ≥10 min. This modified perilymph was designed with the specific aim of minimizing swelling and deformation of the cochlear tissues relative to their initial state. Reduced calcium, relative to actual perilymph, is necessary and sufficient to maintain the integrity of the tectorial membrane (Edge et al. 1998), but not the OHCs, which can maintain their cylindrical shape up to and beyond 2 h postmortem if most of the chloride is replaced by lactobionate (Zeddies et al. 2000). Only data from hemicochlea preparations classified as excellent were included for presentation and analysis here. For a hemicochlea to be classified as being in excellent condition, the following morphological features had to be clearly evident: the tectorial membrane was not lifted away from the reticular lamina, the hair cells were not swollen, and the outer pillar cells were not bent.

A more complete explanation and justification of our use of a perilymph solution containing lactobionate is provided here. In early experiments with the hemicochlea preparation, our bathing solution consisted of Leibovitz L-15 culture medium with the addition of 5 mM HEPES, 24 mM HCO₃, 1.91 mM EGTA, and 40 μM benzamid hydrochloride. The benzamid was added to slow the cells’ deterioration by blocking the hair cell transduction channels (Jürgensen and Ohmori 1988; Rüsch et al. 1994), but the OHCs would nevertheless continue to swell and shorten. In the standard medium (L-15 without benzamid) the lengths of OHCs decreased by 25% in about 30 min. Adding benzamid to the bath increased the time span to 70 min, but did not eliminate swelling and shortening of the OHCs. Blocking acetylcholine receptors with 10 μM strychnine (Doi and Ohmori 1993; Housley and Ashmore 1991) or adjusting solution osmolarity did not improve the situation. A different strategy to reduce distortion of the OHCs was implemented by Zeddies et al. (2000) and was based on the following line of reasoning. Under normal circumstances in vitro, chloride ions can move freely across the OHC basolateral membrane. The flow of these anions into a cell results in an electrically neutralizing influx of cations and a subsequent increase of cytoplasmic osmolarity. Water then flows into the cell to maintain osmotic balance, and the end result is swelling of the cells. Zeddies et al. (2000) demonstrated in an in vitro cochlear preparation that replacing most of the external chloride by lactobionate, an impermeant anion, can virtually eliminate swelling of the OHCs for time periods up to and beyond 3 h. Our own experience in the hemicochlea preparation has confirmed this effect of lactobionate.

**Access to basal turn of cochlea in vivo**

In vivo stiffness measurements were obtained from the cochleae of adult gerbils (Meriones unguiculatus, >60 days after birth) of either sex. Each gerbil was anesthetized by intraperitoneal sodium pentobarbital (80 mg/kg body weight). Maintenance doses of pentobarbital (17 mg/kg body weight) were given whenever the animal showed signs of increasing arousal, as assessed by a paw withdrawal reflex. After the animal was fully anesthetized, breathing was facilitated by inserting a short length of tubing into the trachea, and body temperature was maintained at 38°C using a heating pad. The animal was positioned...
on its back, and its head was stabilized in a heated head holder. The right submandibular gland was exposed by making an incision from the lower right jaw to the right shoulder. The gland was then ligated and removed to reveal the muscles attached to the bulla and to the styloid bone. These muscles were dissected away to expose the portion of the stapedial artery at the medial plane of the bulla. To minimize the risk of bleeding during further manipulations, the artery was tied off at 2 positions as close as possible to the bulla and was cut between the knots. The bulla was then opened to allow access to the cochlea.

Before opening the cochlea itself, an electrophysiological assessment of its function was made, as follows. A silver electrode was hooked onto the bony rim of the round window, and a ground electrode was placed under the skin at the left jaw. The cartilaginous portion of the external auditory meatus was removed, and a brass speculum was cemented with dental acrylic to the bony portion of the external meatus. The animal was then moved onto a vibration isolation table in a soundproof booth, and a high-frequency tweeter (Realistic, model 40-13108) was coupled to the speculum at the ear canal. To document baseline hearing function, an auditory nerve compound action potential (CAP) threshold curve was measured using a modified tracking procedure (Gummer et al. 1987; Taylor and Creelman 1967). The CAP is generated by synchronized activity in the auditory nerve, and the CAP threshold measured at a particular frequency using narrow-band stimulation is an indicator of cochlear function localized to the characteristic place for the measurement frequency (Dallos et al. 1978). CAP thresholds were measured over a 5-octave range between 1.6 and 50 kHz, with a resolution of 6 steps per octave. The stimuli were tonebursts lasting 10 ms (ramped at 1 ms), and the threshold at a given frequency was defined as the sound level required to generate a 10 μV N1-P1 amplitude in the CAP waveform. The contribution of cochlear microphonics was reduced by averaging responses over 32 consecutive toneburst presentations delivered in pairs of opposite phase. It took about 20 min to obtain a single CAP threshold curve with the range and resolution described above.

After determining the baseline CAP thresholds, the casing around the portion of the stapedial artery overlying the cochlea was removed using a sharp Weaver blade, and the artery was gently flipped out of the way. To expose the basilar membrane, an opening was scored in the bone over scala tympani in the basal turn of the cochlea at a location ranging from 2.0 to 2.8 mm from the base (characteristic frequencies: 17.3 kHz over scala tympani in the basal turn of the cochlea at a location ranging from 2.0 to 2.8 mm from the base). To expose the basilar membrane, an opening was scored in the bone using a sharp Weaver blade, and the artery was gently

Hemicochlea versus in vivo

The essential differences between a hemicochlea and the in vivo cochlea preparation used here are the following: 1) the hemicochlea is removed from the living animal; 2) the hemicochlea entails a gross cut through the tissue, whereas the in vivo cochlea is intact except for a small opening in the lateral wall over scala tympani; and 3) in the hemicochlea all of the fluid spaces are breached and filled with perilymph-like solution, whereas in vivo scala media is filled with endolymp. One important consequence of these differences is a significant alteration of the electrical environment that surrounds the cochlear hair cells. Comparison of the initial zero-current potential of OHCs in vivo (Dallos et al. 1982; Russell and Sellick 1983) to that of isolated OHCs (Ashmore 1988; Housley and Ashmore 1992) reveals a reduction of resting potential magnitude on the order of 25–55 mV in the isolated cells. Although the OHCs in the hemicochlea are not physically separated from the surrounding cells of the organ of Corti, preliminary evidence indicates that the resting potentials of OHCs in the hemicochlea is intact except for a small opening in the lateral wall over scala tympani. Moreover, the cut across the scalae combined with the lack of a blood supply results in a complete elimination of the endocochlear potential (EP), which in vivo is a voltage difference of about +80 mV between scala media and the organ of Corti fluid space. The hair cell resting potentials and the EP together, amounting to an electrical gradient as large as 150–170 mV, normally provide the electrical drive for the transducer currents in vivo. Reduction of these currents results in a reduction of the electrically driven shape and stiffness changes of the OHCs (Brownell et al. 1985; He and Dallos 1999), and so the cochlear amplifier is not expected to function nominally in the hemicochlea.

