Direct Visualization of Organ of Corti Kinematics in a Hemicochlea

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INTRODUCTION

The organ of Corti in the mammalian cochlea, situated between the basilar membrane and the tectorial membrane, is an elaborate matrix of sensory cells and supporting cells (e.g., Lim 1986). It is commonly hypothesized that the organ of Corti transforms basilar membrane vibration into a shearing stimulus at the apices of the sensory hair cells. The vibration pattern of the basilar membrane itself is now well understood (Rhode 1971; Robles et al. 1986, 1991; Ruggero et al. 1997; Sellick et al. 1982). Some information on the motion of the reticular lamina is now also available (Ulfendahl et al. 1995). However, motion patterns of the cellular elements within the organ of Corti have not been observed to date, except to a very limited extent. Motion of Hensen’s cells in relation to that of the basilar membrane has been studied with electrical stimulation (Mammano and Ashmore 1993). Electrically evoked motion of outer hair cells and inner hair cell was measured at the apical turn of the gerbil cochlea (Karavitaki et al. 1997). Nevertheless, the transformation from basilar membrane displacement to hair cell stimulation has never been demonstrated experimentally. The widely accepted ter Kuile (1900) model suggests that the organ of Corti rotates about an axis that is different from the axis of rotation of the tectorial membrane. Because both bodies have been assumed to behave as stiff beams at low frequencies, these two different axes of rotation generate shearing motion and the consequent bending of the cilia. This idea has been revived by Davis (1958) and further formalized by Rhode and Geisler (1967). An alternative model (von Bekesy 1953) attributes the shearing motion to the bending of the organ of Corti as a thick elastic plate. Yet other theories suggest that the tectorial membrane and the stereocilia form a distributed resonant system (Allen 1980; Zwislocki 1980).

The organ of Corti has a complex asymmetric structure. Different cell groups within the organ presumably have greatly different mechanical properties (Tohomo and Holley 1996). Explants of half turns from guinea pig cochleae have been used to measure radial and transversal motile responses of the reticular plate of the outer hair cells with transepithelial electrical stimuli (Reuter and Zenner 1990). In this preparation the cochlear explant is placed on the bottom of a Petri dish. Therefore the mechanical suspension of the cochlear partition is different from the in vivo situation. Further, only the motion of the reticular lamina can be studied. The internal kinematics of the organ of Corti remains concealed. In the work of Ulfendahl et al. (1995) confocal laser interferometry was used on the apex of an isolated cochlea preparation to compare reticular lamina and tectorial membrane motions. Making measurements with multiple beam angles permitted the investigators to assess both transversal and radial motion components. They found that, although the reticular lamina had sizable radial motion, the tectorial membrane moved only transversally. In another study, glass beads were placed on the basilar membrane, and Hensen’s cells while extracellular electrical stimulus was applied (Mammano and Ashmore 1993). In this case, motions of the basilar membrane and Hensen’s cells can be compared. However, the displacement of the glass beads was measured with laser interferometry, which limits the measurement to transversal motion only. The shearing motion at the reticular lamina, which is crucial for the transduction process, cannot be detected with this method. A preparation that can render a cross-sectional view of the cochlear partition would provide the most information on the internal kinematics of the organ of Corti. We have developed such a preparation, the hemicochlea, which enables the direct observation of all parts of a given cochlear cross-section (Hu et al. 1995). A video optical flow technique was also developed to measure the motion patterns of elements within the organ of Corti. A similar video technique was used to study the motions of the alligator lizard basilar papilla (Davis and Freeman 1995). De-

* Deceased September 1997.

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termed mechanical behavior between our measurements and the predictions by the classical model. The present work was limited to very low frequencies; hence, dynamical phenomena were not explored.

**METHODS**

**Animal preparation**

Young adult Mongolian gerbils (*Meriones unguiculatus*), 22–25 days of age after birth were used. The gerbils were overdosed with pentobarbital sodium (200 mg/kg) and then killed by rapid cervical dislocation. The auditory bulla was isolated from the temporal bone and trimmed to expose the cochlea. The whole cochlea was mounted on a vibratome (Series 1000, TPI, St. Louis, MO). A commercial Wilkinson razor blade was manually broken into two halves, and one half was mounted on the vibratome. The cochlea was glued on a mounting stub and was oriented so that the sectioning plane of the vibratome would coincide with the midmodiolar plane of the cochlea. The cochlea was then sliced into two hemicochleae. During slicing, the preparation was submerged in L-15 (GIBCO) adjusted to pH 7.35 and osmolarity 310 mOsm. The same medium was also used for immersing the hemicochlea during later measurements.

The viability of the hemicochlea preparation was examined with Live-Dead Assay (Molecular Probes, Eugene, OR). The assay agent consisted of 2 μM calcein-AM and 4 μM ethidium homodimer in Dulbecco’s phosphate-buffered saline (DPBS). The preparation was stained in the dark at room temperature without agitation for 30 min and examined under a scanning confocal microscope without subsequent washing. The various cellular structures of the organ appeared to be viable within 1.5–2 h after dissection (Fig. 1A). After that period the cellular components gradually lost their integrity (Fig. 1B). Red fluorescence, indicating permeable cell nuclear membrane, increased during prolonged observation (2.5–5 h). The usual micromechanical measurements on the hemicochlea take ~1–1.5 h. According to the assay, the cellular components should be in viable condition during such a data collection period.

Twenty-five animals were used in these experiments. Successful sectioning, as judged by a lack of tissue distortion at the cut edge, was achieved for 12 animals. Complete video measurements were obtained for five hemicochleae.

**Imaging**

One resultant hemicochlea was supported by vacuum grease on the bottom of a Petri dish. The Petri dish was then mounted on an upright microscope (Leitz Medilux) equipped with a ×40, 0.75NA, working distance 1.75-mm water-immersion objective (Zeiss). The orientation of the hemicochlea was adjusted so that one cochlear cross-sectional unit (hemi turn) lay in the focal plane of the microscope. A custom-built multiple oblique illuminator (Hu 1998) was fitted onto the microscope to enhance the contrast of the organ of Corti and the tectorial membrane. The orientation and lateral positioning of the light source was adjusted to achieve optimum contrast for each preparation. To maintain a stable illumination level, a regulated DC power source (ripple factor <0.1%) was used to control the microscope illuminator. Image of a chosen turn of the hemicochlea was acquired with a Panasonic MF-602 CCD video camera. The video output of the camera was digitized with an 8-bit LG-3 frame grabber (Scion, MA) at a rate of 30 frames per second. The digitized image had a resolution of 640 by 480 pixels. A customized control program was developed to adjust the amplification ratio and baseline level of the frame grabber. This program could ensure that the full dynamic range of the frame grabber was utilized and no overflow was present. The digitized video sequence was stored in a Macintosh PowerPC 900 computer with 38 MB of RAM.

**Mechanical stimulation**

Micropipettes were pulled from 1.2-mm OD fiber-filled glass tubing to a tip of 20 μm. The tip was then heat polished and fused to a 40-μm spherule. This fabricated glass actuator was positioned below the middle point of the pectinate zone of the basilar membrane (Fig. 2). Placement of the actuator was ~40–50 μm below the plane of sectioning. To ensure good contact between the actuator and the basilar membrane, the former was moved to push the basilar membrane away from its resting position and was then pulled back. The image of the cochlear partition was captured both before and after apposing the driver pipette to the basilar membrane. The resting position of the actuator was adjusted to minimize the difference between these two images. This assured that the basilar membrane was at its resting position for null input to the stimulating actuator. The actuator was driven with a pneumatic micromanipulator (TPI, St. Louis, MO) to vibrate in a direction perpendicular to the plane of the basilar membrane. The vibration amplitude of the actuator was ~0.5 μm peak-to-peak, corresponding to an equivalent sound stimulus of ~107 dB SPL (Ruggiero and Rich 1991). The stiffness of the basilar membrane changes little for this amount of displacement (Olson and Mountain 1991); in other words, the stimulation was within the elastic range of the cochlear partition. There was a DC component in the stimulus (Fig. 3B). This would not affect the measured vibration pattern when only the linear response of the cochlear partition was to be examined.