As has been demonstrated more directly with reversible application of furosemide in vivo, elimination of the EP alone can disrupt the high sensitivity and sharp tuning normally associated with the active cochlea (Ruggero and Rich 1991). Clearly, even in the best case, the hemicochlea can respond no better than a passive cochlea in vivo. Another consequence of the cut across the scalae is that the normal propagation of pressure waves is disrupted. As a result, there is minimal traveling wave behavior in the hemicochlea, but it is still possible to stimulate the tissue mechanically such that local responses can be obtained (Richter et al. 1998b). The cutting and the subsequent maintenance in vitro of a hemicochlea do not seem to induce structural distortions discernable at the light microscopic level: 1) no significant swelling or shrinkage of the tissues occurs with the bathing solution used for the present experiments (Edge et al. 1998), 2) the spatial relationship between the tectorial membrane and the reticular lamina remains well preserved (Edge et al. 1998), and 3) Hensen’s stripe (on the underside of the tectorial membrane) maintains its close relationship to the inner hair cell stereociliary bundle (Richter et al. 1998a). These morphological examinations, in conjunction with the time-series analyses and the possible to stimulate the tissue mechanically such that local responses can be obtained (Richter et al. 1998b). The cutting and the subsequent maintenance in vitro of a hemicochlea do not seem to induce structural distortions discernable at the light microscopic level: 1) no significant swelling or shrinkage of the tissues occurs with the bathing solution used for the present experiments (Edge et al. 1998), 2) the spatial relationship between the tectorial membrane and the reticular lamina remains well preserved (Edge et al. 1998), and 3) Hensen’s stripe (on the underside of the tectorial membrane) maintains its close relationship to the inner hair cell stereociliary bundle (Richter et al. 1998a). These morphological examinations, in conjunction with the time-series analyses and the

Sensor system

A detailed description of the stiffness sensor system is available in Emadi (2001). A stiffness sensor (Fig. 1A) consisted of a stiff steel needle (25–50 μm tip diameter, 200 μm shank diameter, ~1 cm long) attached to a piezoelectric bimorph (1.6-mm-wide series-poled bimorph strip; Piezo Systems, Cambridge, MA) designated as the sensor bimorph. The sensor bimorph was attached end-to-end to another bimorph designated as the driver bimorph (same material as sensor bimorph). The driver bimorph was cemented to a 2-mm-diameter rigid glass rod. Flexible electrical wire was connected to each face of both bimorphs at its proximal end for connection to the stimulus and acquisition hardware. The sensors used here had input stiffnesses on the order of 1–10 N/m. A given sensor (consisting of the needle, sensor and driver bimorphs, glass rod, and electrical connectors) was mounted by its glass connecting rod to a linear actuator (piezo-pusher PZL-007 with drive amplifier PZ 150M; Burleigh Instruments, Victor, NY) used to position the sensor over a 5-μm range. The linear actuator was, in turn, attached to a stage-mounted 3-axis manual micromanipulator (Narashige MMW-203; W. Nuhsbaum, McHenry, IL) used to position the sensor vertically with a precision of 1 μm. For the hemicochlea preparation, a sensor was first positioned with its needle tip in scala tympani, in the plane of the cut edge about 20 μm away from the basilar membrane. The tip

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Stiffness was measured by stimulating the driver bimorph (1,400 ms, 10-Hz sinusoid, 100 ms ON–OFF ramped) to generate motion of the sensor bimorph and needle (about 500 nm peak-to-peak at the needle tip in the no-load condition). It is assumed that the reactance of the sensor bimorph and needle (about 500 nm peak-to-peak at the needle tip in the no-load condition) is negligible, as assessed by examining the signal before and after immersing the tip in the bath fluid. All experiments were controlled with custom stimulus and data acquisition software (Thyme, written by Brian Clark) running on a Pentium PC computer (Midwest Micro). Control and stimulus voltages were delivered with the D/A channels of an arbitrary waveform generator board (Keithley Metabyte AWFG-2).

To derive stiffness values from the sensor signals, the acquired waveforms were further processed off-line, on Apple Macintosh G3 and G4 computers using the analysis and graphing package Igor Pro (WaveMetrics, Lake Oswego, OR). After software filtering (8- to 15-Hz passband) and normalization for the Tektronix 3A9 amplifier gain, a set of “free-field” measurements was selected from the initial steps of the sensor advancement, averaged together, and subtracted from all of the measurements in the series at a given site—this software subtraction removed any inertial signal that was not nulled in hardware. Phase information was inherently included in this subtraction by using the averaged signal itself, rather than only an average of the magnitudes; in any case, it was confirmed experimentally that the free-field signals (mass-dominated) were 180° out of phase with the loaded signals (stiffness-dominated). Finally, for each processed sensor signal from an entire run of measurements at a given site, the peak magnitude was determined using a least-squares fitting algorithm. The peak magnitudes were converted to absolute stiffness values based on the sensor stiffness calibration (described below). All of the data presented here were linearly normalized to a sensor tip diameter of 25 μm by dividing the stiffness values by the factor (d/25 μm), where d is the actual tip diameter of a given sensor; tip diameters ranged from 20 to 50 μm. A linear correction, rather than an area correction, is
based on a parallel beam model for the tissue, for which additional contribution to the return force on the sensor derives from increasing contact along the longitudinal dimension of the cochlea but not from increasing contact across the width of the basilar membrane.

Because the exact position of only the sensor base (proximal end of driver bimorph) was known beforehand, it was necessary to correct for possible compression of the sensor itself before plotting the stiffnesses from a given measurement site against sensor tip position. The position of the sensor tip was estimated from the known sensor base position using an iterative algorithm based on the relative values of the sensor's input stiffness and the tissue stiffness. For each advancement of the sensor base (Δxbase = 1 μm), the next sensor tip position xtip,n+1 was approximated as follows:

\[ x_{\text{tip},n+1} = x_{\text{tip},n} + \Delta x_{\text{tip}} \]

\[ \Delta x_{\text{tip}} = (\Delta x_{\text{base}} k_{\text{sensor}})/(k_{\text{sensor}} + k_{\text{tissue}}) \]

where \( k_{\text{sensor}} \) was the input stiffness of the sensor as determined from the sensor calibration (described below), and \( k_{\text{tissue}} \) was the stiffness of the tissue measured at the previous sensor tip position \( x_{\text{tip},n} \). It was assumed that before contacting the tissue, \( \Delta x_{\text{tip}} = \Delta x_{\text{base}} \).