The driving signal was a 1 to 2 Hz sinusoidal command generated with a Wavetek waveform generator. The video acquisition timing was synchronized with this driving signal. However, the signal period produced by the Wavetek generator was not an integral multiple of the video frame period. As discussed below, this produces some quantitative inaccuracies for sampling frames at the beginning and at the end of the acquisition period. The driving signal was first amplified 10-fold with an AC amplifier and then fed to a speaker. A piston was glued on the speaker to generate a pressure wave. This pressure wave was coupled to the pneumatic micromanipulator to vibrate the glass pipette. The stimulus waveform was digitized at 30 Hz in synchrony with the RS-170 video signal. The total data collection time for each video sequence was 32 cycles of the stimulus. The video images were accumulated in the same frame buffer and then divided by 32 to obtain the averaged video frames.

Figure 3A shows a view of the stimulus actuator in contact with the pectinate zone of the basilar membrane. The vibration waveforms for the actuator and that of a selected location within the organ of Corti are shown in Fig. 3B. Aside from distortions caused by our analysis at the endpoints, both showed sinusoidal waveforms. Figure 3, C and D, displays two opposite phase patterns of the spatial distribution of the vibration around the basilar membrane. Note that the starting point of data acquisition is at the peak or valley of the stimulus waveform (due to the characteristics of the Wavetek waveform generator). Therefore all the displacements are relative to the starting point and thus are of a single sign, although they represent a complete cycle. The optical flow algorithm (see Optical flow analysis) computes the displacement of one frame based on the selected frame and the five preceding and the five following frames. If the stimulus period is not an exact integer multiple of 1/30 s, then this computation produces an end-effect that distorts the beginning and end of the cycle, affecting the first and last
five points. Thus even if a waveform is a pure sinusoid, the analysis itself introduces waveform distortion. Any individual flow-field other than the first and last five frames, is accurate (as in Fig. 3, C and D); errors only occur at the endpoints. This artifact can be avoided by increasing stimulus frequency so that a complete vibration period can be obtained even when the first and last five sampling points are discarded.

Optical flow analysis

The digitized video sequence of the image of the vibrating cochlear partition was analyzed using the optical flow technique (Barron et al. 1994). This technique can generate two-dimensional displacement measurements for all the objects within the field of view that possess sufficient contrast.

The illumination of the preparation is maintained at a constant level with the regulated DC power supply. In addition, the light transmittance of the cochlear structure remains constant within the short period of data acquisition (30–60 s). Therefore any temporal modulation of the video image intensity is a result of the motion of the structures under observation. Under this condition, the gradient constraint equation (Barron et al. 1994) can be used to estimate the two-dimensional velocity field of the structures within the video frame.

\[
\nabla I(\vec{r}, t) \cdot \vec{v}(\vec{r}, t) + \partial I(\vec{r}, t)/\partial t = 0
\]

where \( \vec{v}(\vec{r}, t) \) is the velocity for pixel \( \vec{r} \) at time \( t \), \( I(\vec{r}, t) \) is the spatial gradient of video image \( I(\vec{r}, t) \), \( \nabla I(\vec{r}, t) = [\partial I(\vec{r}, t)/\partial x]\hat{x} + [\partial I(\vec{r}, t)/\partial y]\hat{y} \); \( \hat{x}, \hat{y} \) are the unit vectors along the \( x \) and \( y \) axes, respectively. \( \nabla I(\vec{r}, t) \cdot \vec{v}(\vec{r}, t) \) is the vector dot product of \( \nabla I(\vec{r}, t) \) and \( \vec{v}(\vec{r}, t) \). \( \partial I(\vec{r}, t)/\partial t \) denotes the partial time derivative of the image \( I(\vec{r}, t) \). The instantaneous displacement for a given location \( \vec{r} \) is \( \vec{v}(\vec{r}, t)\Delta t \), where \( \Delta t \) is the

FIG. 1. Live-dead assay of the cochlea. A: 1½ h after dissection. The outer hair cells (OHCs), inner hair cell (IHC), and supporting cells in the organ of Corti exhibit green fluorescence. However, some cells in the spiral ligament and elsewhere show red fluorescence in their nuclei. B: 2½ h after dissection. The OHC and IHC cytoplasm turned yellow. This is possibly a mixture of red fluorescence from dead cells and green fluorescence from some cells that are still alive.
time interval between two consecutive video frames (33.4 ms in our system). In all the analysis results, the displacement vector is used instead of velocity vector.

There are two unknowns in Eq. 1: \( v_x(\vec{r}, t) \) and \( v_y(\vec{r}, t) \). The two-dimensional velocity \( \vec{v}(\vec{r}, t) \) cannot be solved solely based on Eq. 1, and an additional constraint needs to be introduced. In our analysis, a group of optical flow equations for a 5 × 5 pixel neighborhood was used to estimate the velocity for one pixel. To ensure that reliable velocity vectors were computed, an eigenvalue threshold (\( \lambda_1 \)) of value 0.2–1.0 was imposed on the group of the optical flow equations (Barron et al. 1994). This threshold eliminates the optical flow field for those regions that either have too poor contrast or the motion of which is too small to be reliably measured. Therefore in our experiments the interior of the tectorial membrane and the center of the modiolus usually did not contain any flow field vectors.

Digitized video images often contain various noises such as camera thermal noise, electronic circuit noise, and frame grabber quantization noise. Because the optical flow field is based on the differentiation of raw video images, the optical flow computation is relatively sensitive to noises present in the input image sequence. The input image sequence was passed through a low-pass filter to extract signal structure of interest and to enhance signal-to-noise ratio. In its current implementation, a spatiotemporal Gaussian filter is used. The Gaussian filter has a standard deviation of 1.5 unit. It takes the form

\[
F(x, y, t) = \frac{1}{\sqrt{2\pi\sigma_x\sigma_y\sigma_t}} \exp \left\{ -\frac{1}{2} \left[ \frac{(x - \mu_x)^2}{\sigma_x^2} + \frac{(y - \mu_y)^2}{\sigma_y^2} + \frac{(t - \mu_t)^2}{\sigma_t^2} \right] \right\}
\]

where \( \mu_x, \mu_y, \mu_t \) designate the center position of the filter in the \( x, y \) direction and on the time axis \( t \). Parameters \( \sigma_x, \sigma_y, \sigma_t \) are the standard deviations of the Gaussian filters in the \( x, y, \) and \( t \) directions, respectively. Standard deviations \( \sigma_x, \sigma_y \) were set to 1.5 pixel interval. Correspondingly, \( \sigma_t \) was set to 1.5 frame interval. The filters are sampled out to three standard deviations. This translates to five frames on both sides of the selected frame in the temporal dimension. As determined by Eq. 2, the filter kernel is (0.00102838, 0.00759876, 0.0360008, 0.109361, 0.213006, 0.266012, 0.213006, 0.109361, 0.0360008, 0.00759876, 0.00102838).

Before being applied to the actual hemicochlea preparations, the optical flow analysis was first calibrated with both digital and physical methods. In the digital method, one video frame was numerically extrapolated to generate a sequence of frames, each of which was translated by 1/32 to one pixel with respect to the preceding frame. One pixel has a physical size of 8 µm at the focal plane of the camera and corresponds to 0.2 µm in the cochlear tissue when using a × 40 objective. The displacement field was faithfully reproduced down to 1/32 pixel or an actual displacement of 6 nm. In the physical calibration, a sequence of video frames was captured of a micropipette vibrating with predetermined amplitudes. Vibrations as small as 20 nm were reliably quantified. The higher physical threshold is possibly due to the noise introduced by the video camera and the frame grabber.