A sensor was calibrated by using it to measure a series of glass test fibers covering a range of stiffnesses. The sensor response amplitudes for the different test fibers were plotted against the stiffnesses of the test fibers. Figure 1B shows an example of calibration data for one sensor. Such data were fitted with the following equation:

\[ n[k_i] = n_{\text{max}} k_i/(k_i + k_0) \]

where \( n[k_i] \) is the sensor response amplitude to a load with stiffness \( k_i \), \( n_{\text{max}} \) is the maximum voltage response of the sensor, and \( k_0 \) is the input stiffness of the sensor system. This equation is based on modeling the sensor system as a displacement source \( D_\text{x} \) (fixed at a single amplitude for all calibration and tissue measurements) in series with a linear spring \( K_\text{c} \). The parameter \( n_{\text{max}} \) was determined experimentally by measuring the sensor response to a very stiff load, and a least-squares fitting algorithm was used to determine \( K_\text{c} \). Load stiffness \( k_0 \) could then be readily computed from the sensor response voltage \( n[k_i] \) as follows: \( k_0 = K_\text{c} n[k_i]/(n_{\text{max}} - n[k_i]) \). The stiffnesses of the glass test fibers were measured with a “string instrument” (Fig. 1C), modeled after a design published by Zwischenloch and Cefaretti (1989). In our instrument, a tungsten wire (about 70 cm length, 25 μm diameter) is suspended horizontally with one end fixed and the other end free to move. A mass attached to the free end provides tension within the wire. A test fiber is mounted horizontally in a micromanipulator, and its tip is lowered onto the wire (crossing perpendicular to the wire) by moving the base of the fiber in small known increments. The deflections \( \Delta y_i \) of the portion of the wire in front of the objective are measured with an eyepiece reticle. Zwischenloch and Cefaretti (1989) computed the stiffness of a fiber from the tension in the wire (known from the attached mass) and the angular deflection of the wire (determined geometrically). In our implementation, the string instrument was calibrated directly and then the deflection \( \Delta y_i \) was used to determine the force on, and deflection of, the fiber tip. A displacement calibration of the string instrument (\( \Delta y_i/\Delta F_j \)) was obtained by applying a very rigid probe at location \( x_j \), (the location at which the test fiber contacted the wire). A force calibration of the string instrument (\( \Delta y_i/\Delta F_j \)) was obtained by hanging known weights at this same location. The deflection \( \Delta y_i \) and the force \( F_j \) applied to the fiber tip, in conjunction with the known motion \( \Delta y_j \) of the base of the test fiber, were then used to calculate the effective stiffness \( k_{\text{fiber}} \) at the tip of the fiber: \( k_{\text{fiber}} = F_j/\Delta y_{\text{base}} - \Delta y_j \). The test fibers had stiffnesses ranging on the order of 0.3–30 N/m.

R E S U L T S

Figure 1D shows a representative example of a stiffness–deflection curve. Stiffness is plotted as a function of sensor tip position. The zero for the tip displacement is assigned to designate the last measurement that was still in the noise floor. In this way, positions greater than zero can be interpreted as the static deflection of the tissue itself. Just beyond zero deflection, the tissue exhibits an initial rise of stiffness. At intermediate deflections, the tissue exhibits an approximately constant stiffness termed the “plateau” stiffness. For larger tissue deflections, the stiffness increases monotonically to the maximum applied deflection. In accordance with a beam model for the basilar membrane (Allaire et al. 1974; Guummer et al. 1981), stiffness–deflection curves of this type have been quantified by fitting with a quadratic function \( k = k_0 + a(x - x_0)^2 \) over a selected portion of the curve, as indicated in Fig. 1D. The fitted constant \( k_0 \) is a measure of the plateau stiffness. Because the plateau could extend over several microns of tissue deflection, only the portion of the plateau immediately preceding the final rise of stiffness is included for the curve fit. In general, measurements of the type shown in Fig. 1D were obtained in vivo and in the hemicochlea preparation.

In vivo measurements

To assess the viability of the cochlea for the in vivo stiffness measurements, CAP thresholds were measured with stimulus frequencies ranging from 2 to 50 kHz, which encompasses the characteristic frequencies of the stiffness measurement locations. Figure 2, A and B show CAP data from one experiment for which cochlear function was assessed at multiple stages of the preparatory surgery. For this experiment, there was <15 dB loss of sensitivity at the measurement location after the stiffness measurements were completed (arrow in Fig. 2B). Note that, even if the CAP thresholds do not shift at all through the course of an in vivo experiment, measurements of stiffness are still expected to represent the passive mechanical situation. There should be little, if any, contribution from the active process because the measurements were obtained with a stimulus frequency (10 Hz) that is far below the characteristic frequencies for these measurement locations. Accordingly, these stiffness data are important primarily for the fact that the tissue is in its native in situ position.

Figure 1D showed an example of stiffness measured in vivo. These data were obtained at the middle of the pectinate zone of the basilar membrane approaching from the scala tympani side. The plateau stiffnesses were determined for all of the in vivo midpectinate measurements and are shown in Fig. 3A (circles) as a function of exact longitudinal position. As additional confirmation that these measurements reflect the passive mechanics of the cochlea, the effect of death on the stiffness response was examined. Basilar membrane midpectinate stiffness was recorded at the same measurement site at multiple times before and after euthanizing an animal (Fig. 2C). The average plateaus before death (including the points near 0 min) and after death are 0.93 N/m \((n = 7)\) and 0.90 N/m \((n = 6)\), respectively. The plateau stiffnesses measured before and after death are not statistically different \((P = 55\%; \ t\text{-test})\). Even if the lowest point in the premortem data is considered to be an outlier and excluded from the analysis, the difference between the pre- and postmortem data remains statistically insignificant \((P = 57\%)\), and the total scatter of the stiffnesses is <2 dB. As an additional metric of any changes with time in situ, the data were fitted with a linear regression (in log space). The slope of this line is \(-0.00032\ dB/min\), equivalent to a total change of \(-0.04\ dB\) (equivalent to a decrease by a factor

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of 1.005) over 2 h. These results demonstrate not only that the in vivo stiffness measurements do not include contributions from the active process but also that death by itself does not affect the passive mechanical response properties of the tissue, neither immediately nor up to at least 2 h postmortem. As is the case with the hemicochlea (see following text) and with any invasive process, there remains the possibility of some unavoidable damage that occurs on contact with the tissue. Fitting these time-series data with a linear regression yields a slope of 0.00047 (N/m)/min and a correlation coefficient of 0.27. Over a 2-h time course, this slope extrapolates to a total stiffness change of 0.056 N/m, equivalent to 8% or 0.7 dB. All other hemicochlea measurements reported in this study were obtained earlier than 2 h postmortem.