**RESULTS**

Two sets of the optical flow analyses are presented in Fig. 4. Figure 4, A–C, is for the middle turn and Fig. 4, D–F, for the basal turn of the cochlea. In Fig. 4, A and B, motion patterns of the middle turn are shown for two opposite phases of the stimulus cycle. The source of stimulation was a 1.96-Hz sinusoidal vibration of 0.55-µm peak-to-peak amplitude delivered at the middle point of the basilar membrane, peripheral to the outer pillar foot (outside the image shown). The stimulus frequency was 0.94 Hz for the basal turn measurement (Fig. 4, D–F). The basilar membrane exhibited large scale bending at its upper surface. At the lower surface of the pectinate zone of the basilar membrane, a certain amount of local indentation was present due to the direct contact with the stimulation probe.

Several elements exhibit distinct motion patterns. The foot of the outer pillar cell and the arcuate zone of the basilar membrane (in Fig. 4, A–C) move perpendicular to the plane of the basilar membrane. Along the length of the outer pillar cell, the displacement vectors are in line with the pillar’s longitudinal axis. At the reticular lamina, the cuticular plates of the three rows of outer hair cells follow mainly radial motion. The inner hair cell stereocilia as well as its cuticular plate translate largely parallel to the plane of the basilar membrane. The apparent shearing stimuli to both inner and outer hair cells are of the same polarity. When the basilar membrane moves toward scala media, the input to the hair cells is in the excitatory direction ( Hudspeth and Corey 1977), inasmuch as the cuticular plate moves toward the modiolus. The Deiters’ cells and outer hair cells together form a physical connection between the reticular lamina and the basilar membrane. The motion vectors of these cells constitute a gradient field, which changes from transversal motion near the basilar membrane to radial motion near the reticular lamina. In addition, the tectorial membrane vibrates perpendicular to the plane of the basilar membrane with little radial component evident. Finally, the foot of the inner pillar cell shows little motion, indicating that the organ of Corti is constrained at the peripheral border of the spiral lamina, as expected. The characteristics discussed above also delineate the motion pattern of the basal turn, as shown in Fig. 4, D–F. The tectorial membrane has very high contrast around its boundary in Fig. 4, D–F. However, very few velocity vectors are measurable around this boundary, and most that are present appear to be of random orientation. This is a result of the aperture limitation present in the optical flow technique (Barron et al. 1994). For a linear boundary or a boundary with small curvature, the local sets of optical flow equations degenerate. Therefore such a set of optical flow equations has a very small \( \lambda_1 \) value and is thresholded out. This makes it impossible
to estimate two-dimensional velocity vectors for straight boundaries.

More quantitative assessment of the cochlear micromechanism can be obtained from Fig. 5, A and B, where vibration trajectories for one full cycle are plotted for some salient components of the middle turn cochlear partition. Corresponding plots for the basal turn are shown in Fig. 6, A and B. According to these data, the radial motion of the tectorial membrane is only 1/15th to 1/6th of the radial motion of the reticular lamina. The tectorial membrane exhibited different vibration patterns at its upper surface and undersurface (Figs. 3 and 4). It is the motion at the undersurface of the tectorial membrane that determines the shearing input to the hair cells. Therefore locations on the TM that are on its undersurface are used to represent the motion of the TM. The transversal motion of the tectorial membrane is also relatively small, about one-half to one-fifth of the amplitude of the basilar membrane vibration (Fig. 5B, d and f; Fig. 6B, d and f). The mechanical transmission ratio between radial reticular lamina displacement and basilar membrane motion can be calculated. Because the motion of the basilar membrane differs for different radial positions, the motion at the foot of the outer pillar is chosen as the basilar membrane input. At the foot of the outer pillar cell, the displacement is not purely transversal (Fig. 5Bd and Fig. 6Bf). In Fig. 6Bf, the outer pillar cell has a larger radial component, possibly because this measuring point is further away from the basilar membrane. Both the transversal and radial components are considered as the input motion. For outer hair cells this ratio is 0.7–1.1 (Figs. 5B, b and c, and 6B, a, b, and e). The corresponding ratio for inner hair cells is 0.7–0.9 (Fig. 5Ba and Fig. 6Bc).