**Edge effect.** Because a hemicochlea is prepared by making a cut across the scale of a cochlea, it is necessary to determine a sufficient depth away from the cut edge for obtaining useful measurements. To this end, stiffness measurements were made at the middle of the basilar membrane pectinate zone at increasing distances from the cut edge (Fig. 4B). The tissue was clearly deflected during the measurement at the cut edge (as

**FIG. 2.** A: compound action potential (CAP) threshold curves from one in vivo experiment, obtained before opening the cochlea (cyan curves), at various stages of preparatory surgery (black curves), and after stiffness measurements were completed (red curves). Characteristic frequency at the stiffness measurement location is indicated by the arrow. B: shift of CAP thresholds relative to averaged baseline. Data indicate that there is <15 dB loss of sensitivity at the stiffness measurement location. C: plateau stiffnesses measured in one experiment in situ (different experiment than depicted in A and B), before and after euthanizing the animal. Line is a fit in log space: \( k = 0.91 \text{ N/m} - 0.00032 \text{ dB/min} \times t \). These data demonstrate that there is negligible change of stiffness up to 2 h postmortem.

**FIG. 3.** A: in vivo (circles) and hemicochlea (dashes) midpectinate plateau stiffnesses as a function of distance from the base of the cochlea. Dashed line is a fit to the hemicochlea data (computed on logarithm of stiffness): \( k = a + bx \), where \( a = 2.10 \pm 1.79 \text{ N/m}, b = -4.43 \text{ dB/mm}, x \) is in mm, and \( Pr = 0.95 \). Arrow indicates the plateau stiffness obtained from the in vivo experiment depicted in Fig. 2. A and B: residuals (in dB) for the in vivo and hemicochlea midpectinate plateau data, relative to the fitted line of A.
monitored visually), but its stiffness could not be discriminated from the noise floor. This low stiffness is most likely attributable to the cut through the tissue. At 80 μm from the cut edge, the stiffness is almost an order of magnitude larger than that measured at 20 μm from the cut edge. For depths greater than about 80 μm, the tissue stiffness asymptotes toward a constant value. These results suggest that, at a sufficient distance from the cut edge, the tissues in a hemicochlea exhibit stable mechanical properties that are relevant to the normal physiology of the cochlea. Based on the results of Fig. 4D, all other hemicochlea stiffness measurements reported in this study were made ≥100 μm away from the cut edge.

EFFECT OF LARGE TISSUE DEFLECTION. In some cases, it was useful to take repeated measurements at a single measurement site. Figure 4C demonstrates that measurements could be obtained repeatedly without a gross change of stiffness unless the tissue was pushed to very large deflections. Measurements obtained after a large (45 μm) excursion exhibit decreased plateau stiffnesses (0.082 ± 0.003 N/m, n = 3) relative to those obtained before the large excursion (0.129 ± 0.015 N/m, n = 4). It is unlikely that there is a simple threshold deflection that determines whether the tissue is damaged. Instead, it is probably the case that larger deflections result in greater damage. To minimize the tissue damage caused by the measurements themselves, the basilar membrane was not deflected more than 25 μm when multiple data traces were to be obtained at the same site or at closely adjoining sites. Note that damage to the stereociliary complex may occur even for submicron deflections (Dallos 2003). This particular damage may be an avoidable consequence of the point stiffness measurements. A more thorough discussion of point measurements in the context of normal physiology is provided below.

ANISOTROPY: DEPENDENCY ON SENSOR APPROACH ANGLE. A series of measurements was made in the hemicochlea with the sensor approaching at multiple angles relative to the transverse axis (Fig. 4D). The solid line in Fig. 4D is a cosine fit through the plateau stiffness data. The largest stiffness does not appear exactly along the designated 0° axis. The fit yields a peak stiffness of 0.86 ± 0.11 N/m at an approach angle of −33 ± 10° (χ² = 0.40), which is directed through the organ of Corti along the long axis of the third row of outer hair cells. This result has several implications. First, it supports the contention that the “point” stiffness measured at a particular structure has contributions from all of the connected structures. Consistent with this notion, Naidu and Mountain (1998) demonstrated a decrease in basilar membrane stiffness after removing the organ of Corti. Second, because the cochlear tissues in general are not isotropic structures (and because the partition as a whole has inherent directionality in its architecture), the point stiffness is not simply a function of position but is also a function of the direction in which force is applied. As a consequence, micromechanical models must account for the directional dependency of mechanical impedances to generate accurate reproductions of motion patterns within a cochlear cross section.

**Basilar membrane stiffness profiles**

Figure 5A shows all of our hemicochlea stiffness data (excellent preparations only) as a function of tissue deflection for different radial positions on the basilar membrane. As indicated by the diagram above each column, the data were ob-

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**FIG. 4.** A: plateau stiffness as a function of time post-mortem in a hemicochlea, demonstrating little change of stiffness over the time course of a typical experiment. Data were obtained in the basal turn of the middle of the basilar membrane pectinate zone and have been fitted with a linear regression: \( k = a + bt \), where \( a = (0.685 \pm 0.035) \) N/m, \( b = (0.000467 \pm 0.000416) \) (N/m)/min, and \( Pr = 0.270 \). B: plateau stiffness as a function of distance from the cut edge in a hemicochlea. Data were obtained in the middle turn at the middle of the basilar membrane pectinate zone. Data have been fitted with an exponential curve. C: plateau stiffness before and after a relatively large (45 μm) tissue deflection. Data were obtained in the upper basal turn of a hemicochlea at the middle of the basilar membrane pectinate zone. Arrow indicates the last measurement before the large deflection. D: plateau stiffness as a function of sensor approach angle, defined in the inset. Data were obtained in the basal turn of a hemicochlea at the middle of the basilar membrane pectinate zone. Solid line is a cosine fit with a maximum at −33°.
FIG. 5. A: basilar membrane stiffness traces shown as a function of sensor tip excursion. As indicated by the diagram above each column, the data were obtained at the following radial positions: middle of the pectinate zone, halfway between midpectinate and the outer pillar foot, the outer pillar foot, and the middle of the arcuate zone. Within each graph, red lines indicate data obtained from the basal turn location (2.9 mm from base), green lines indicate data obtained from the upper basal turn location (5.5 mm from base), and blue lines indicate data obtained from the middle turn location (7.3 mm from base). B: data from A, replotted in log–log coordinates. C: scatter diagrams of slopes in dB/dB of curves in B, computed within a sliding 5-point window, shown as a function of tissue deflection at the middle of the window. Color coding for measurement location is the same as in A and B. D: averages and SDs of stiffnesses averaged within several different 5-μm tissue deflection ranges, shown as a function of distance from the base of the cochlea. Dashed line in each graph is the fitted line determined by Naidu and Mountain (1998) for their equivalent radial position on the basilar membrane.
tained at the middle of the pectinate zone, midway between the midpectinate site and the foot of the outer pillar cell, at the outer pillar foot, and at the middle of the arcuate zone below the fluid-filled tunnel of Corti. Within each graph, stiffness data are shown for multiple locations along the length of the cochlea, as distinguished by line color. To provide further perspective on the variation of stiffness with radial position, data from several individual hemicochleae are shown in the 3-dimensional scatter plots in Fig. 6. Measurements could be made all the way to the osseous spiral lamina (modiolar side) but, because of inaccessibility, typically not all the way to the spiral ligament (outer wall side). The data in Fig. 6 show that, from the middle of the pectinate zone, the stiffness is either relatively constant or drops slightly until nearing the outer pillar foot, where the stiffness shows a large increase. Although in some cases a trough is seen, the stiffness in the arcuate zone is typically comparable to that observed below the outer pillar foot. Near the margins of the basilar membrane (i.e., at the spiral lamina and the spiral ligament), there is a dramatic increase of stiffness, as expected.

**FIG. 6.** Four examples of 3-dimensional scatter plots of stiffness data. A and B: examples from the basal turn location (2.9 mm from the base). C: example from the upper basal turn location (5.5 mm from the base). D: example from the middle turn location (7.3 mm from the base). In each panel, the vertical axis indicates stiffness in units of N/m. The x-axis (running approximately from left to right in each panel) indicates the radial position along the width of the basilar membrane. Zero on this axis represents the position at the middle of the pectinate zone, and the “OP” label marks the position below the outer pillar foot. The y-axis (running approximately from “front” to “back”) indicates the sensor tip excursion in μm, with zero representing initial contact with the tissue at any given radial position. Note that the various axis limits are not the same across all panels.


**Discussion**

**Point stiffness measurements**

Under physiological conditions, the stimulus driving the basilar membrane is a distributed fluid pressure, ultimately derived from incoming sound waves. In contrast, for the basilar membrane stiffness measurements described in this and previous studies, the stimulus is a focal point force applied by a probe at a single radial position on the basilar membrane. Associated with this difference in stimulation mode is a difference in the magnitude of basilar membrane deflection. For the point stiffness measurements, the tissue is deflected on the order of 10s of microns at the measurement site. Normal physiological basilar membrane motion is in the submicron range, even for high-level sound stimulation (e.g., Ruggero et al. 1997). The stiffness measurements clearly involve tissue deflections that are significantly larger than sound-induced motion in vivo, and it is important to consider some potential pitfalls with these measurement methods. Previous investigators (Gummer et al. 1981; Miller 1985; Naidu and Mountain 1998; Olson and Mountain 1991) have argued that the physiologically relevant return force from the basilar membrane is attributed to the embedded radial fibers and that the measured plateau stiffness represents the stiffness of these fibers. We also have adopted this interpretation, but there is the possibility that the relevant stiffness occurs at much smaller tissue deflections and may be buried in the noise. Another distinct possibility is that the large deflections during the stiffness measurements incur immediate damage to the stereociliary complex. If this complex normally contributes to the stiffness at the basilar membrane, its contribution will not easily be observed with the point stiffness measurements. Acknowledging that some caution is necessary, we assume for our discussion here that the point force measurements provide a useful indication at least of spatial gradients of mechanical properties within the cochlea. Further caution is likely necessary when comparing these measurements directly to values of in vivo response properties.

With respect to our choice of using a quadratic function to fit the stiffness–deflection curves at the midpectinate site (as depicted in Fig. 1D), some further discussion and justification are necessary. Our use of the quadratic fit follows previous work in which the basilar membrane is approximated by parallel beams sustaining transverse deflections (Allaire et al. 1974; Gummer et al. 1981). A recent immunohistochemical study in the rabbit cochlea has demonstrated that Type II collagen is an important constituent of the fibrous components of the basilar membrane (Dreiling et al. 2002). Type II collagen is a constituent also of hyaline cartilage and vitreous body of the eye (Fung 1993). Although the biomechanical properties will, of course, depend on the exact configuration of the collagen fibrils and on the nature of other proteins in the tissue, to a first approximation we may expect response properties of the basilar membrane to be generally similar to that seen in these other types of tissue. In cartilage, for example, the stress–strain relation at small deformations has been modeled using a power law function. For our purposes, however, we maintain use of the quadratic fitting function because the primary purpose of our fit is to obtain values for the plateau stiffness (i.e., the constant term), which has been observed consistently in our data and in previous work. Although ultimately it may be more appropriate to fit the rising portion of the stiffness–deflection curves with a nonquadratic function (see Fig. 7 and accompanying discussion), we believe that for the purpose of obtaining plateau values, the quadratic fit is accurate and sufficient.