The various components of the organ of Corti vibrate in synchrony and have negligible phase difference. In other words, the phase behavior of the cochlear mechanism agrees with the prediction of the ter Kuile kinematic model for the very low frequencies used in these experiments. However, the top of the inner hair cell seems to have a transversal motion that is in the opposite direction to that of the basilar membrane motion (Fig. 5Ba). This transversal motion is close to zero for the basal turn measurement (Fig. 6Bc). When the basilar membrane moves toward the scala media, the top of the inner hair cell moves toward the scala tym-
FIG. 4. Vibration patterns of the cross-section of the cochlear partition. A: middle turn, basilar membrane is fully displaced toward scala tympani. B: middle turn, basilar membrane is fully displaced toward scala media. C: outline sketch of the middle turn cochlear partition, corresponding to A and B. TM, tectorial membrane; IHC, inner hair cell; OHC, outer hair cell; IP, inner pillar cell; OP, outer pillar cell; BM, basilar membrane; DC, Deiters’ cell. D: basal turn, basilar membrane is fully displaced toward scala tympani. E: basal turn, basilar membrane is fully displaced toward scala media. F: outline sketch of the basal turn cochlear partition, corresponding to D and E. Legend is as in C. Vibration patterns are represented by overlaying the optical flow diagram onto the video image of the hemicochlea. Every thin yellow arrow points in the direction of the instantaneous displacement of the pixel where the arrow originates. Length of each yellow arrow represents the magnitude of the displacement for that pixel. Black calibration bar represents 100 nm of displacement for the yellow flow arrows. Calibration bar also represents 4 μm anatomic dimension. To display the small mechanical motion (0.1–0.5 μm), the flow arrows are magnified by 40 times relative to the anatomic structures. Large red arrows are added manually to summarize the overall features of the vibration patterns.
pani, and vice versa. This phenomenon, however, does not contradict the ter Kuile model. The ter Kuile model only requires that different components of the organ of Corti rotate as a single rigid body when the basilar membrane is displaced. In other words, the angular displacement of the different structures of the organ must be in phase. In Figs. 5B and 6B, the top of the inner hair cell moves toward the modiolus when the basilar membrane moves toward the scala media. This is in accord with the ter Kuile model. In addition, the transversal component at the top of the inner hair cell is toward scala tympani when its radial component is toward the modiolus.

FIG. 5. Two-dimensional trajectories of middle turn cochlear structures. Vibratory paths of different components of the cochlear partition are plotted for one full vibration cycle. Plus signs on the video image in A represent the anatomic locations of the structures for trajectories in Ba–Bf, respectively. A corresponds to the middle turn as shown in Fig. 4, A–C. The animal is a 21-day-old (DAB) gerbil. The stimulus frequency is 1.96 Hz. Each trajectory is composed of 16 concatenated arrows, each of which represents the displacement from one video frame to the next. Coordinates for the chosen cochlear structures were found using the National Institutes of Health Image software. The motion vectors for a given structure were then calculated by averaging the optical flow results for a $5 \times 5$ pixel neighborhood centered at the predetermined coordinates. Displacement units for both $x$ and $y$ axes are in $\mu$m. The $x$ axis is in the plane of the basilar membrane. The $y$ axis is perpendicular to the plane of the basilar membrane. All panels have the same scale.
DISCUSSION