**Comparison of hemicochlea to in vivo data**

Because there are important differences between a hemicochlea and a cochlea in vivo, it is necessary to examine the extent to which the hemicochlea is useful in the study of normal physiology. For the present work, the crucial question is whether the cutting procedure results in material property changes that could impede proper measurement of driving point stiffness. We can demonstrate, by a direct comparison of point stiffness data from the hemicochlea and from the in vivo cochlea, that there is no statistical difference of local elastic properties between these preparations. In Fig. 3A, stiffnesses measured at the middle of the basilar membrane pectinate zone were shown as a function of distance from the basal end of the basilar membrane for both preparations. The hemicochlea data were fitted with a line (in log-space), and the deviation from this line has been computed for each point from both preparations (Fig. 3B). These residuals were then grouped by type of preparation, and the 2 populations of residuals were compared and found to be statistically the same ($P = 62\%$, Student’s t-test). The fact that the 2 sets of residuals are not statistically different implies that the stiffnesses measured in the 2 preparations are not quantitatively different. We recognize that this analysis and comparison assumes that the underlying model of stiffness as a function of longitudinal location is accurate. Moreover, it may make more sense to compute the fitted line from the in vivo data, rather than the hemicochlea data. Unfortunately, the in vivo data are tightly clumped in terms of measurement location, and the resulting fit would be very sensitive to small perturbations of the data. Consequently, we chose to use the hemicochlea data for the curve fit and to acknowledge the potential pitfalls of this analysis.

**Comparison of stiffness data to previous work**

Previous data on basilar membrane stiffness as a function of tissue deflection are available for the guinea pig (Gummer et al. 1981; Miller 1985) and for the gerbil (Naidu and Mountain 1998; Olson and Mountain 1991, 1994). Cross-species differences complicate a direct quantitative comparison between the present data and the guinea pig data, but at least a qualitative comparison can be made. In terms of the initial rise, plateau, and quadratic increase, our stiffness–deflection curves at the midpectinate site (Fig. 5A, first column) of the gerbil are similar to those measured by Gummer et al. (1981) in the guinea pig. The present data are somewhat different from those reported by Miller (1985) for the guinea pig. Whereas Miller reported observing 3 distinct plateaus, our gerbil hemicochlea data reveal only one plateau that can clearly be discerned from the noise floor.

Our stiffness measurements can be more directly compared with the previously reported stiffnesses in the gerbil cochlea. Our data are qualitatively similar to those of Olson and Mountain (1991) except for their initial plateau, which we did not regularly observe. The data of Naidu and Mountain (1998) obtained on the gerbil basilar membrane below the Hensen cells are used for a quantitative comparison to our data. This
radial position is most equivalent to the middle of the basilar membrane pectinate zone, and the plateau stiffnesses were determined in the same manner used here. We compare our plateau data to their second plateau data. Based on their reported equation for stiffness as a function of position along the length of the cochlea (their Fig. 5), their data yield a value of...
2.23 N/m for the plateau stiffness at a longitudinal location equivalent to the basal turn location of a hemicochlea (25% from base to apex). At this location in the hemicochlea, the average plateau stiffness is 0.79 N/m. After a linear one-dimensional correction for the sensor tip diameters (25 μm here compared with 10 μm for their sensor), their values are larger than those reported here by a factor of 7.0. Correcting for probe diameter (as opposed to probe surface area) is based on the beam model for the basilar membrane; a correction based on probe surface area increases the discrepancy to a factor of 17.7. This difference in magnitude between the gerbil hemicochlea data and the gerbil cochlear turn preparation (Naidu and Mountain 1998) needs to be addressed more thoroughly.

There are several possible reasons for the difference. The cochlear turn preparation was bathed in Leibovitz L-15 medium (similar in ionic composition to perilymph). The hemicochlea was bathed in an artificial perilymph containing less calcium than L-15 (which has about 1.3 mM Ca2+; Gibco BRL) and with most of the chloride replaced by lactobionate, an impermeant anion. This solution was designed with the specific aim of reducing swelling and deformation of the tissue (the tectorial membrane, in particular) relative to its initial state (Edge et al. 1998). In an isolated cochlear preparation bathed in standard L-15, both the tectorial membrane and the OHCs are expected to swell and deform within 1 h (see METHODS, regarding bathing solution). The structures in the hemicochlea were monitored throughout the experiments to ensure that at least no visible deterioration occurred. In addition to different bathing solutions, the stiffness measurements were obtained with slightly different sensor configurations. The sensor used by Naidu and Mountain (1998) was of the same design as that described by Olson and Mountain (1991). The sensor system used for the present work was of generally similar design, but was configured to have an input stiffness comparable to that of the measured tissue (see METHODS). Our sensor system had an input stiffness on the order of 1–10 N/m, depending on the particular sensor being used, whereas the sensor used by Naidu and Mountain had an input stiffness on the order of 3,500 N/m (Olson and Mountain 1991). The sensors used in the present project were calibrated against test stiffnesses in the range of stiffnesses measured for the tissue. The sensor used by Naidu and Mountain was calibrated against stiffnesses larger than those typically measured in the tissue. Furthermore, they assumed a linear relationship between sensor voltage and load stiffness, whereas our calibration corrects for nonlinear effects attributed to the finite sensor input stiffness. If the sensors are nonlinear, as suggested here, but a linear calibration is assumed, then a mismatch between the sensor and tissue stiffnesses can result in a significant distortion of the reported stiffness values. It is possible that, when loaded against the physiological range of stiffnesses, their sensor reported results that were skewed to higher values. In summary, calibration errors and tissue condition are possible sources of discrepancy between the 2 sets of stiffness data.

**Longitudinal coupling**

Plateau stiffnesses measured at increasing distances from the middle turn cut edge of a hemicochlea are shown in Fig. 4B. The change of stiffness very close to the cut edge is used here to quantify the longitudinal coupling within the basilar membrane itself. The reasoning is that the extent to which abnormal conditions at the cut edge manifest themselves further along the spiral can be exploited as a measure of the longitudinal coupling. Because the scalae in the hemicochlea are hydrodynamically shunted, the effects of fluid pressure differentials should be negligible. Consequently, the coupling space constant computed here is expected to reflect an inherent property of the basilar membrane/organ of Corti complex. To compute the space constant, the plateau stiffnesses in Fig. 4B are mapped into logarithmic coordinates and then fitted with an exponential rise to an asymptote. The following function is used to achieve both the remapping and the exponential fit

\[
\ln[\kappa(z)] = \ln[\kappa_0] + (\ln[\kappa_0] - \ln[\kappa_0])(1 - e^{-z/\zeta})
\]

where \(\ln[\cdot]\) is the natural logarithm operator, \(\kappa(z)\) is the stiffness in N/m at depth \(z\) from the cut edge, \(\kappa_0\) is the stiffness in N/m at \(z = 0\), \(\kappa_0\) is the asymptotic stiffness in N/m, and \(\zeta\) is an exponential space constant for the change of stiffness away from the cut edge. The curve fit yields the following coefficients: \(K_0 = 0.00048 \pm 0.00167\) N/m, \(K_0 = 0.06930 \pm 0.00407\) N/m, and \(\zeta = 20.6 \pm 7.6\) μm. \(K_0\) is approximately an order of magnitude below the noise floor and so is effectively 0, as would be expected. The space constant \(\zeta\) has a value of 21 μm, comparable to the width of 2 OHCs and supporting previous evidence that there is relatively little longitudinal coupling within the pectinate zone of the basilar membrane (e.g., Miller 1985; Richter et al. 1998b; Völfrich 1978). For comparison, Naidu and Mountain (2001), who pushed on the basilar membrane with a rigid probe and optically measured deflections of nearby structures, reported a value of \(\approx 40\) μm for the longitudinal coupling space constant at the equivalent of our middle turn location (based on their Eq. 3).

**Radial stiffness variations**

As indicated by our data in Figs. 5A and 6, the overall magnitude of the stiffness–deflection curves changes with radial position along the width of the basilar membrane. In general, the stiffness at the middle of the pectinate zone (0 on the radial axis of the surface plots) is lower than that at the foot of the outer pillar cell (marked with “OP” in each panel of Fig. 6). There is sometimes a slight decrease of stiffness from the middle of the pectinate zone toward the outer pillar foot, before the large increase near the outer pillar foot is seen. Stiffness measured at the modiolar edge of the basilar membrane (i.e., next to the osseous spiral lamina) is dramatically larger than for other radial positions. This large stiffness makes sense in light of the close proximity to the attachment zone at the bone. At the spiral ligament, which is the outer attachment zone of the basilar membrane, it was difficult to obtain data for all but the basilar turn. The available data demonstrate a large increase of stiffness near this margin, similar to the increase seen on the modiolar side. These results are, for the most part, similar to previous measurements of this type (Naidu and Mountain 1998; Olson and Mountain 1994; von Békésy 1960).

Plateau stiffness values, based on an underlying beam model, have been used previously and in the present work to quantify the midpectinate site of the basilar membrane. Here, we examine whether this same model is appropriate for other radial sites on the basilar membrane. The stiffness–deflection curves of Fig. 5A have been replotted in log–log coordinates.
The slopes of these curves in log–log space provide an indication of the “growth order” of the stiffness as a function of tissue deflection (Fig. 5C). In a first approximation, a slope of 0 dB/dB corresponds to constant stiffness, 1 dB/dB corresponds to linear growth, 2 dB/dB corresponds to quadratic growth, and so forth. Figure 7 shows histograms of the slopes computed over selected 5-μm ranges of deflections for the 4 measured radial sites in the basal turn location. For both pectinate zone sites (middle and halfway toward the outer pillar foot) the growth order is centered between 0 and 1 for deflections ≤20 μm. For the largest deflection range (20–25 μm), the stiffness growth at the midpectinate site spreads to higher orders centered near 2, corresponding to quadratic growth; no data were obtained at the largest deflections for the other pectinate site. The growth of stiffness at the midpectinate site is consistent with the beam model (Allaire et al. 1974; Gummer et al. 1981)—constant stiffness followed by quadratic growth. In contrast, the slope histograms for the outer pillar foot and the midarcuate sites demonstrate a shift to higher-order stiffness growth at smaller deflections. Moreover, at the larger deflections, the growth is spread more evenly among the higher terms rather than centered around 2. We conclude that the beam model, along with the plateau stiffnesses derived from it, is not appropriate for all regions of the basilar membrane; this conclusion is not inconsistent with previous modeling of biological tissue, for which nonquadratic growth of stiffness with deflection has been demonstrated (Fung 1993). Note that this slope analysis provides exact results only for monominal functions passing through the origin. For polynomial functions or for functions displaced along either axis, the growth values can become skewed. In spite of this potential complication, the data were not rescaled or offset before computing the slopes in log–log space because the intent was to examine the raw data without assuming any underlying model. Because the data in Figs. 5C and 7 provide only an approximation of the growth of stiffness with deflection, they cannot be used to determine accurately an appropriate underlying model for the different parts of the basilar membrane. Instead, the histograms are intended only to verify a qualitative change in basilar membrane behavior in going from the pectinate zone to the outer pillar foot and on to the arcuate zone. As described below, a difference is seen also in the estimated magnitudes of the longitudinal gradients for the different radial sites.

**Longitudinal stiffness gradients**

Gradients of stiffness along the length of the cochlea are of interest for several reasons. In conjunction with changes of tissue dimension and associated changes of inertia, the stiffness gradients may play an important role in the place-dependent tuning of the basilar membrane; von Békésy (1960), for example, demonstrated that adding a gradation in stiffness to a synthetic basilar membrane could induce a separation of sound frequencies along its length. The stiffness gradients probably contribute to the varying phase response along the length of the cochlea and thus to the origin of traveling wave behavior. The gradients may be important for the proper functioning of the cochlear amplifier by influencing the locations at which boosting occurs for a given input sound frequency. Figure 5A demonstrates that, for any given radial position on the basilar membrane, the absolute stiffness decreases toward the apex of the cochlea. This result is in qualitative agreement with previous studies on the basilar membrane (Naidu and Mountain 1998; von Békésy 1960). In this section, the stiffness gradients are quantified and compared with the previous work.

It is assumed in a first approximation that there is a constant logarithmic gradient of stiffness along the length of the cochlea. For the midpectinate site of the basilar membrane, an estimate of this gradient can be computed from the plateau stiffnesses, already shown as a function of longitudinal position in Fig. 3A. The line is a best fit to the plateau stiffnesses, computed in log coordinates and extrapolated to both ends of the cochlea. The slope of this line is −4.43 dB/mm, corresponding to a total stiffness change by a factor of 335 (−50.5 dB) when extrapolated over the entire 11.4-mm gerbil cochlea. This stiffness change is 8.4 times larger than the 41-fold (−32 dB) change reported by Naidu and Mountain (1998) in their gerbil cochlear turn preparation at the “Hensen cell” position, which is most analogous to our midpectinate position.