To relate data obtained from hemicochlea experiments to the functioning of the cochlea in vivo, the differences and similarities between these two situations need to be addressed. One important difference between the hemicochlea and the cochlea in vivo is the loss of endocochlear potential (EP) and the replacement of endolymph with a fluid containing high sodium. As demonstrated experimentally, this replacement does not produce significant distortion (swelling or shrinking) of any structures within the cochlear partition, as long as Ca$^{2+}$ levels remain low (endolymph-like) (Edge et al. 1998). The absence of EP will decrease the receptor potential and therefore the driving force for outer hair cell electromotility (Ashmore 1987). Assuming the normal EP as +80 mV (von Békésy 1952) and the resting potential of outer hair cells as −70 mV (Dallos et al. 1982), the elimination of EP will reduce the receptor potential by ~50%. The outer hair cell receptor potential is further reduced by the depolarization of these cells due to the low intracellular [K$^+$] and high [Na$^+$] in vitro (Ashmore 1987). The response of the basilar membrane at the characteristic frequency can be reduced by >30 dB (Ruggero and Rich 1991) when EP is eliminated. It is therefore expected that the cochlear amplifier (Davis 1983) would be nonfunctional in the hemicochlea.

In our experiments the cochlea is sectioned into two halves. The coupling of acoustic energy via the cochlear fluids, which

![Two-dimensional trajectories of basal turn cochlear structures. A: basal turn location of a 21 DAB gerbil. B: basal turn responses for locations marked in A. Manner of plotting is the same as in Fig. 5B. The stimulus frequency is 1.7 Hz.](image-url)
is critical for the establishment of the traveling wave in the cochlea in vivo, is altered due to the open fluid space. Each unit of the hemicochlea partition, composed of the tectorial membrane, basilar membrane, and organ of Corti cross-section (a few cell widths in length), is poorly coupled to neighboring units via the surrounding fluid, especially at the cut edge. As measured in the hemicochlea, the vibration of the basilar membrane decays longitudinally at a rate of $-58$ dB/mm (Richter et al. 1998). This supports the notion of weakly coupled longitudinal cochlear units. Therefore the results from the hemicochlea study describe the passive and local behavior of the cochlear unit under observation. Such results provide the basis on which a databased integrated cochlear model can be established.

Another potential difference is the form of mechanical stimulus used to drive the basilar membrane. Here a glass micropipette was brought into contact with the middle point of the basilar membrane. Therefore the stimulus takes the form of a concentrated point stimulus. This concentrated point stimulation is different from the in vivo case, where a uniformly distributed pressure drives the basilar membrane into vibration. The mode of vibration for the cochlear partition might be different due to such discrepancy. As shown in Fig. 3, $C$ and $D$, the displacement of the basilar membrane was distributed along the width of the membrane. It was not limited to the contact point of the stimulation. This approximates the in vivo situation. However, this observation is not sufficient to rule out the possibility of a difference in the radial patterns of basilar membrane displacement between hemicochlea and the live cochlea. One possible method to study this issue is to place the point stimulator at different radial locations along the basilar membrane. If the measurement results remain consistent for the different locations of the input force, then the hemicochlea motion pattern can be regarded as a reasonable representation of the in vivo vibration mode. Otherwise, the prediction of the in vivo behavior of the cochlear partition can only be obtained through a synthesis of the motion patterns for different radial locations.

The cochlear partition was driven by a probe that vibrated with a sinusoidal waveform. Therefore the various other structures of the cochlear partition should follow a sinusoidal motion. At very low frequencies, as used in these experiments, the phase difference between the horizontal component and vertical component of the vibration is negligible. Therefore the vibration trajectories for all the structures should follow a straight line. As shown in Figs. 5$B$ and 6$B$, most of the trajectories do exhibit nearly linear paths. However, some loop paths are displayed by the trajectories of the tops of the outer and inner hair cells. The factors that produced these loop paths are as yet unknown.