As discussed previously, use of the plateau stiffness value is based on a beam model for the basilar membrane, and we have argued that this model is not necessarily valid for all radial sites on the basilar membrane. Without assuming any underlying model, longitudinal gradients could be estimated for all of the measured radial sites on the basilar membrane, as follows. For each available stiffness–deflection curve from a given radial site at a given longitudinal location, the stiffness values were averaged (after conversion to log units) over consecutive points within several 5-μm tissue deflection ranges. These “segmental” averages are shown in Fig. 5D, where the abscissa in each graph indicates distance from the base of the cochlea; the different symbols and line colors indicate averages computed over different tissue-deflection ranges. Several estimates of the stiffness gradient for each radial site were computed by fitting lines to the segmental averages as a function of longitudinal location. For both pectinate zone sites, the gradients are similar to the value of −4.4 dB/mm at the midpectinate site computed using the plateau stiffnesses. The stiffness gradient estimates are shallower for the other radial sites, particularly for the midarcuate position. Included in each graph of Fig. 5D is the fitted line determined by Naidu and Mountain (1998) for a corresponding radial position on the basilar membrane in their gerbil cochlear preparation. We have not normalized their data to account for differences in size of the sensor probe tips because a constant scaling will shift these lines up and down but will not affect their slopes. Note that, whereas our gradients in Fig. 5D were based on stiffnesses averaged over particular deflection ranges, the gradients reported by Naidu and Mountain were all based on plateau stiffnesses derived from their stiffness–deflection curves from each radial site. According to Fig. 5D, their reported gradients are shallower than the present results for the pectinate zone sites, but are similar for the other sites. The conclusion for the midpectinate site is the same as was obtained by the direct comparison of our plateau stiffnesses against theirs.

**Role of stiffness in frequency–place mapping**

To examine whether our measured gradient of stiffness could account for the known change of best frequency along the length of the cochlea, we used 2 models of basilar membrane motion. The first is a relatively simple lumped-parameter
model incorporating a one-dimensional spring–mass resonance. The second is a fluid model presented by Steele and Zais (1983). As shown below, the result of our analyses from both of these models is that the stiffness gradient does, in fact, yield the known change of best frequency along the length of the cochlea. This result is in contrast to that presented by Naidu and Mountain (1998), whose measured stiffness gradient did not account for the known frequency–place map.

**MODEL 1: SPRING–MASS RESONANCE.** Here we examine the effect of the measured stiffness gradient in conjunction with estimated changes of tissue mass. In this analysis, some gross simplifications of the cochlea’s mechanical behavior are applied. First, the cochlea is conceptually divided along its longitudinal dimension into many short segments, with each segment modeled as a friction-free lumped-parameter system having a single mass suspended by a single stiffness attached to a rigid support. Such a system exhibits a resonance at a radial frequency equal to \( k (m)^{0.5} \), where \( k \) is the stiffness and \( m \) is the mass (Rossing and Fletcher 1995). Second, the lumped-stiffness parameter of the entire cochlear cross section at a given longitudinal location is approximated with the basilar membrane plateau stiffness at the midpectinate site. The measured best frequencies at the basal and middle turn locations of the hemicochlea are 9.4 and 2.0 kHz, respectively (Richter and Dallos 2003), corresponding to a decrease by a factor of 4.7 (2.2 octaves) between these 2 locations. If the stiffness gradients for different positions across the width of the basilar membrane imply either that more elements are required for an accurate reproduction of the motion patterns in the passive cochlea or that the roles of various regions of tissue may shift in importance along the length of the cochlea. It is interesting in this regard that motion measurements at many individual points within a cochlear cross section indicate that the best frequency is the same for all structures at a given longitudinal location (Richter and Dallos 2003). This latter result suggests that all elements separately have similar resonance characteristics or that a subset of the cochlear structures dominates the frequency response and the remaining structures are entrained to this subset. A third point regarding our analysis is that the lumped model assumes motion along a single linear axis, whereas rotational motion patterns may play an important role in the normal function of the cochlea. A fourth point is that we have not taken into account any contribution of mass from the fluid layers above and below the basilar membrane/organ of Corti complex. Finally, it should be clear that, although it is informative on a basic level, the spring–mass model used here is inherently insufficient to aid in understanding the details of either the active response of the cochlea (i.e., action by the outer hair cells) or the stimulation of the hair bundles of the hair cells.

**MODEL 2: 3-DIMENSIONAL FLUID.** Steele and Zais (1983) quantified the motion of the basilar membrane using a 3-dimensional model that, in contrast to the spring–mass model above, incorporates effects of the fluid layers surrounding the cochlear partition (see also Steele and Taber 1979, 1981). In this model, the basilar membrane is approximated by a hinged plate that has as its compliance the compliance of the basilar membrane pectinate zone. A frequency–place map can be derived from their formulation for the “transition point” along the length of the basilar membrane as a function of frequency: \( 2 \rho \omega^2 = \pi^2 l/(120C) \), where \( \rho \) is the fluid density, \( \omega \) is the radian frequency \((2\pi)\), and \( C \) is the volume compliance at the transition point (Steele and Zais 1983; their Eq. 2). The volume compliance can be expressed as \( C = L^2/(120D) \), where \( D \) is the effective plate bending stiffness and \( L \) is the width of the basilar membrane pectinate zone ( Olson and Mountain 1991; their Eq. A5b). For a probe loaded at the center of the basilar membrane, assuming simply supported edges and a small probe diameter relative to the pectinate zone width, the point stiffness can be approximated from the plate bending stiffness:

\[
k \approx 48dD/L^3,
\]

where \( k \) is the point stiffness and \( d \) is the probe diameter. Combining these expressions, the best frequency can be computed from the point stiffness at a given location: \( \omega^2 = \pi^2 k/(96L^2pd) \). From this derivation based on the Steele and Zais (1983) model, we can compute the expected best frequencies for our basal and middle turn measurement locations. Our probe diameter \( d \) was \( 25 \times 10^{-6} \text{ m} \), and the fluid density \( \rho \) is \( 10^3 \text{ kg/m}^3 \). From our present results, the midpectinate plateau stiffness \( k \) is 0.79 N/m for the basal turn and 0.084 N/m for the middle turn. The pectinate zone width \( L \) is \( 168 \times 10^{-6} \text{ m} \) for the basal turn and \( 192 \times 10^{-6} \text{ m} \) for the middle turn ( Edge et al. 1998; their Table 2). The computed best frequencies are then 9.5 kHz for the basal turn and 2.7 kHz for the middle turn. These estimated values are in reasonable agreement with the experimentally measured values of 9.4 and 2.0 kHz, respectively, for the basal and middle turn locations in the hemicochlea (Richter and Dallos 2003). The conclusion from this
analysis is the same as that obtained above using the spring-mass resonance model: that is, our measured stiffness gradient can account for the change of best frequency along the length of the cochlea.

DISCLOSURES

This work was supported by the National Institute for Deafness and Other Communication Disorders (DC-00708) and by the National Science Foundation (IBN-0077476). G. Emadi was supported in part by a grant from the Hugh Knowles Center, Northwestern University.

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