Our conclusions do not support the hypothesis of opposite phase shearing for inner hair cell and outer hair cells as suggested by the analogy with a bending plate (Tonndorf 1974; von Békésy 1953). In such a model, the inner and outer halves of the reticular lamina undergo opposite shearing due to the bilateral fixation of the basilar membrane and the organ of Corti. The present work suggests that the organ is not restrained on both sides. The reticular lamina is possibly rigidly connected with the tunnel of Corti structure, which has high strength due to the inner pillar–outer pillar cell complex. The Hensen’s cell side is not constrained and simply follows the force exerted by the pillar complex. The shearing input to both inner and outer hair cells is toward the taller stereocilia when the basilar membrane is displaced toward scala vestibuli. This accords with the commonly accepted polarity of excitation (Davis 1958; Flock et al. 1962; Hudspeth and Corey 1977). Because the effective stimulus to the hair cell is the relative radial shearing between reticular lamina and undersurface of the tectorial membrane, this stimulus is clearly dominated by the motion of the reticular lamina. The material stiffness of the fibrillar main body of the tectorial membrane has been measured to be seven times smaller than the stiffness of the stereocilia of the outer hair cells (Zwislocki et al. 1988; Zwislocki and Cefaratti 1989). However, the hair bundles of the outer hair cells only interact with the undersurface of the tectorium, Hardesty’s membrane (Lim 1986). Strong attachment between Hardesty’s membrane and outer hair cell stereocilia has been shown (Lim 1986). It is possible that Hardesty’s membrane has considerably greater radial stiffness than the body of the tectorial membrane. Consequently, the shearing motion between the reticular lamina and the tectorial membrane may effectively drive the hair bundles of the outer hair cells. Due to the low excitation frequency of 1–2 Hz, these experiments do not address Zwislocki’s suggestion of mass-loading by the tectorial membrane and its resonant behavior (Zwislocki 1980; Zwislocki et al. 1988; Zwislocki and Cefaratti 1989).

According to Rhode and Geisler’s geometric model (Rhode and Geisler 1967), the ratio of outer hair cell shearing to basilar membrane displacement is $\sim 1.0$. In their model, the basilar membrane motion is defined as the displacement of the middle of the basilar membrane span. In our measurements, the basilar membrane input is defined as the motion of the junction of the arcuate and pectinate zones of the basilar membrane. The similarity of theoretical and experimental transmission ratios confirms the principal assumptions of their model. Our results argue against the existence of a 10-fold mechanical gain between the hair cell shearing and basilar membrane displacement (Russell et al. 1986), at least at low frequencies. Of course, it is conceivable that local resonances exist within the organ of Corti that would provide higher gain at appropriate frequencies. Our present results do not address this possibility.

Radial displacement at the reticular lamina was observed in a cochlea explant study (Reuter and Zenner 1990). However, such displacement was induced by electrical stimulation applied across the organ of Corti. The physiological basis for this radial displacement was hypothesized to be the electromotility of the outer hair cells. In case of the hemicochlea, the stimulation is simple mechanical. In addition, the absence of endo-cochlear potential, together with low outer hair cell resting potential, greatly reduces the effect of outer hair cell electromotility. Thus radial displacement is produced at the reticular lamina by both mechanical and electrical stimulation. Radial displacement of the reticular lamina was also observed by Ulfendahl et al. (1995), who also emphasize a lack of such component in tectorial membrane motion.

Hemicochlea experiments provide comprehensive information on the low-frequency passive mechanical behavior of the organ of Corti. From the optical flow analysis of the video motion sequences, it is revealed that the vertical vibration of basilar membrane is effectively transformed into the shearing motion at the interface between reticular lamina and tectorial
membrane. The polarity of the effective shearing is excitatory when the basilar membrane moves toward scala vestibuli. Such shearing motion is a result of the radial motion of the reticular lamina, with little contribution from the tectorial membrane. The pillar cells and Deiters’ cells follow the basilar membrane movement at their lower ends, and their upper ends exhibit both radial and transversal motion components. The inner hair cell body vibrates nearly parallel to the plane of the basilar membrane. The reticular lamina, as well as the top of the outer hair cells embedded in it, shows predominantly radial motion and a small amount of transverse motion. Our results provide experimental support for the main features of the classical paradigm of sensory hair cell stimulation in the cochlea (Davis 1958; ter Kuile 1900), at least at low frequencies.

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